Augmentation Potency of Ethanol Seed Extract of *Ricinodendron heudelotii* on Some Male Reproductive Hormones in Wistar Albino Rats

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Abstract: The quest for the use of plants in the management of reproductive hormone dysfunction prompted this research. This study explored the effects of *Ricinodendron heudelotii* seed ethanol extract on some male reproductive hormones. Twenty five male albino rats were used. The animals were grouped into five and each group had five (5) albino rats. The extract was processed from the seeds of *Ricinodendron heudelotii*. Group A served as control, group B animals were administered with 300mg/kg.bw, while groups C, D and E animals were administered 450, 600 and 750 mg/kg.bw of the extract respectively for twenty one days. The animals were sacrificed and their blood samples were collected for the assay of testosterone, Follicle Stimulating hormone (FSH) and Luteinizing hormone (LH). From the results obtained, the serum testosterone level of the experimental animals showed a concentration dependent increase in all the extract administered groups when compared with the value of the control group. FSH and LH levels showed an increase statistically (p≤0.05) in comparison to the level of the control. *Ricinodendron heudelotii* seed extract effectively increased the reproductive hormones of the animals by increasing the serum Testosterone, FSH and LH levels, hence suggests its effectiveness in enhancement of the male reproductive function.

Keywords: *Ricinodendron heudelotii*, Testosterone, Follicle Stimulating Hormone, Luteinizing Hormone

1. Introduction

Current increase in the rate of infertility in men has raised concern in the globe as it has led to various problems ranging from the home to the society at large. The use of synthetic conventional pharmaceuticals in the treatment of illnesses and biochemical disorders has been reported to be expensive [1], almost not readily accessible and with side effects. The presence of some metabolites found in plants [2, 3], enables them to carry out their therapeutic function through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process [4], hence the need to evaluate the plant of study for its assumed therapeutic value for the enhancement of reproductive functions in male wistar albino rats. The plant *Ricinodendron heudelotii* as revealed from study [5] contains phytochemicals that aids its potency to be used as a medicinal plant. Some of the medicinal properties of *Ricinodendron heudelotii* include; antimicrobial, anti-ulcer, antihyperlipidemic, and antioxidant biomarkers enhancer in diabetic oxidative stressed wistar albino rats [6].

Hormones play a vital role in semen production and men’s fertility [7]. The male reproductive hormones of concern in this study includes; testosterone, follicle stimulating hormone and luteinizing hormones.

Testosterone is a male hormone that has significant impact on spermatogenesis [8], maintains and produces the secondary sexual characteristics of the male, made in the
testes and enters systemic circulation in relatively constant concentration in a healthy and reproductive age male. Testosterone production enhances masculine, facial and body hair, and enlargement of the genital; it is also responsible for sex drive and works with FSH to stimulate sperm production. Testosterone levels are strongly correlated with libido and therefore testosterone is one of the prescriptions given to men with reduced sex drives [9].

Follicle stimulating hormones in males stimulates the production of sperm in the testes of men, are synthesized and secreted by the gonadotropic cell. They also regulate the growth, pubertal maturation and reproductive processes of the body [10]. Low frequency of gonadotrophin-releasing hormones increases FSH in ribonucleic acid level of rats, but is not directly correlated with an increase in circulating FSH.

Luteinizing Hormones (LH) causes the interstitial cells of the testes to produce testosterone and is produced in response to gonadotrophin-releasing hormone by the anterior pituitary gland. It stimulates the Leydig cell (testicular cells). Changes in LH and Testosterone blood levels and pulse secretion are induced by changes in sexual arousal in human males [11].

This study investigates the augmentation potency of *Ricinodendron heudelotii* ethanol seed extract on some male reproductive hormones in Wistar albino rats.

## 2. Materials and Methods

### 2.1. Plant Material

The dried seeds of *Ricinodendron heudelotii* were purchased from a local market in Port Harcourt, identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt. The clean seeds were pulverized into powder.

### 2.2. Preparation of Extract

The ground powder was subjected to extraction using 99% ethanol in the ratio of 1:3 ground powders to ethanol. After 72 hours, the mixture was filtered using whatman number 1 filter paper (Whatman qualitative filter paper No. 1, Camlab UK) and the extract was allowed to concentrate in a water bath to obtain a pure extract. The extract was stored at freezing temperature prior to its use for administration to the experimental animals.

