Sperm Parameters and Testicular Histology of Male Wistar Rats Treated with Phoenix Dactylifera after Consumption of Local Mmahi Salt

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ABSTRACT

Phoenix dactylifera (date fruit) is one of the nutritious and healthiest fruits due to its excellent nutritional properties, and many beneficial health and medicinal properties. The aim of this study was to evaluate sperm parameters and testicular histology of male Wistar rats treated with Phoenix dactylifera after consumption of a dose of local Mmahi salt (local salt of Ebohnyi State origin, in Nigeria). Fresh date fruits, Mmahi salt, rat feed, clean drinking water, and 24 male wistar rats were the main materials for this study. The rats were divided into 6 groups: Group 1 (negative control group with 0% Mmahi salt (MS) and date fruit exposure), Group 2 (positive control I that received 75mg/kg of MS for 4 weeks), Group 3 (positive control II that received 75mg/kg of MS for 4 weeks followed by 2 weeks of self-recovery), Group 4 (that received 75mg/kg of MS for 4 weeks followed by 2 weeks of 200mg/kg of date fruit), Group 5 (that received 75mg/kg of MS for 4 weeks followed by 2 weeks of 400mg/kg of date fruit), and Group 6 (that received 75mg/kg of MS for 4 weeks followed by 2 weeks of 600mg/kg of date fruit). Actively motile spermatozoa in this study had a mean SEM in G1 (65.00±2.89), G2 (55.00±0.00), G3 (45.00±2.89), G4 (63.75±2.39), G5 (55.50±2.04), and G6 (58.75±2.39). Slightly motile spermatozoa were low in the groups treated with higher doses of date fruit extract, but least in G1. Non-motile spermatozoa were high in G2 and G3, but lower in G1, G5, and G6. G4, G5, and G6 had higher sperm counts compared to G2 and G3, although not significant. Histological examination showed in G1 - normal histology, in G2 - moderate spermatogenic arrest, in G3 - severe distortion of testicular architecture, in G4 - enhanced spermatogenesis, in G5 - moderate distortion of the seminiferous tubules, and G6 - enhanced spermatogenesis. A high dose of Mmahi salt is detrimental to sperm parameters and testicular histology. However, this study has shown that date fruit extract can improve sperm parameters and testicular histology in male wistar rats fed with Mmahi salt.

INTRODUCTION

Gametes are ova and sperm cells that are haploid and have one copy of each type of chromosome, i.e., 1–22 X or 1–22 Y (Ikwuka, 2023a). The sperm cell must fertilize an ovum in vivo or in vitro for conception (pregnancy) to occur. Human viral infections such as COVID-19 can affect women's pregnancy course (Okeke, 2023a; Okeke, 2023b). SCD is a hereditary hemoglobinopathy and can cause hemolytic anemia which is characterized by reduction in the number of circulating erythrocytes and/or hemoglobin due to increased destruction of erythrocytes (Ikwuka, 2023e). Even in fertility, pregnancy can be complicated by anemia due to SCD and despite the significant need for effective treatment options for SCD patients, current treatments both traditional and newly developed, only ameliorate acute and chronic SCD manifestations without addressing the underlying cause (Musa, 2023).

Infertility is a condition associated with psychological, economic, and medical implications resulting in trauma, stress, particularly in a social set-up in some countries, with a strong emphasis on child-bearing (Sharma, 2017). According to the International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO), infertility is a disease of the reproductive system defined by failure to achieve clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse (Zegers-Hochschild, 2009).

Infertility is one of the main issues with human reproduction that has gained global recognition (Baysah, 2023). Male infertility could be primary if a male has never successfully impregnated a woman or secondary if a male had previously impregnated a woman (Ikechebelu, 2003). Infertility is estimated to be 6% and 10% in the UK and the USA respectively (Ugwuja, 2008). The rate in Nigeria and some sub-Saharan African countries may surpass 30% (Larsen, 2000), an observation supported by (Ikechebelu, 2003), that stated that primary and secondary infertility had prevalence rates of 65% and 35% respectively in South-Eastern Nigeria (Ikechebelu, 2003). Male fertility is dependent on semen volume, count, motility, and morphology (Baysah, 2023).

