BIOCHEMICAL PARAMETERS AND SPERM CHARACTERISTICS IN MALE RATS GIVEN PLANTAIN STEM JUICE IN CYCLOSPHOSPHAMIDE-INDUCED REPRODUCTIVE TOXICITY

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ABSTRACT

Aim: This study was aimed at determining the efficacy of plantain stem (Musa paradisiaca) juice in reversing the cyclophosphamide (CP) induced reproductive toxicity in rat model. Study design: The twenty male rats (Wistar strain) were divided into 4 groups with each group having 5 rats. The rats in group 1 and 2 (negative and positive control) were administered with distilled water and cyclophosphamide (6mg/kg body weight) respectively, while Group 3 was given cyclophosphamide orally at the dose of 6 mg/kg body weight. However, group 3 was treated with plantain stem juice (2 mg/kg body weight) 1 hour after CP administration once daily for 21 days. Group 4 was treated with plantain stem juice alone also for 21 days. Methodology: Semen characteristics, serum testosterone, FSH, LH, testicular anti-oxidant enzymes, lipid peroxidation and histopathology of testes were the parameters assayed using Light microscopy and biochemical methods.

Results: The degree of sperm cell mortality; sperm count was significantly decreased (p<0.05) with an increase (p<0.05) in the abnormal and dead sperm in animals administered with CP (Group 2) compared to animals in the control group. Also serum FSH and LH levels were significantly reduced (p<0.05) with increase serum testosterone level in the rats which received a co-treatment of plantain stem juice and CP (6mg/kg) when compared to the rats which took CP alone. A significant increase in MDA concentration and decreases (p<0.05) in superoxide dismutase and glutathione peroxidase activities was noted in group 2 compared to the control group. Notably, plantain stem juice co administration and plantain stem juice alone compared favorably with the negative control in all the parameters. Testes of Animals
co-treated with plantain stem juice displayed nearly normal histoarchitecture and signs of recovery when compared with the control. **Conclusion:** These findings indicate that plantain stem juice is protective against CP-induced reproductive toxicity.

**KEYWORDS:** *Musa paradisiaca* biochemical parameters, oxidative disturbances, sperm characteristics.

**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]

According to Jordan *et al.*[7] Plantain is one of the most common herbs found. It can literally be seen growing out of the cracks of sidewalks and roads, found on the majority of homeowners lawn cultivated or waste ground or even in places where there is little sun.

Reports have shown Plantain (*Musa paradisiaca*) to be beneficial in the management of several disease conditions, including atherosclerosis, diabetes mellitus, hypertension, hyperlipidaemia and thyroid dysfunctions[8,9] and also offers protective effects on organs of the body, such as the kidneys, in certain clinical conditions.[10]

Studies conducted on natural diets like plantain showed that its consumption by man could improve reproductive functions and also ameliorate certain reproductive dysfunctions.[8][11]
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
Cyclosphoshamide 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
Plantain stem (Musa paradisiaca) was collected in a village bush called Amassoama, in Southern Ijaw Local Government Area of Bayelsa State. The plants were 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. The plantain was collected and outer green part of the stem was peeled off and its white inner portion was cut into small pieces (1000g). The pieces were crushed mechanically and the juice about 600ml was extracted. The juice was immediately stored at 4°C for later use.

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 21 days).
Group 3: Plantain stem juice (2ml/kg bwt) + (6mg/kg) Cyclophosphamide cp orally for 21 days
Group 4: Plantain stem juice (2ml/kg bwt). 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.[12]

The dilution of epididymal sperm was used to determine the sperm count using the hemocytometer by the method of Zambrano et al.[13]

Sperm morphology was determined by the method as described by Shalizar et al.

