



**EFFECT OF *COSTUS AFER* ON FERTILITY PARAMETERS IN
CYCLOPHOSPHAMIDE-INDUCED REPRODUCTIVE TOXICITY IN MALE ALBINO
RATS.**

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ABSTRACT

CP is an anticancer and immunosuppressive agent commonly used in men of reproductive age. This study was aimed at determining the effect of the ethanolic extract of *Costus afer* on the reproductive system of rats treated with CP. The Twenty rats used for the experiment were of albino strain, and were divided into four groups of five rats each. Group one (control) received 2ml/kg body weight of distilled water, group two were given CP at a dose of 6mg/kg, while group 3 (50mg/kg) and group 4 (100mg/kg) of ethanolic extract of *Costus afer* and CP one hour after extract administration once daily for 21days The CP treated group showed significant decrease in the body and organ weights and spermatogenic activities as well as many histological alterations. CP treatment also caused significant decrease in sperm count and motility with an increase in dead and abnormal sperms. Moreover significant decrease in serum levels of testosterone and increased concentrations of FSH and LH. Also Significant decrease in SOD and GPx activities and increase MDA level were observed in CP-treated rats, while co administration of *C.afer* caused a significant increase in SOD and GPx activities and decrease MDA level ($P < 0.05$). Notably, *C.afer* co-administration minimized the effect in the above-mentioned parameters. These findings indicated that *C.afer* is protective against CP-induced reproductive toxicity.

KEYWORDS: *Costus afer* biochemical parameters, reproductive toxicity, spermatogenic activities.

INTRODUCTION

Cyclophosphamide is an alkylating agent used widely in the treatment of cancer and nephritic syndrome. A wide range of adverse effect including reproductive toxicity has been demonstrated in human and animals.^[1] It induces oxidative stress and has cytotoxic effect on normal cells, especially in the reproductive organ. A major side effect of cyclophosphamide is the alteration of male reproductive function.^[2]

The use of plants as medicine by people dates as far back as the beginning of civilization. Plants are important sources of many biologically active compounds. Plants used in traditional medicine provide an interesting and still largely unemployed source for the development of new drugs.^[3] Globally about 85% of all medications for health care are derived from plants.^[4] Medicinal plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, cardioprotectives, antibacterials, antivirals and antiprotozoals. However, this study focuses on remedies for fertility. Conversely, a number of herbs used in the management of reproductive disorders were *Sphenocentrum jolyanum* for loss of libido in men, *Telferia occidentalis* and *Aframomum melegueta*

for low sperm counts.^[5] Others include *Boehiva diffusa*^[3], *Crataegus monogyna*.^[6]

Costus afer, of the family Costaceae, a perennial rhizomatous herb, is commonly called "spiral ginger", „ginger lily" or „bush cane"^[7]. „eti" by the Isokos and Urhobos and „monkey sugarcane" in Warri and most parts of Delta State, Nigeria. Most rural dwellers use this medicinal plant to treat upper respiratory tract and gastro-intestinal infections,^[8] gonorrhoeal,^[9] and syphilis.^[10] *Costus afer* is rich in phytochemical constituents. The leaves have been shown to contain an abundance of alkaloids and flavonoids and trace amounts of saponins, tannins and glycosides.^[10] Flavonoids and alkaloids have been reported to be antibacterial, antiviral, anti-inflammatory and antineoplastic.^[11]

Studies conducted on natural diets like plantain showed that its consumption by man could improve reproductive functions and also ameliorate certain reproductive dysfunctions.^{[12][13]}

The aim of this study therefore is to determine the effect of administration of plantain stem juice on fertility

parameters in cyclophosphamide induced reproductive toxicity in male albino rats.

MATERIALS AND METHODS

Animals

Twenty (20) adult healthy sexually matured male (3 months of age weighing between 180 and 260g) albino rats of Wistar strain were used in this study. The rats were obtained from the animal house of the Niger Delta University, College of Health Sciences, Bayelsa State and housed in standard cages. They were then allowed free access to standard feed (growers mash) and water for a period of two weeks to acclimatize to the cage environment prior to the commencement of the experiment. All the protocols were performed in accordance with the Institutional Animal Ethical committee (IAEC) as per the directions of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Chemicals

Cyclophosphamide was a product of CELON LAB Ltd INDIA. Kits from Teco diagnostics Ltd. USA, Sigma-Aldrich Ltd., U.S.A. PerkinElmer, USA were used. All other reagents/chemicals obtained from standard suppliers were of analytical grade.