The major causes of male infertility are identified to be testicular failure, testicular obstruction, cryptorchidism, low semen volume, sperm agglutination, idiopathic infertility, varicocele, erectile or ejaculatory dysfunction, abnormal viscosity, endocrine disorder, high density...
of sperm, congenital abnormalities like hydrocele, environmental causes, occupational hazards, pesticide exposure, tight underwears, sleep deprivation, alcohol and tobacco consumption (Bayasgalan, 2004; Baysah, 2023). Male infertility has also been associated with oxidative stress and different systemic immune inflammatory processes. In inducing oxidative stress, the major free radicals that are of physiological significance are superoxide anion, hydroxyl radical, and hydroperoxy radical, while non-radical is hydrogen peroxide (Ikwuka, 2023b).

Numerous environmental factors have been implicated in the global decline in male fertility. Many compounds implicated are ubiquitous in our modern society; and men may routinely encounter them daily (Krzastek, 2020). During the past 50 years, the rapid expansion of chemical industries in both developed and developing countries has resulted in releasing a plethora of xenobiotics into the environment (Singh, 2015). These toxic substances include heavy metals such as cadmium, lead, mercury, and arsenic (Krzastek, 2020) and a high dose of Mmahi salt from Okposi Salt Lake in Ohaozara Local Government Area of Ebonyi State, Nigeria (Ogbanshi, 2016), all pose a threat to spermatogenesis.

In addition, the interplay, role and effects of metabolic syndrome diseases on male fertility are still being investigated by different researchers (Baysah, 2023). Metabolic syndrome diseases, MSDs (Hypertension, Adiposity, Diabetes mellitus and Dyslipidemia) are interrelated diseases with very high morbidity and mortality rates (Ikwuka, 2015; Ikwuka, 2017a; Ikwuka, 2017c; Ikwuka, 2023c; Ikwuka, 2023f; Virstyuk, 2016). Results from different studies have shown that high levels of blood pressure, glucose and lipid metabolic disorders, asymptomatic hyperuricemia, activation of systemic immune inflammation and fibrogenesis, contribute to kidney damage (Ikwuka, 2017d; Ikwuka, 2017e; Ikwuka, 2018a; Ikwuka, 2018c; Ikwuka, 2018d; Ikwuka, 2019a; Ikwuka, 2019c; Ikwuka, 2022; Ikwuka, 2023d; Virstyuk, 2017a; Virstyuk, 2018a; Virstyuk, 2019; Virstyuk, 2021a; Virstyuk, 2021b). Adiposity, diabetes mellitus and dyslipidemia have also been linked with erectile dysfunction (Baysah, 2023).

Studies have recently concentrated on medicinal plants or their compounds in response to the growing need to decrease or stop the impacts of environmental factors on spermatogenesis. Due to the proximity of these medicinal plants to traditional healers, rural residents who live in locations where these harmful substances are released choose traditional remedies (Mbuni, 2020). Moreover, the concentration of conventional healthcare facilities in towns has made access to these facilities in rural regions difficult, making rural dwellers opt for traditional remedies (Maroyi, 2015).

Plant medicine has continuously been practised for centuries beginning from the Chinese era in the field of medicine (Van De Graff, 2001). Plants have served as a basis of many pharmaceuticals used today (Ekechi, 2023b; Ekechi, 2023b; Madukwe, 2013), and they make enormous secondary metabolites which offer defence against environmental stress (Nnam, 2012). Rauwolfia vomitoria has a neuroprotective ability at it elevates antioxidants and suppresses lipid peroxidation (Ekechi, 2023a).