Biochemical parameters
a) Hormones
Enzyme-linked immunosorbent assay (ELISA) as described in the instructions provided by manufacturer’s kits (Perkin Elmer, 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.\textsuperscript{[14]} Super oxide dismutase (SOD) activity was measured by the method of Misra and Fridovich.\textsuperscript{[15]} The Gluthathione peroxidase (Gpx) activity was measured by the method of Chance and Maehly\textsuperscript{[16]} as provided by Sigma-Aldrich Ltd., U.S.A. The assay method of Hunter et al.\textsuperscript{[17]} as modified by Gutteridge and Wilkins.\textsuperscript{[18]} 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.\textsuperscript{[19]}

### 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 Plantain stem juice were significantly increased (\( p < 0.05 \)) when compared to CP treated group (control) (Table 1.) The body and testes weights were significantly increased in the group administered P.S alone when compared to the CP treated group (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Final body weight (BW)(g)</th>
<th>Testes weight (TW)(g)</th>
<th>Testes weight as % Body weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Distilled water (normal)</td>
<td>244.40 ±8.73\textsuperscript{a}</td>
<td>1.19±0.04\textsuperscript{a}</td>
<td>0.49±0.08\textsuperscript{a}</td>
</tr>
<tr>
<td>2.</td>
<td>CP (Positive Control)</td>
<td>191.20±8.42\textsuperscript{b}</td>
<td>0.84±0.10\textsuperscript{b}</td>
<td>0.43±0.03\textsuperscript{b}</td>
</tr>
<tr>
<td>3.</td>
<td>P.S + C.P</td>
<td>244.20±7.59\textsuperscript{a}</td>
<td>1.08±0.03\textsuperscript{c}</td>
<td>0.35±0.06\textsuperscript{c}</td>
</tr>
<tr>
<td>4.</td>
<td>P.S only</td>
<td>247.60±4.03\textsuperscript{a}</td>
<td>1.20±0.03\textsuperscript{a}</td>
<td>0.47±0.01\textsuperscript{a}</td>
</tr>
</tbody>
</table>

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 P.S 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 decreased (p<0.05) while that of testosterone increased (p<0.05) significantly for the group administered P.S. alone when compared with the CP treated group (Table 2).

Table 2. Effect of plantain stem juice on serum concentrations of sex hormones in cyclophosphamide-induced reproductive toxicity in male wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>FSH (iu/L)</th>
<th>LH (iu/L)</th>
<th>Testosterone(ng/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water(normal)</td>
<td>6.86±0.38a</td>
<td>2.40±0.68a</td>
<td>6.34±0.66a</td>
</tr>
<tr>
<td>2</td>
<td>CP (positive control)</td>
<td>12.62±0.78b</td>
<td>7.04±0.91b</td>
<td>2.64±0.59b</td>
</tr>
<tr>
<td>3</td>
<td>P.S + C.P</td>
<td>9.72±1.50c</td>
<td>5.28±0.69c</td>
<td>8.62±0.42c</td>
</tr>
<tr>
<td>4</td>
<td>P.S only</td>
<td>8.30±0.66a</td>
<td>3.78±0.94a</td>
<td>6.10±0.92a</td>
</tr>
</tbody>
</table>

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 P.S 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 P.S. alone when compared to the CP treated group. (Table 3).

Table 3. Effect of plantain stem juice on epididymal sperm characteristics in cyclophosphamide-induced male reproductive toxicity in male wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sperm count (10⁶/ml)</th>
<th>Motility (%)</th>
<th>Dead sperms (%)</th>
<th>Abnormal sperms(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Distilled water(normal)</td>
<td>75.40±5.41a</td>
<td>83.60±1.94a</td>
<td>7.24±0.86a</td>
<td>5.46±0.50a</td>
</tr>
<tr>
<td>2.</td>
<td>C.P (control)</td>
<td>14.80±1.92b</td>
<td>40.60±4.15b</td>
<td>38.60±2.70b</td>
<td>31.80±2.38b</td>
</tr>
<tr>
<td>3.</td>
<td>P.S + C.P</td>
<td>41.40±3.50c</td>
<td>48.20±5.71c</td>
<td>21.00±6.04c</td>
<td>19.60±4.15c</td>
</tr>
<tr>
<td>4.</td>
<td>P.S only</td>
<td>49.60±2.19d</td>
<td>65.40±6.18d</td>
<td>17.80±1.92d</td>
<td>13.40±3.64d</td>
</tr>
</tbody>
</table>
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 P.S 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 when compared to the CP treated group. (Table 4).