Preparation of extracts

Fresh leaf of *Costus afer* were collected from a residential farmyard in Niger Delta University (N.D.U), Amassoma, Wilberforce Island, Bayelsa State, Nigeria and was botanically identified and deposited at the Herbarium of department of biological science, in Niger Delta University (N.D.U), Amassoma, Wilberforce Island, Bayelsa State, Nigeria. These were washed with distilled water, shade dried and pulverized. The leaves of *Costus afer* were thoroughly washed with distilled water to remove debris and contaminants, they were then dried in an oven at 40°C until a constant weight was reached and then pulverized using an electric blender (Blender, 462 Nakai Japan). 200g of the powdered of *Costus afer* was extracted in 600ml of absolute ethanol for 24 hours at room temperature with constant shaking using a flask shaker (Model, Denly A-500). The extract was filtered with Whatman No 1 filter paper and the resulting filtrate evaporated to dryness using a rotatory evaporator at 40°C, the resultant concentrate was then reconstituted in distilled water to give the required doses used in the study.

Experimental design and procedures

Experimental design

Twenty (20) adult male albino rats of average weight 200g were used in this research work. The animals were divided into four groups:

Group 1: Negative Control (2ml/kg body weight (bwt) distilled water orally for 21 days)

Group 2: Positive Control (6mg/kg Cyclophosphamide (cp), orally for 21days).

Group 3: *Costus afer* (50mg/kg) + (6mg/kg) Cyclophosphamide cp orally for 21 days

Group 4: *Costus afer* (100mg/kg) + (6mg/kg) Cyclophosphamide cp orally for 21 days

Cyclophosphamide(6mg/kg) in aqueous solution was administered orally an hour before the administration of extract consecutively for 21 days.

Sample Collection and Biochemical Analysis

After the experimental period, animals in all groups were sacrificed. By the end of each experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. 5ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 minutes before centrifuging at 800g for 5 minutes. The supernatant was used for the biochemical analysis. The testes and epididymis were excised using a midline abdominal incision. The testes were immediately weighed and the epididymis transferred into sterile bottles containing 10ml of normal saline for semen analysis. The testes were also transferred into 10% neutral buffered formalin for histopathological examination. Testes were excised and washed in cold saline, Ten percent tissue homogenates were prepared in 0.1M Tris -HCL buffer (pH 7.4).

Sperm characteristics

The sperm motility was evaluated from the caudal epididymis using a microscope according to the method of Selvakumar *et al.*^[14]

The dilution of epididymal sperm was used to determine the sperm count using the hemocytometer by the method of Zambrano *et al.*^[15]

Sperm morphology was determined by the method as described by Shalizar *et al.*^[16]

Biochemical parameters

a) Hormones

Enzyme-linked immunosorbent assay (ELISA) as described in the instructions provided by manufacturer's kits (PerkinElmer, USA) was used for the determination of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone concentrations.

b) Markers of oxidative stress/ disturbances

Catalase activity was determined by the method of Cohen *et al.*^[17] Super oxide dismutase (SOD) activity was measured by the method of Misra and Fridovich^[18] The Glutathione peroxidase (Gpx) activity was measured by the method of Chance and Maehly.^[19] as provided by Sigma-Aldrich Ltd., U.S.A. The assay method of Hunter *et al.*^[20] as modified by Gutteridge and Wilkins.^[21] was adopted for the assay of Malondialdehyde (MDA) concentration

Histopathological study

Small pieces of testes tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6µm in thickness were cut and stained with hematoxylin and eosin.^[22]

STATISTICAL ANALYSIS

Data was expressed as mean ± SD of five estimations. The statistical significance was evaluated by one way ANOVA using SPSS (Statistical Package for Social Sciences) version 16.0 and the individual mean compared by post Hoc LSD and Tukey method. Values were considered statistically significant when $p < 0.05$.