Phoenix dactylifera (date fruit) is grown in many tropical regions of the world. It is chewy with a sweet flavour, and the fruit has become quite popular recently. Date fruit is high in antioxidants which protect cells from free radicals. The free radicals are unstable molecules and may cause harmful reactions in the body, leading to disease (Ekechi, 2023a). The three most potent antioxidants in date fruit are flavonoids, carotenoids, and phenolic acid, which together with other constituents of the fruit have many health benefits (Rahmani, 2014; Yun, 2006) such as lowering inflammatory markers, such as interleukin-6 (IL-6) whose high level is associated with neurodegenerative diseases e.g. Alzheimer's disease (Essa, 2016; Hüll, 1996), reduce the activity of amyloid beta proteins, which can form plaques in the brain (Essa, 2016), promote cervical dilation during pregnancy labor (Kordi, 2014), improve sperm quality, and prevent sperm damage (Chen, 2013). Nevertheless, Metabolic Syndrome Diseases also require new and effective treatment regimens. Dapagliflozin which is a Sodium-Glucose Linked Transporter 2 (SGLT-2) inhibitor and Liraglutide which is a Glucagon-like Peptide 1 Receptor Agonist (GLP-1 RA) have been found to increase the effectiveness of treatment and improve the clinical course of type 2 diabetes mellitus and hypertension in patients with such comorbidities (Ikwuka, 2017b; Ikwuka, 2018b; Ikwuka, 2019b; Ikwuka, 2021; Virstyuk, 2017b; Virstyuk, 2018b; Virstyuk, 2018c).

Exposure to chemicals or compounds has been demonstrated to make organisms infertile or in some circumstances, enable organisms to pass on the pathology to subsequent generations (Janardhanan, 2016). Literature is very scarce on new substances, in this case, Mmahi salt. This study therefore aimed to investigate the toxicity of Mmahi salt on the testes of male wistar rats and the ameliorating effects of Phoenix dactylifera on sperm motility and testicular histology.

MATERIALS AND METHODS

Date Fruit Collection and Extraction

Dried date fruits were purchased at Abakaliki and later identified by a taxonomist at the Herbarium of the Department of Applied Biology at Ebonyi State University, Abakaliki, Nigeria. The date fruits were soaked in clean water for 24 hours at 4°C after which the edible flesh was gently peeled, as described by (Akunna, 2012). The wet flesh was blended with a little water added in the process to form a homogenous product that was then filtered to remove large particles, and finally, a high concentration of smooth date fruit extract was obtained.

Preparation of Mmahi Salt Stock Solution

The Mmahi salt was gotten in Okposi community from the Okposi Salt Lake which is one of the salt lakes in

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Ebonyi State. The other lake is the larger Uburu Salt Lake in Uburu community, which produces salt sometimes (Omotayo, 2021). Both salt lakes are in Ohaezara Local Government Area of Ebonyi State, Nigeria. The Okposi Salt Lake is locally known as Mmahi Ezii (Agwu, 2021), and the salt lakes served as the inspiration for the Ebonyi State’s slogan as “Salt of the Nation” (Osisiohgu, 2020). The process used in forming the stock solution as well as its administration was based on the study carried out by (Ogbanshi, 2016) in which 7.5g of Mmahi salt was dissolved in 100ml of deionised water to form 75mg/ml stock solution.

**Experimental Design**

A total of twenty-four adult male wistar rats were procured from the animal house in the Department of Anatomy, Ebonyi State University, Abakaliki, Nigeria. The study was conducted in the same location, as such no acclimatization was required. These rats were separated into six (6) groups with four (4) rats in each group as shown in Table 1.

**Histological Examination**

All the animals were sacrificed by cervical dislocation at the end of their respective experimental period, and their testes were excised and fixed in 10% neutral buffered formalin. The testicular tissues were prepared using the routine histological technique as adopted by (Udeh, 2023a, Udeh, 2023b; Ekechi, 2023a).

