Original Article

EFFECT OF AQUEOUS EXTRACT OF PUMPKIN LEAF (TELFAIRIA OCCIDENTALIS) AND BITTER LEAF (VERNONIA AMYGDALINA) ON SOME SPERMATOZOA CHARACTERS OF MALE WISTAR RATS

1Otamere HO, 1Osifo UC, 1Nwaogwugwu CC, 1Balogun JJ, 1Akpamu U, 2Ujaddughe MO.

1Department of Physiology, 2Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria

Abstract

Aim: This study examined the effect of Telfairia Occidentalis and Vernonia Amygdalina on spermatozoa characteristics of adult Wistar rats.

Method: The twenty four (24) adult Wistar rats used in these experiments were randomly assigned to groups A, B, C, and D. Group A served as control and received normal rat chow and water throughout the experiment while group B, C and D, served as experimental groups. Group B and C received 500mg/kg of aqueous extract of Pumpkin Leaf and Bitter leaf respectively and group D received 500mg/kg body weight of the combined aqueous extract of both Leaves for 14 days. At the end of the experiment, semen examination was done to evaluate sperm parameters.

Result: Result showed that Telfairia Occidentalis has an increasing effect on sperm count, but a reducing effect on sperm motility. Similarly, aqueous leaf extract of Vernonia Amygdalina was observed to increase sperm count, improve sperm morphology but reduce sperm motility. On the other hand, the administration of the combined aqueous extracts of Telfairia Occidentalis and Vernonia Amygdalina produces significant improvement in sperm motility, count and morphology compared to group A, B and C.

Conclusion: The results suggests that Telfairia Occidentalis and Vernonia Amygdalina Leaves might improve some sperm functions, however, their combination has synergistic potential on sperm function and may improve fertility in male wistar rats.

Keywords: Infertility, Telfairia occidentalis, Vernonia amygdalina, Spermatozoa.

Introduction

Infertility is the failure of a couple to achieve pregnancy after one year of regular, unprotected intercourse.1 It can as well be described as “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (and there is no other reason, such as breastfeeding or postpartum amenorrhoea). Over the years, infertility has been on the increase in both males and females and has become an issue of global concern. Infertility may be caused by infection in the male or female, but often there is no obvious underlying cause. Research findings shows that a third each of infertility problem is as a result of male, female and both factors, while the remaining is unclear.2
More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. The remaining cases of male infertility can be caused by a range of conditions including anatomical problems, hormonal imbalances, and genetic defects. Sperm abnormalities are a critical factor in male infertility. These abnormalities include: low sperm count, poor sperm motility (movement) and abnormal sperm shape. Risk factors for male infertility include: varicocele (an enlarged varicose vein in the spermatic cord that connects to the testicle), aging (which can reduce sperm counts and motility and decrease the genetic quality of sperm), sexually transmitted diseases (which can cause scarring in the male reproductive system or impair sperm function), lifestyle factors (such as smoking and substance abuse), long-term or intensive exposure to certain types of chemicals, toxins, or medications.

Herbs have been used over the years for treatment of various diseases. According to Modi et al.; fluted pumpkin seed oil (FPSO) has been reported to possess some essential properties (vitamin A, tannins, linoleic acid, oleic acid and alkaloids) which suppress lipid peroxidation, hence, improving testicular function. Telfairia occidentalis has distinctive nutritional and phytochemical properties which can elicit varied morphological, physiological and biochemical effects.

*Vernonia amygdalina* is a valuable medicinal plant that is widespread in West Africa, it is known as bitter leaf due to its characteristic bitter taste and flavour, and can be used as an active anticancer, antibacterial, antimalarial and antiparasitic agent. This plant contains complex active components that are useful pharmacologically. In ethno medicine, the roots and the leaves are used to treat fever, hiccups, kidney problems and stomach discomfort.

Despite the fact that *Telfairia occidentalis* leaf and *Vernonia amygdalina* leaves are widely consumed by man in the Southern Nigeria and other parts of West Africa in addition to their versatile ethno medical usage, little is known about these leaves on the male reproductive system except for some unverified claims by herbal practitioners. The few reported studies on this subject present a plethora of apparently conflicting results, supposedly due to their largely limited scope.