RESULTS

Body and testes weights were significantly decreased ($p < 0.05$) by cyclophosphamide treatment. After 21 days when compared with the normal rats. However, body and testes weights of rats exposed to CP and ethanolic leaf extract of *Costus afer* (50mg/kg) were significantly increased ($p < 0.05$) when compared to CP treated group (positive control) (Table 1.) The body and testes weights were also significantly increased in the group administered CP and ethanolic leaf extract of *Costus afer* (100mg/kg) when compared to the CP treated group (Table 1).

Table 1. Effect of ethanolic leaf extract of *Costus afer* (CA) on body weight (BW)g and weight of testes (TW)g in cyclophosphamide-induced male reproductive toxicity in male wistar rats.

Group	Treatment	Final body weight (BW)(g)	Testes weight (TW)(g)	Testes weight as % Body weight (%)
1.	Distilled water (normal)	244.40 ± 8.73 ^a	1.19 ± 0.04 ^a	0.49 ± 0.08 ^a
2.	CP (Positive Control)	191.20 ± 8.42 ^b	0.84 ± 0.10 ^b	0.43 ± 0.03 ^b
3	50mg/kg C.A + 6mg/kg CP	231 ± 7.60 ^a	0.94 ± 0.04 ^c	0.41 ± 0.01 ^c
4	100mg/kg C.A + 6mg/kg CP	240 ± 2.59 ^a	1.10 ± 0.31 ^a	0.46 ± 0.21 ^a

Values are expressed as mean ± SD (n=5). Means in the same column with different superscript Letter (s) are significantly different; $p < 0.05$ one-way ANOVA followed by post-hoc and Tukey.

The serum levels of FSH and LH was significantly increased ($p < 0.05$) and serum concentration of testosterone decreased significantly by treatment with CP alone when compared to the normal (Table 2). But the administration of *Costus afer* (50mg/kg) along with CP significantly decreased ($p < 0.05$) the FSH and LH levels

while that of testosterone increased significantly when compared to the positive control. The FSH and LH levels were also decreased ($p < 0.05$) while that of testosterone increased ($p < 0.05$) significantly for the group administered *Costus afer* (100mg/kg) when compared with the CP treated group (Table 2).

Table 2. Effect of ethanolic leaf extract of *Costus afer* (CA) on serum concentrations of sex hormones in cyclophosphamide-induced reproductive toxicity in male wistar rats.

Groups	Treatment	FSH (iu/L)	LH (iu/L)	Testosterone(ng/ ml)
1	Distilled water(normal)	6.86 ± 0.38 ^a	2.40 ± 0.68 ^a	6.34 ± 0.66 ^a
2	CP (positive control)	12.62 ± 0.78 ^b	7.04 ± 0.91 ^b	2.64 ± 0.59 ^b
3	50mg/kg C.A + 6mg/kg C.P	7.24 ± 0.36 ^a	2.82 ± 0.65 ^a	6.84 ± 0.42 ^a
4	100mg/kg C.A + 6mg/kg CP	7.06 ± 0.56 ^a	2.64 ± 0.46 ^a	6.46 ± 0.50 ^a

Values are expressed as mean ± SD (n=5). Means in the same column with different superscript letter(s) are significantly different; $p < 0.05$ one-way ANOVA followed by post-hoc and Tukey.

Treatment of male rats with CP caused a significant ($P < 0.05$) decrease in the Sperm count and motility, while dead and abnormal spermatozoa increased significantly ($p < 0.05$) when compared to the normal. But the administration of *Costus afer* (50mg/kg) along with CP significantly caused a decrease in dead and abnormal spermatozoa, it caused a significant increase ($P < 0.05$) in

semen quality when compared to CP treated group. There were also significant decreases ($P < 0.05$) in dead and abnormal spermatozoa and significant increase ($P < 0.05$) in semen quality by the group administered *Costus afer* (100mg/kg) when compared to the CP treated group.(Table 3).

Table 3. Effect of ethanolic leaf extract of *Costus afer* (CA) on epididymal sperm characteristics in cyclophosphamide-induced male reproductive toxicity in male wistar rats.