Table 4. Effect of plantain stem juice on superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Malondaldehyde (MDA).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SOD (u/mg protein)</th>
<th>GPx (u/mg protein)</th>
<th>MDA (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Distilled water (normal)</td>
<td>3.41±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.84±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.</td>
<td>C.P (control)</td>
<td>1.68±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.42±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.88±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.</td>
<td>P.S + C.P</td>
<td>2.46±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.50±0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.60±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.</td>
<td>P.S only</td>
<td>2.75±0.98&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.91±0.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.49±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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, vacoulization and fibrosis in intersttital and peritubular tissue (figure 2). Administration of Plantain stem juice along with CP restored these changes towards normalcy (Fig. 3). Testicular section of rats given plantain stem juice alone 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.

Fig. 2: Photomicrograph of testicular section of cyclophosphamide treated rats reveals markedly shrunken seminiferous tubules with severe germ cell aplasia and basement membrane thickening.
DISCUSSION

The serious social implications of infertility have made its prevalence a reason for public health interest in most developing nations.\textsuperscript{[20]} Social, economic and personal effects which go beyond childlessness can be caused by fertility problems.\textsuperscript{[21]} This is a major reason for marital problems in some locality.\textsuperscript{[22]} Studies conducted on diets of natural origin like
plantain has revealed that consumption could improve reproductive functions and also ameliorate certain reproductive dysfunctions.[8][11]

In the present study, there were decreases in body and testis weights, histological changes in testis which are indications of CP induced toxicity. This decreases might be due to the mass of the differentiated spermatogenic cells which depends on the weight of the testes[23]. 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 the reason for the decrease in the organ weights in CP-treated rats. [24] 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.[25] 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.[26] Moreover, while the disturbance in Leydig cell function could be said to be the cause of the significant increases in serum LH level[27], 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.[28] In a previous report by Higuchi et al.[29] 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. Comparing the Dead sperm it was 38.6% for CP alone as against 7.24% for the normal and 17.8% for PS only (though a bit high it is significantly lower than the CP alone which was 38.6%) a similar trend was observed for the abnormal sperm. 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.[30] 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.\textsuperscript{[31]} 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.\textsuperscript{[32]}

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 P.S 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 \textit{et al}\textsuperscript{[33]} and Ghosh \textit{et al}\textsuperscript{[34]} 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.\textsuperscript{[35][36,37]} Therefore, oxidative stress could play a critical role in the induction of sperm abnormalities through DNA denaturation and fragmentation.\textsuperscript{[38]}

The result of this study shows that the co-administration of plantain stem juice was effective in protecting or attenuating testicular damage following CP exposure. Yakubu \textit{et al}\textsuperscript{[8]} 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\textsuperscript{[11]} it was reported that animal models given methanolic extract of \textit{Musa paradisiaca} (MEMP) had their testicular damage reversed in diabetic-induced testicular disorders. The result obtained from this research showed that the administration of low dose of plantain (\textit{Musa paradisiaca}) stem juice had protective effect against cyclophosphamide-induced reproductive toxicity which is in agreement with the work of Alabi \textit{et al},\textsuperscript{[39]} on beneficial effects of low dose \textit{Musa paradisiaca} on the semen quality of male wistar rats. Although P.S might be cytotoxic from the results of the abnormal and dead sperm which were a bit high when compared to the normal. However other parameters assessed were even significantly statistically closer when comparing the PS to the normal. Collectively, the results of this study showed that CP administration can cause testicular damage tissue through oxidative stress, while co-administration of plantain stem juice minimized the adverse effect of CP exposure based on
parameters of testicular toxicity. However more work has to be done to investigate the possible cytotoxicity of the plantain stem juice alone.

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