**Materials and Methods**

**Extract Preparation:** *Telfairia Occidentalis* (Pumpkin Leaf) and *Vernonia Amygdalina* (Bitter Leaf) were procured from Ekpoma main Market in Esan West L.G.A, Edo state, Nigeria. They were taken to the herbarium unit of the Department of Botany of Ambrose Alli University, Ekpoma for identification and authenticication. The leaves were washed in distilled water and allowed to dry on the bench at room temperature for 5 days and then chopped into bits. 12.5grams of the chopped form was thoroughly blended in 24 mls of distilled water. The aqueous extract was obtained following filtration of the blended mixture into a conical flask using filter paper. The concentration of the filtrate (aqueous extract) was 500mg/ml and it was maintained throughout the study at refrigeration temperature at 1.6°C.

**Animals for the Study:** Twenty four (24) male albino rats (Wistar strain) was used for the study and was procured from the Animal House, College of Medicine, Ambrose Alli University, Ekpoma, Edo state Nigeria. They were kept in the animal holdings in the...
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laboratory of the Department of Physiology, Ambrose Alli University, Ekpoma, Nigeria.

Acclimatization: Animals were kept in cages that are adequately ventilated and equipped with convenient feeding and drinking through. They were allowed to acclimatize for at least two weeks before commencement of the experiment and were fed with normal rat chow and tap water ad libitum.

Semen Analysis: The semen was placed in 1ml of physiological solution (normal saline) after which a drop was placed on a clean glass slide, then covered with a glass cover-slip and it was observed under the light microscope (×40 and ×100 magnifications) for motility.

Animal Grouping: The animals were grouped into four (4) groups (A-D) and each group contains six (6) male rats. Group A served as control and fed normal rat chow and water only. Group B, C and D served as the experimental groups. Group B received Pumpkin leaf extract, w C Bitter extract and group D received both.

Experimental Procedure: 2mls syringe were used for measurement and oral gastric canula was used to administer the filtrates of Pumpkin and Bitter leaves to the rats that were used in this study. Group A (control) received distilled water while group B received 0.5ml (500mg/kg of aqueous extract of Pumpkin leaves) and group C received 0.5ml (500mg/kg of aqueous extract of Bitter leaf) respectively for 14 days.

1. Sample Collection and Analysis

Semen and Testes Collection: The rats were anaesthetized with chloroform for about 5 minutes and placed on dorsal recumbent. The testicles were surgically removed through a lower abdominal incision. The epididymis was trimmed off the testes and sample was collected from the caudal region through an incision made with a scalpel using a Pasteur pipette.

Sperm Count: This was achieved using the new improved Neubauer’s counting chamber (Haemocytometer). The semen fluid was diluted with physiological (normal) saline by adding 1.0 ml to the semen. The counting chamber was covered with a cover-slip until a rainbow picture is seen at the edges. This chamber was filled with sperm fluid and place under a binocular light microscope using an adjustable light source. The rule part was focused and the number of spermatozoa was counted in five 16-celled squares. The sperm concentration was calculated and multiplied by $10^6$ and expressed as $(X) \times 10^6$/ml, where $X$ is the number of sperm in a 16-celled square.

Statistical Analysis: The data obtained was analyzed using statistical package (SPSS), version 17. The statistics include mean ± Standard error of mean and analysis of variance (Anova), $p<0.05$ was considered significant.

Results

Sperm count showed a significant increase in group B, C, and D compared to the control ($p<0.05$). There was a statistically significant
increase in sperm motility of group D, while group B and C showed a significant decrease compared to the control (p<0.05). Result shows a significant improvement (P<0.05) in sperm morphology in group B, C and D compared with control.