Group	Treatment	Sperm count (10 ⁶ /ml)	Motility (%)	Dead sperms (%)	Abnormal sperms(%)
1.	Distilled water(normal)	75.40 ± 5.41 ^a	83.60 ± 1.94 ^a	7.24 ± 0.86 ^a	5.46 ± 0.50 ^a
2.	C.P (control)	14.80 ± 1.92 ^b	40.60 ± 4.15 ^b	38.60 ± 2.70 ^b	31.80 ± 2.38 ^b
3	50mg/kg C.A + 6mg/kg CP	64.00 ± 3.39 ^c	72.20 ± 3.83 ^c	9.60 ± 2.50 ^c	8.00 ± 2.44 ^c
4	100mg/kg C.A + 6mg/kg CP	71.80 ± 2.59 ^a	78.60 ± 2.07 ^a	8.40 ± 2.30 ^a	6.20 ± 1.92 ^a

Values are expressed as mean ± SD (n=5). Means in the same column with different superscript letter(s) are significantly different; $p < 0.05$ one-way ANOVA followed by post-hoc and Tukey.

Administration of CP alone significantly decreased the antioxidant activity of SOD, GPx but increased significantly ($p < 0.05$) the MDA level (Table 4) but co administration of *Costus afer* (50mg/kg) alongside CP caused significant ($p < 0.05$) decrease in the MDA concentration and increases ($p < 0.05$) in SOD and GPx

activities when compared to the CP treated group. There were also significant decreases ($P < 0.05$) in MDA concentration and significant increases ($P < 0.05$) in SOD and GPx activities by the group administered *Costus afer* (100mg/kg) and CP when compared to the CP treated group.(Table 4).

Table 4. Effect of ethanolic leaf extract of *Costus afer* (CA) on superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Malondaldehyde (MDA).

Group	Treatment	SOD(u/mgprotein)	GPx(u/mg protein)	MDA(nmol/g)
1.	Distilled water (normal)	3.41±0.12 ^a	4.84±0.25 ^a	0.50±0.01 ^a
2.	C.P (control)	1.68±0.16 ^b	2.42±0.11 ^b	0.88±0.05 ^b
3	50mg/kg C.A +6mg/kg CP	3.07±0.15 ^c	4.12±0.44 ^c	0.62±0.04 ^c
4	100mg/kg C.A + 6mg/kg CP	3.37±0.18 ^a	4.69±0.24 ^a	0.56±0.05 ^a

Values are expressed as mean ± SD (n=5). Means in the same column with different superscript letter(s) are significantly different; $p < 0.05$ one-way ANOVA followed by post-hoc and Tukey.

Histopathological findings. Testes from group 1 showed a normal feature of seminiferous epithelium and Interstitial tissue with active spermatogenesis (figure 1). Testes from those treated with cyclophosphamide revealed a markedly shrunken seminiferous tubules with severe sperm cell aplasia and basement membrane thickening as well as rupture, vacuolization and fibrosis in interstitial and peritubular tissue (figure 2). Administration of *Costus afer* (50mg/kg) alongside CP restored these changes towards normalcy (Fig. 3). Testicular section of rats administered *Costus afer* (100mg/kg) and CP shows showing reversible atrophized seminiferous tubules of various sizes and shape. The interstitial space is widen with leydig cells.(fig. 4).

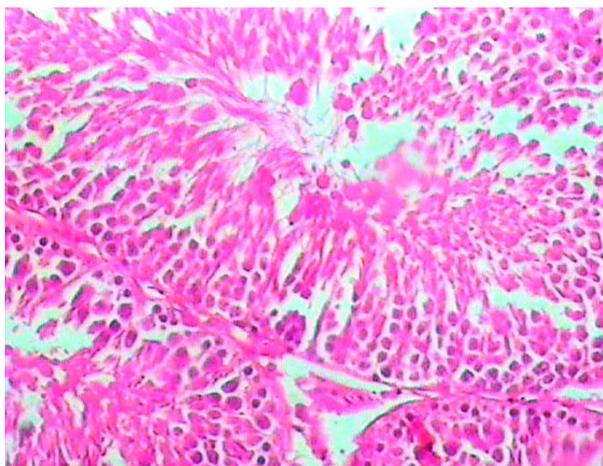


Fig. 1: Photomicrographs of testicular sections of control rats. Testes exhibiting a normal feature of seminiferous epithelium and interstitial tissue with active spermatogenesis (Mag x40).