**Epididymal Sperm Preparation and Tests for Sperm Count and Sperm Motility**

Epididymal sperm preparation was carried out after animal sacrifice. The right caudal part of each epididymis was excised from the rats, cut and placed in a beaker containing 5ml of physiological saline solution. The epididymis was rocked for 10 minutes to liberate their spermatozoa into the saline solution to form a suspension. Sperm count was done using the modified method of (Yokoi, 2004). The sperm suspension was diluted with 40% sodium bicarbonate formalin fluid at a ratio of 1:100. A Neubauer-ruled counting chamber (hemocytometer) was assembled and 10 microlitres of the diluted sperm suspension was aspirated, introduced into the counting area of the hemocytometer and examined under a light microscope. Three metrics were used to assess motility: proliferating cells, non-proliferating cells, and dead cells. According to observation, the rapidly motile, slowly motile, and non-motile cells were noted and expressed in percentages. The sperm viability was estimated by adding the progressive and non-progressive motile cells. Non-motile cells were the dead cells with no visible movement seen during microscopic examination.

**Data Analysis**

Collected data were cleaned, entered, hardcoded and analyzed with one-way analysis of variance (ANOVA) in the International Business Machine (IBM) Statistical Package for Social Sciences (SPSS) version 25 software. Results were presented as Mean±Standard Error of Measurement (SEM). p<0.05 was used as the significance level for comparisons.

**Ethical Clearance and Approval**

Ethical clearance and approval for this study was sought from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, Ebonyi State University. The Ethical Approval Code Number for this study is EBSU/FBMS/02/2018/22/002.

**RESULTS AND DISCUSSIONS**

**Result**

Microscopic Findings of Testicular Tissues

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Table 1: Experimental Design Involving Mmahi Salt

<table>
<thead>
<tr>
<th>Groups</th>
<th>Designation</th>
<th>Administration / Dosage</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>Rat feed and water</td>
<td>6 weeks</td>
</tr>
<tr>
<td>2</td>
<td>Positive control I</td>
<td>75mg/kg of Mmahi Salt only</td>
<td>4 weeks</td>
</tr>
<tr>
<td>3</td>
<td>Positive control II</td>
<td>75mg/kg of Mmahi Salt for 4 weeks, followed by 2 weeks of self-recovery</td>
<td>6 weeks</td>
</tr>
<tr>
<td>4</td>
<td>Mmahi Salt + Date fruits</td>
<td>75mg/kg of Mmahi Salt for 4 weeks, followed by 200mg/kg of date fruits for 2 weeks</td>
<td>6 weeks</td>
</tr>
<tr>
<td>5</td>
<td>Mmahi Salt + Date fruits</td>
<td>75mg/kg of Mmahi Salt for 4 weeks, followed by 400mg/kg of date fruits for 2 weeks</td>
<td>6 weeks</td>
</tr>
<tr>
<td>6</td>
<td>Mmahi Salt + Date fruits</td>
<td>75mg/kg of Mmahi Salt for 4 weeks, followed by 600mg/kg of date fruits for 2 weeks</td>
<td>6 weeks</td>
</tr>
</tbody>
</table>

Source: Field Work, 2022
Figure 1: (A & B) Photomicrographs of the section of testis of rats in group 1 (negative control) that received only rat feed and drinking water. Displayed are typical testicular architecture with active seminiferous tubules bordered with Sertoli cells (SC), interstitial cells of Leydig (ICL) between the lobules, and well-enhanced stages of spermatogenesis (WES). [H&E: x400].

Figure 2: (A & B) Photomicrographs of the section of testis of rats in group 2 (positive control I) that received 75mg/kg of Mmahi salt only for 4 weeks. The histoarchitecture shows moderate degeneration with moderate spermatogenic arrest (SA) as the inner portion of the seminiferous tubules is almost eroded (blue ring and black arrows) and pyknotic Sertoli cell (PSC) (yellow ring). [H&E: x100].