Discussion

A wide range of medicinal plant sources have been reported to have various physiological effects due to their phytochemical and nutritional potentials. The present study on aqueous extract of *Telfairia Occidentalis* or *Vernonia Amygdalina* leaves or their combinations demonstrated various effects on sperm physiological parameters. Specifically, *Telfairia Occidentalis* at a dose of 500mg/kg has a reducing effect on sperm motility and a significant improving effect (p<0.05) on sperm count and morphology compared to control (Tables 1, 2 and 3). In accordance with this findings, has previously reported *Telfairia Occidentalis* to reduce motility of spermatozoa at 500mg/kg dosage and Saalu et al reported higher doses (400 mg/kg/day/oral and 500mg/kg/day/oral) to provoke varying degrees of testicular degeneration, deranged sperm parameters and worsened testicular oxidative status. Although the present study observed improved sperm morphology (such as increased normal sperm cells and reduced immature and disfigured sperm cells) in *Telfairia Occidentalis* treated group compared to the control, this is contradicting to the report of Saalu et al. Most of the active ingredients in *Telfairia Occidentalis* have well documented spermatogenic activities. The administration of this extract could therefore be regarded as a steady supply of additional nutrient to the treated rats over the control rats. As antioxidants, the flavonoids and vitamins in *Telfairia Occidentalis* Leave extract could maintain sperm morphology, sperm survival and sperm function. The reduction in spermatozoa motility may be subject to the degenerative changes caused by the extract in the seminiferous epithelium and may be similar to the type reported in several organs by Ajayi et al. The etiology of this pathology have been traced to be the presence of alkaloids, which have been observed with *Telfairia Occidentalis*. These alkaloids are bioactivated to release reactive metabolites, which bind to cell molecules and cross-link DNA to cause cellular damage. The relationship of the observed findings also may be explained by the fact that reduced fluid secretion and tubular contraction is seen with many testicular toxicants. In many case this is a secondary consequence of germ cell loss, but in others it appears to be an early event and probably presents disturbed sertoli cell function.

Since tubular fluid secretion is an androgen dependent function, compound that cause significant reductions in testicular testosterone levels will reduce fluid secretion and reduce tubular diameter as a secondary effect. Studies have shown the use of the concoction of fresh Pumpkin Leaves as a high-value health tonic for impotent men. Again, 500mg/kg aqueous leaf extract of *Vernonia Amygdalina* was observed to reduce sperm motility, increase sperm count and improve sperm morphology significantly (p<0.05) compared to control (Table 1, 2 and 3). In contrast to these findings, Longe et al and Oyeyemi et al have reported significant improvement in all sperm parameters in *Vernonia Amygdalina* ingestion. Specifically, Saalu et al showed that low doses (50mg/kg and 100mg/kg) of *Vernonia Amygdalina* increased sperm qualities.
Table 1: Percentage Spermatozoa Motility of Male Wistar Rat fed with Pumpkin Leaf and Bitter Leaf Extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>% Sperm Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - Control</td>
<td>87.50±2.74</td>
</tr>
<tr>
<td>B – Rats fed Pumpkin Leaf</td>
<td>84.17±8.01</td>
</tr>
<tr>
<td>C - Rats fed Bitter Leaf</td>
<td>84.17±3.76</td>
</tr>
<tr>
<td>D - Rats fed combined leave</td>
<td>91.33±3.83</td>
</tr>
</tbody>
</table>

n=6

Table 2 Sperm Count of Male Wistar Rat fed with Pumpkin Leaf and Bitter Leaf Extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sperm count (Million/ML)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - Control</td>
<td>57.50±4.18a</td>
</tr>
<tr>
<td>B - Pumpkin Leaf</td>
<td>66.33±5.47b</td>
</tr>
<tr>
<td>C - Bitter Leaf</td>
<td>68.33±5.16b</td>
</tr>
<tr>
<td>D - Pumpkin Leaf and Bitter Leaf combined</td>
<td>69.50±7.71b</td>
</tr>
</tbody>
</table>