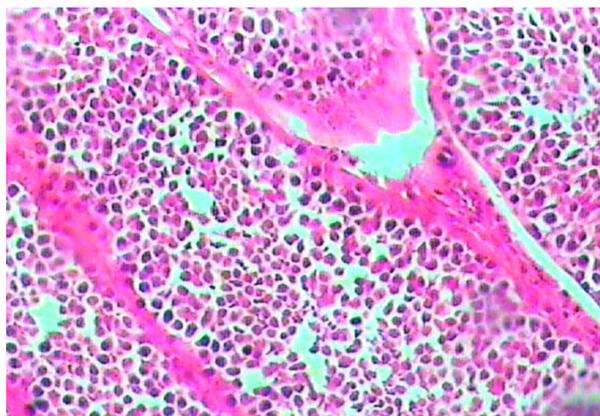


Fig. 2: Photomicrograph of testicular section of rats treated with cyclophosphamide reveals markedly shrunken seminiferous tubules with severe germ cell aplasia and basement membrane thickening showing numerous dilated seminiferous tubules with altered architecture. (Mag x40).

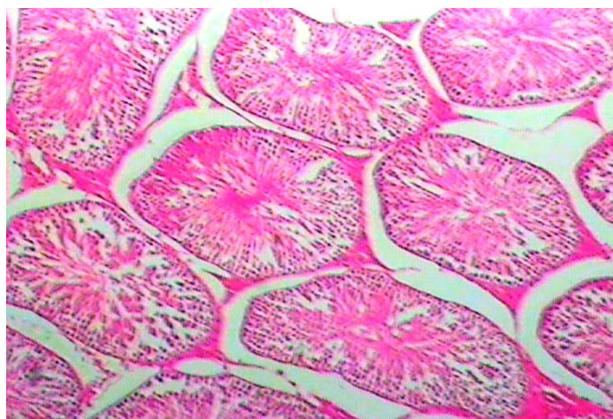


Figure 3: Photomicrograph of testicular section of rat treated with 50mg/kg *costus afer* and cyclophosphamide (6mg/kg). Showing numerous seminiferous tubules with intact membrane and leydig cells. The lumen of the seminiferous tubules is filled with sperm cells. (Mag x40).

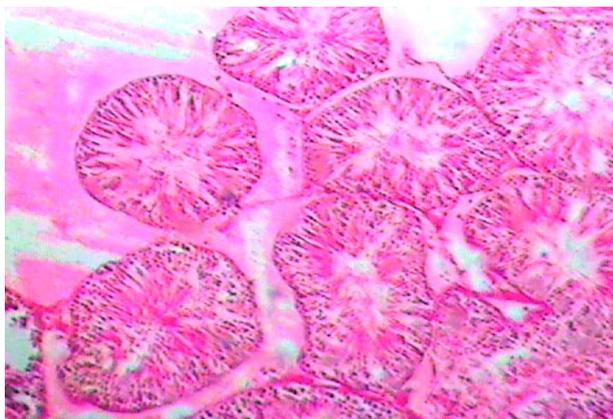


Figure 4: Photomicrograph of testicular section of rat treated with 100mg/kg *costus afer* and cyclophosphamide (6mg/kg). Showing reversible atrophied seminiferous tubules of various sizes and shapes. The interstitial space is widened with reduced Leydig cells. (Mag x40).

DISCUSSION

The serious social implications of infertility have made its prevalence a reason for public health interest in most developing nations.^[23] Social, economic and personal effects which go beyond childlessness can be caused by fertility problems.^[24] This is a major reason for marital problems in some localities.^[25] Studies conducted on diets of natural origin like plantain has revealed that consumption could improve reproductive functions and also ameliorate certain reproductive dysfunctions.^{[12], [13]}