Figure 3: (A & B) Photomicrographs of the section of testis of rats in group 3 (positive control II) that received 75mg/kg of Mmahi salt for 4 weeks, followed by 2 weeks of self-recovery. Severe distortion due to tissue degeneration was seen. Severe spermatogenic arrest (SA) and focal area of hemorrhage (FAH) were also evident. [H&E: x100].
Figure 4: (A & B) Photomicrographs of the section of testis of rats in group 4 that received 75mg/kg of Mmahi salt for 4 weeks, followed by 200mg/kg of date fruit extract for 2 weeks. Histoarchitecture shows moderate regeneration (ES) with mild pyknotic (P) Sertoli cells. [H&E: x100].

Figure 5: (A & B) Photomicrographs of the section of testis of rats in group 5 that received 75mg/kg of Mmahi salt for 4 weeks, followed by 400mg/kg of date fruit extract for 2 weeks. Histoarchitecture shows moderate distortion of the seminiferous tubules as there was mild regeneration. Pyknotic (P) Sertoli cells and interstitial cells of Leydig had mild degeneration. [H&E: x100].

Figure 6: (A & B) Photomicrographs of the section of testis of rats in group 6 that received 75mg/kg of Mmahi salt for 4 weeks, followed by 600mg/kg of date fruit extract for 2 weeks. Histoarchitecture shows moderate regeneration with well-outlined testicular cells and well-enhanced spermatogenesis (WES). [H&E: x100].
Results for Sperm Motility and Sperm Count

Actively motile sperm cells for the six experimental groups, expressed in mean percentage±SEM were – G1(65.00±2.89), G2(50.00±0.00), G3(45.00±2.89), G4(63.75±2.39), G5(55.00±2.04), and G6(58.75±2.39). The positive control groups (G2 and G3) had low mean percentages for actively motile sperm cells. Groups 4, 5 and 6 had increased mean percentages, while the negative control group (G1) had the highest mean percentage of actively motile sperm cells. Table 2 showed that only group 3 showed a significant difference (p<0.05) when group 1 was compared to other groups, while groups 3, 4, 5, and 6 were not significantly different (p>0.05) when compared to group 2.

Table 2: Mean Percentage Values for Sperm Motility and Sperm Count

<table>
<thead>
<tr>
<th>Groups</th>
<th>AM (%)</th>
<th>SM (%)</th>
<th>NM (%)</th>
<th>Sperm count (x 10⁵/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>Mean±SEM</td>
<td>Mean±SEM</td>
<td>Mean±SEM</td>
</tr>
<tr>
<td>1</td>
<td>65.00±2.89</td>
<td>13.33±1.67</td>
<td>21.67±1.67</td>
<td>61.33±3.53</td>
</tr>
<tr>
<td>2</td>
<td>55.00±0.00</td>
<td>20.00±0.00</td>
<td>25.00±0.00</td>
<td>50.00±0.00</td>
</tr>
<tr>
<td>3</td>
<td>45.00±2.89</td>
<td>28.33±4.41</td>
<td>26.67±3.33</td>
<td>53.33±4.84</td>
</tr>
<tr>
<td>4</td>
<td>63.75±2.39</td>
<td>15.00±2.04</td>
<td>21.25±1.25</td>
<td>63.25±3.07</td>
</tr>
<tr>
<td>5</td>
<td>55.50±2.04</td>
<td>21.25±2.39</td>
<td>23.75±1.25</td>
<td>58.00±1.87</td>
</tr>
<tr>
<td>6</td>
<td>58.75±2.39</td>
<td>21.25±1.25</td>
<td>20.00±2.04</td>
<td>62.25±3.09</td>
</tr>
</tbody>
</table>

Source: Field Work, 2022

AM, SM and NM represent actively motile, sluggishly motile and non-motile respectively. a* denotes a significant difference (p<0.05) when each of groups 2, 3, 4, 5 and 6 were compared to group 1; a# denotes no significant difference (p>0.05) when each of groups 2, 3, 4, 5, and 6 were compared to group 1; b# represents no significant difference (p>0.05) when each of groups 3, 4, 5, and 6 were compared to group 2.