n=6  *p<0.05

Table 3: Percentage of Dead Sperm Cells, Immature Sperm Cells and Normal Spermatozoa Morphology of Male Wistar Rat fed with Pumpkin Leaf and Bitter Leaf Extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Dead Cells (%)</th>
<th>Total Immature Cells (%)</th>
<th>Total Normal Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - Control</td>
<td>12.50±2.74ab</td>
<td>32.50±4.18a</td>
<td>56.50±5.82a</td>
</tr>
<tr>
<td>B - Pumpkin Leaf</td>
<td>15.83±8.01a</td>
<td>19.17±9.17b</td>
<td>73.33±8.16b</td>
</tr>
<tr>
<td>C - Bitter Leaf</td>
<td>18.83±3.76a</td>
<td>18.33±5.16b</td>
<td>75.00±5.48b</td>
</tr>
<tr>
<td>D - Pumpkin and Bitter Leaf combined</td>
<td>8.67±3.83b</td>
<td>16.33±7.12b</td>
<td>77.83±4.71b</td>
</tr>
</tbody>
</table>

n=6  *p<0.05
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Thus the findings of the present study may be due to the higher dose ingested (500mg/kg) which is asserted to have provoked sperm quality and possible testicular histology alteration. In view of the assertion, tannins (which is a constituent of Vernonia Amygdalina), though classified as antioxidant; at high dose has been reported to act as pro-oxidant and increase lipid peroxidation. Studies also, shows that treatment with antioxidants improves steroidogenesis by enhancing the primary effect of leydig cell endocrine function along with increased circulatory testosterone production and stimulation of spermatogenesis. The present study observations with Vernonia Amygdalina are in consistent with this report.

Furthermore, the administration of the combined aqueous extracts of Telfairia Occidentalis and Vernonia Amygdalina was observed to produce an increase in sperm motility, significant improvement (p<0.05) in sperm count and sperm morphology compared to A (control), B (Pumpkin leaf) and C (Bitter leaf) (Table 1, 2 and 3). Following intense literature search, this study appears to be first which examined the effect of the combined leaf extracts of both Pumpkin and Bitter leaves on spermatozoa qualities of Wistar rats. The findings appear stimulating. It is possible that improvements in all the sperm parameter seen in the rats treated with combined leaves extracts is due to dosage used (250m/kg extract of Pumpkin leaf and 250m/kg extract of Bitter leaf). The presence of oleic acid (a mono unsaturated fatty acid) in the Pumpkin leaves extracts reduces the susceptibility of testis to lipid peroxidation. This probably explains the improved sperm parameters as seen in the rats treated with combined leaves extracts. Even the antioxidative properties of vitamin A present in the combined extracts protects the testis against lipid peroxidation, hence, promotes spermatogenesis and improves structural differentiation of epithelia cells of the epididymis.

**Conclusion**

The leaf extracts of Telfairia Occidentalis and Vernonia Amygdalina have ability to improve some sperm functions and their combination

### Table 4: Mean Values of Abnormal Sperm Cells of Male Wistar Rat fed with Pumpkin Leaf and Bitter Leaf Extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tailless Head (%)</th>
<th>Headless Tail (%)</th>
<th>Bent-Tail (%)</th>
<th>Curved Tail (%)</th>
<th>Curve/Mid Piece (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - Control</td>
<td>6.67±5.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.67±2.25</td>
<td>1.17±2.04</td>
<td>0.83±2.04</td>
<td>1.33±2.16</td>
</tr>
<tr>
<td>B - Pumpkin</td>
<td>3.33±2.58&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.83±2.04</td>
<td>0.83±2.04</td>
<td>0.00±0.00</td>
<td>1.67±2.58</td>
</tr>
<tr>
<td>C - Bitter</td>
<td>1.33±2.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.83±2.04</td>
<td>1.17±2.04</td>
<td>0.83±2.04</td>
<td>0.83±2.04</td>
</tr>
<tr>
<td>D - combined</td>
<td>2.83±2.48&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.17±0.41</td>
<td>1.33±2.16</td>
</tr>
</tbody>
</table>

n=6
has synergistic potential on spermatozoa activity deserving of continuing research.

REFERENCES

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