### 2.3. Ethical Consideration in Animal Handling

The use of animals in this article was performed in accordance with the public health policy on Human Care and Use of Laboratory Animals of National Institute of Health [12].

### 2.4. Experimental Animals

A total of twenty-five male Wistar albino rats were purchased from the animal farm, Department of Biochemistry, University of Port-Harcourt and brought into the animal house, Department of Biochemistry, Rivers State University. The rats were weighed and allowed to acclimatize for 14 days.

#### 2.4.1. Administration Procedure

The twenty-five (25) male albino rats were divided into five (5) groups of five (5) rats per group. The treatment design was as follows:

1. **Group 1**: The rats were fed with normal feed and water only. It served as the control.
2. **Group 2**: The rats were fed with normal feed, water and were administered with a dose of 400mg/kg/bw of aqueous seed extract.
3. **Group 3**: The rats were fed with normal feed, water and were administered with a dose of 600mg/kg/bw of aqueous seed extract.
4. **Group 4**: The rats were fed with normal feed, water and were administered with a dose of 800mg/kg/bw of aqueous seed extract.
5. **Group 5**: The rats were fed with normal feed, water and were administered with a dose of 1000mg/kg/bw of aqueous seed extract.

#### 2.4.2. Duration of Administration/Bioassay

Administration was carried out daily for a period of 21 days after which the rats were sacrificed and their blood sample was collected for assay of testosterone, follicle stimulating hormone and Luteinizing hormone using the chemiluminescence immunoassay techniques using kits in accordance with standard protocols outlined by the kits producer Autobio diagnostics CO., Ltd. Zhengzhou, China [13]. Samples were collected from the animals via cardiac puncture with a 5.0ml syringe; this process was repeated for each of the rats.

### 2.5. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Student’s *t*-tests using a commercially available statistics software package (SPSS for Windows, V. 15.0) program. Results were presented as means±SD. *P* values≤0.05 were regarded as statistically significant.

## 3. Results

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Percentage Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>72</td>
<td>83</td>
<td>15.3</td>
</tr>
<tr>
<td>B</td>
<td>75</td>
<td>93.4</td>
<td>24.5</td>
</tr>
<tr>
<td>C</td>
<td>85</td>
<td>102.2</td>
<td>20.2</td>
</tr>
<tr>
<td>D</td>
<td>93</td>
<td>110</td>
<td>18.27</td>
</tr>
</tbody>
</table>
Table 2. Mean Weight of wistar albino rats before and after administration of extract and percentages differences for male reproductive function.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Percentage Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>83</td>
<td>115</td>
<td>39</td>
</tr>
<tr>
<td>B</td>
<td>93.4</td>
<td>146</td>
<td>56</td>
</tr>
<tr>
<td>C</td>
<td>102.2</td>
<td>152</td>
<td>50</td>
</tr>
<tr>
<td>D</td>
<td>110</td>
<td>158</td>
<td>45</td>
</tr>
<tr>
<td>E</td>
<td>123</td>
<td>188</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 3. Effect of ethanol seed extract of Ricinodendron heudelotii on some male reproductive hormones of wistar albino rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Testosterone</th>
<th>Follicle Stimulating Hormone</th>
<th>Luteinizing Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.75±0.6400°</td>
<td>0.29±0.1900°</td>
<td>0.29±0.0200°</td>
</tr>
<tr>
<td>2</td>
<td>1.95±0.6400°</td>
<td>0.55±0.2300°</td>
<td>0.43±0.0300°</td>
</tr>
<tr>
<td>3</td>
<td>2.40±0.2800°</td>
<td>0.81±0.0800°</td>
<td>0.63±0.0500°</td>
</tr>
<tr>
<td>4</td>
<td>2.65±0.0700°</td>
<td>0.92±0.0900°</td>
<td>0.94±0.0300°</td>
</tr>
<tr>
<td>5</td>
<td>3.10±0.7070°</td>
<td>2.25±0.2100°</td>
<td>1.25±0.0700°</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±Standard deviation.
Values with different superscripts show significant difference at p≤0.05 level.
Values with the same superscripts show no significant differences.

Figure 1. Photomicrograph of testes of experimental rats.

a. Slide for control group showing no histological change in the testes.
b. Slide for 400mg/kg bw showing normal sperm cells in the tubules and leydig cells.
c. Slide for 600mg/kg bw showing no obvious change in the anatomy of the testes.
d. Slide for 800mg/kg bw showing normal sperm cells.
e. Slide for 1000mg/kg bw showing normal sperm cells.