Discussion

Reproduction is a crucial biological characteristic for creating new individual organisms and is essential for both an individual's life and the survival and evolution of the species. Infertility is one of the world's top public health concerns because it affects 15% of couples who are of reproductive age and is caused by environmental and occupational exposure to hazardous substances (Baysah, 2023; WHO, 2020). Some substances have been documented to be extremely toxic, whereas others are not. The effects of the toxic components at low levels of exposure on the structure and function of the male reproductive system are well shown by the doses or exposures to the toxic compounds (Akunna, 2012; El Arem, 2014; El-Kott, 2014; El-Neweshy, 2012; Zare, 2020). The use of plants in the treatment of associated sickness has persisted. However, new pharmacological entities have been created due to studies into natural products such as date fruit. This study, therefore, focused on how date fruit ameliorated the toxic effects of Mmahi salt.

In G2 (positive control I) group, after being exposed to Mmahi salt for four weeks with no self-recovery or treatment with date fruit extract, photomicrograph of their testes revealed a mild degeneration with a moderate spermatogenic arrest, pyknotic Sertoli cells, and interstitial cells of Leydig. This finding agrees with the findings of (Fang, 2018) which highlighted that testicular morphological changes were discovered when the reproductive health of rams on a high-salt diet was examined. Moreover, a deformed structure is likely promoted by increased salt intake, which likely increases reactive oxygen species (ROS) in male hamster renal medulla cells and viable horse sperm (Burnaugh, 2010). It has been also reported that high salt dose leads to testicular distortions in wistar rats because salt intake impacts the...
male reproductive system negatively (Nwangwa, 2015). Distortion of the testicular architecture caused by severe degeneration of testicular tissues, spermatogenic arrest, and focal area of hemorrhage was noticed in the photomicrograph of group 3 testicular section which ingested Mmahi salt for four weeks, followed by 2 weeks of self-recovery. This shows that the effect of salt on testicular histology does not reverse even if the administration of salt stops. In the results of the semen analysis, the mean percentage of actively motile sperm cells for group 3 was significantly lower (p<0.05) when compared to G1 (negative control group), while sluggishly motile sperm cells (p<0.05) and non-motile sperm cells (p>0.05) were higher when compared to the negative control group. The sperm counts for groups 2 and 3 (positive control groups) were lower than the value in the negative control group 1. This shows that high dose of salt negatively affects sperm motility and sperm count. A significant decrease in epididymal sperm motility as a result of high salt diet (8%) compared with the control condition has been reported in rats (Adekunbi, 2016). In addition, a significantly high percentage of abnormalities (p<0.05) was noted in treated rats with 8% high salt diet relative to control rats (Adekunbi, 2016).

Another study observed that diet-fed rats with high salt (8%) had a significant increase (p<0.05) in sperm abnormalities, while no significant effects were detected in sperm motility with a high or low salt diet (Iranloye, 2013). The findings in these studies support this present study. However, contradictory results were obtained by (Lins, 2018), who demonstrated that moderate level of salt in the drinking water of pre-pubertal male sheep exhibited beneficial influences on sperm functions such as sperm motility, sperm concentration, and sperm vigour. This contradictory result might be attributed to different animals being studied. The date palm’s widespread use as a botanical and medicinal plant indicates how important it is to maintaining human health. Clinical studies have also outlined several advantages of date fruits (Echegaray, 2021). It has been experimentally shown to improve testicular parameters (Agwu, C. (2021)).

REFERENCES


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online: https://art-of-medicine.ifnmu.edu.ua/index.php/aom/article/view/179/150