In the present study, there were decreases in body and testis weights, histological changes in testis which are indications of CP induced toxicity. These decreases might be due to the mass of the differentiated spermatogenic cells which depends on the weight of the testes.^[26] Confirming our findings, the significant decrease in organ weight by CP might be due to diminished number of germ cells, atrophy of Leydig cells and a significant lower rate of spermatogenesis. Also the reduction in the availability of androgens might be the reason for the decrease in the organ weights in CP-treated rats.^[27] The serum concentrations of FSH and LH were significantly increased ($p < 0.05$) and serum level of testosterone decreased significantly by treatment with CP alone when compared to the normal (Table 2). Rezvanfar *et al.*^[28] reported no significant difference in LH and FSH levels between groups whereas CP decreased plasma testosterone concentration when compared to the control. In Satureja khuzestanica essential oil (SKEO) co-administration in cyclophosphamide induced reproductive toxicity in rats. Confirming our findings, A Shalizar Jalali *et al.*,^[6] found that serum concentrations of FSH and LH were significantly elevated, while serum testosterone decreased by CP in cyclophosphamide induced reproductive toxicity in rats.

The marked reduction in serum testosterone might be due to increased generation of free radicals which is one of the possible mechanisms involved in CP-induced

Leydig cell degeneration.^[29] Moreover, while the disturbance in Leydig cell function could be said to be the cause of the significant increases in serum LH level^[30], the elevation of FSH could be attributed to failure of spermatogenesis caused by a number of factors which include: testicular failure; genetic abnormalities and toxic exposure such as radiation, chemotherapy and heat.^[31] In a previous report by Higuchi *et al.*,^[32] CP induced an epididymis specific effect on sperm count and motility confirming the result of the present study where treatment of male rats with CP caused a significant ($P < 0.05$) decrease in the Sperm count and motility, while dead and abnormal spermatozoa increased significantly ($p < 0.05$) when compared to the normal. The damage of germ cells, spermatozoa and mature sperm has been shown to be as a result of the impairment of membrane fluidity and permeability.^[33] It has also been reported that CP causes cell death which might be responsible for the decrease in epididymal sperm count observed in CP-treated rats.^[34] The production of abnormal and dead sperms may be as a result of direct toxicity of CP activity on the spermatogenesis in the seminiferous tubules.^[35]

In our study, testicular SOD and GPx activities were significantly reduced and MDA concentration elevated in CP-administered rats when compared to the control. But co-administration of *Costus afer* (50mg/kg and 100mg/kg) alongside CP caused significant ($p < 0.05$) decrease in the MDA concentration and increases ($p < 0.05$) in SOD and GPx activities when compared to the CP treated group. (Table 4).

Confirming our point of view Das *et al.*^[36] and Ghosh *et al.*^[37] found that ascorbic acid and alpha-tocopherol-succinate respectively reduced cyclophosphamide induced reproductive toxicity. There are several reports on the benefit of antioxidants in protecting male reproductive system from deleterious effects of reactive oxygen species and other free radicals generated during CP exposure. There are other studies that have shown the improvement in the reproductive toxicity of CP through reduction of oxidative stress.^{[38] [39,40]} Therefore, oxidative stress could play a critical role in the induction of sperm abnormalities through DNA denaturation and fragmentation.^[41]

The result of this study shows that the co-administration of *Costus afer* (50mg/kg and 100mg/kg) was effective in protecting or attenuating testicular damage following CP exposure. This is in agreement with work by Yakubu *et al.*^[12] who reported that plantain fruits can be used in the management of sexual dysfunctions as seen in the present study, though the exact mechanism of action or how it enhances this is not known. Similarly in a study conducted by Ojewole and Adewumi,^{[11] [13]} it was reported that animal models given methanolic extract of *Musa paradisiaca* (MEMP) had their testicular damage reversed in diabetic-induced testicular disorders. The result obtained from this research showed that the

administration of *Costus afer* (50mg/kg and 100mg/kg) had protective effect against cyclophosphamide-induced reproductive toxicity which is in agreement with the work of Alabi *et al.*,^[42] on beneficial effects of low dose *Musa paradisiaca* on the semen quality of male wistar rats. This is also in agreement with work by Arhoghro *et al.*,^[43] who reported that co-administration of plantain stem juice minimized the adverse effect of CP exposure. Collectively, the results of this study showed that CP administration can cause testicular damage through oxidative stress, while co-administration of *Costus afer* at the various concentrations administered minimized the adverse effect of CP exposure based on parameters of testicular toxicity.

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