4. Discussion

The result on table 1 shows the effects of the ethanol seed extract of Ricinodendron heudelotii on the male reproductive hormone. There was an observed increase in the weight of the rats. Also the volume of feaces of the rats increased, with increase in their feed consumption. This suggests that the rats were in a healthy state. Table 2 shows the mean and percentage difference of animals before and after administration of ethanol seed extracts of Ricinodendron heudelotii. A significant increase in the weight of animals administered seed extract at 400mg/kg,bw (group B), 600mg/kg,bw (group C), 800mg/kg,bw (group D) and 1000mg/kg,bw (group E) was observed. The increase in weight appeared to be concentration dependent when
compared to the control group. This is expected because *Ricinodendron heudelotii* seed like the melon (egusi) seed is an oily seed [14], hence are also high in fatty acids. The increment in weight of the rats could be as a result of the high level of Poly Unsaturated Fatty Acids (PUFAs) [15] and alpha Eleostearic Fatty Acid which is a conjugated Linoleic acid [16].

Table 3 shows the effect of the seed extract on the male reproductive hormone. The table showed that serum Testosterone which serves to produce and maintain the secondary sexual characteristics of the male had a concentration dependent increase when compared with the control group. However, the increase was not statistically significant at \( p \geq 0.05 \). This increase in the groups as concentration increases could be attributed to the high content of Crude Fibers, Protein, Potassium in *Ricinodendron heudelotii* which makes it rich in nutrients [17] and serves as a potential nutritious food to improve the health status of its consumer. Also the high levels of Poly Unsaturated Fatty Acids (PUFAs) in the seed [18] could be suggested to have led to the increase. Some poly unsaturated fatty acids (PUFAs) are essential fatty acids and functions to aid disease conditions such as cardiovascular diseases. Long chain Poly Unsaturated Fatty Acids have been detected in the sperm cells of males, that gives the sperm Plasma Membrane the fluidity it needs to participate in the membrane fusion events associated with fertilization [19]. The seeds of *Ricinodendron heudelotii* are high in High Density Lipoprotein (HDL) [20].

HDLs are complex particles of multiple proteins, they are referred as good cholesterol, that functions to gets rid of cholesterol in the wall of the arteries and takes it to the liver to be removed from the body through the Intestines [21] and the synthesis of these sex steroid hormones require cholesterol as substrate [22].

The serum follicle stimulating hormone (FSH) and the luteinizing hormones (LH) that functions to regulate the growth, pubertal maturation and reproductive processes of the body of the animals administered with *Ricinodendron heudelotii* seed ethanol extract showed an increasing variation. The increase was observed to be concentration dependent as shown in table 3 and was significant at \( p \leq 0.05 \). The increase in serum FSH and LH levels can be attributed to the presence of phytochemicals such as Tannins, Flavonoids, Oxalate, Phenol, Phytate, Alkaloid and Saponin [5]. Flavonoids are found in a considerable amount in *Ricinodendron heudelotii* seed extract and functions as Anti-oxidant, Enzyme inhibitors, Immune or Hormones modulators [23] and specific Flavonoids are identified to have a wide range of biological properties that has good effects.

The photomicrographs of the testes as shown in Figure 1 showed that for group A which is the control group, there was no obvious histological change in the testes. The photomicrographs of groups B and C which were administered with 400mg/kg and 600mg/kg.bw respectively, normal sperm cells in the tubules and Leydig cell. The photomicrograph of groups D and E which were administered with 800mg/kg and 1000mg/kg.bw respectively showed no obvious changes in the microscopic anatomy of their testes. Therefore, as observed in Figure 1, at lowered concentrations there was no distortion in the testicular functions and sperm cell of the rats, at increased concentrations the testes also showed its normal integrity and no obvious histological changes was observed.

5. Conclusion

Based on the results obtained from this research study, the ethanol seed extract of *Ricinodendron heudelotii* possess some properties that gives the seed the potency to be used for the enhancement of the male reproductive hormones while still serving as a good source of nutrient, thereby agreeing with the statement made by Hippocrates, the Father of medicine “Let food be thy medicine and medicine thy food”.

Conflict of Interest

The authors declare that they have no competing interest.

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