Changes in Sperm Morphology and Characteristics of Experimentally-induced Hypertensive Wistar Rats Treated with Lagenaria breviflora Roberty or Xanthosoma sagittifolium Exell

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Authors’ contributions

This work was carried out in collaboration among all authors. Author OAO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author OOA carried out the study and statistical analyses. Author TSL managed the animal study and managed the literature searches. Author ABS designed the study and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: This study was designed to evaluate the male reproductive toxicity that may accompany treatment of hypertension in Wistar rats with methanol extracts of whole fruit of Lagenaria breviflora Roberty or corm of Xanthosoma sagittifolium Schott.

Place and Duration of Study: The study was carried out at the Animal House of the Department of Veterinary Pharmacology and Toxicology between November, 2016 and January, 2017.

Methodology: Antihypertensive study was carried out in 40 adult male Wistar rats equally and randomly distributed into 8 groups. First group was normotensive rats administered with distilled water, while hypertension was induced in groups 2-7 intraperitoneal administration of DOCA-salt...
1. INTRODUCTION

Infertility in the male is the inability to produce fertile sperm cells or semen for fertilization of fertile ovum, which does not lead to conception of the female bred [1]. Male infertility may be induced by many factors which include diseases, exposure to environmental conditions, adverse drug effect or plants [2]. One lifelong non-infectious disease with infertility as one of its sequels is hypertension. Several studies examining hypertensive men demonstrated a significant inverse relationship between blood pressure and total serum testosterone, which could be associated with impaired reproductive potential, free testosterone and sex hormone-binding globulin [3,4,5,6].

Clinically, various classes of antihypertensive drugs such as Angiotensin Converting Enzymes Inhibitors, calcium channel blockers, loop diuretic have been used to manage hypertension and to alleviate symptoms [7,8]. However, the efficacy of these drugs is only 40-60% usually with a combination of two or more antihypertensive drugs from different categories and this invariably increases the cost of treatment and accompanying side effects [9]. Frequently observed adverse effects of synthetic antihypertensive drugs include emotional distress, gastrointestinal disturbance, dry mouth, dizziness, cough, visual disorders, headache, peripheral circulatory symptoms like cold hands and feet, pedal oedema [10,11].

Herbal medicines have been commonly used as alternative therapies and remain so instead of synthetic drugs because of their possible fewer side effects [12]. Medicinal plants reported to be used in the treatment of hypertension include Colocasia esculenta [13], Guiera senegalensis leaves [14]. The focus of this study was on the possible adverse effects that may accompany treatment of hypertension with Lagenaria breviflora whole fruit or Xanthosoma sagittifolium corn, with particular emphasis on the reproductive toxicity in experimentally-induced hypertensive male Wistar rats. These medicinal plants have been traditionally recommended to hypertensive patients with some unverified result. L. breviflora (family Cucurbitaceae) is locally used for treatment of diseases of inflammatory origin and known to possess potent antioxidant, anti-inflammatory and analgesic properties [15,16,17]. It is reported to lower fertility in normal Wistar rats [18]. X. sagittifolium (family Araceae) is a very close relative of C. esculenta which is widely used as a replacement source of carbohydrate for diabetics [19]. It has potent antioxidant property as well [20], but there is a sparsity of information on its effect on the male reproductive system. Male reproductive toxicity will be assessed by the changes in the spermatozoa morphology and characteristics.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extracts

Fresh fruits of Lagenaria breviflora Roberts and corms of Xanthosoma sagittifolium were purchased from Bodija Market, Ibadan, Nigeria in July 2015 and identified by botanists at the Department of Botany, University of Ibadan. L. breviflora was dried at room temperature. The dried extract was ground to fine powder using a mill. Dried extract of X. sagittifolium was prepared by milling the corms and dried at room temperature. The dried extract was ground to fine powder using a mill.
**2.2 Experimental Animals**

Forty normotensive male Wistar rats (150 - 200g) were used for the study. The rats were housed at the Experimental Animal House of the Department of Veterinary Pharmacology & Toxicology, University of Ibadan. They were allowed access to feed (commercially available rat pellets) and water *ad libitum*. The animals were humanely handled in accordance with the guidelines for the Animal Care and Use Regulation Ethical Committee of the University of Ibadan, Ibadan, Nigeria.

**2.3 Experimental Procedure**

The animals were randomly and equally divided into eight groups with 5 rats each. Rats in Group 1 were maintained as control normotensive, while hypertension was induced and maintained in groups 2-8 by intraperitoneal injection of 11-Deoxycorticosterone (DOC) Salt (25 mg/kg) twice weekly and daily inclusion of 2% sodium chloride in drinking water. The blood pressure of the rats was determined before commencement of treatment.

Group 1 was normotensive rats fed with commercial rat pellet and allowed access to clean water *ad libitum*. Group 2 were hypertensive untreated rats, groups 3 and 4 were hypertensive rats treated with Lisinopril 5 mg/70 kg or Hydrochlorothiazide 12.5 mg/70 kg body weight. Groups 5 and 6 were also hypertensive rats treated with *L. breviflora* Roberty methanol extract at 100 mg/kg or 200mg/kg body weight, while groups 7 and 8 were treated with *X. sagittifolium* methanol extract at 100mg/kg or 200mg/kg body weight. All treatment was done orally using oro-pharyngeal cannula. The hypertensive rats were also fed with commercial rat pellet but allowed access to clean drinking water with 2% NaCl *ad libitum*.

**2.4 Sample Collection and Analysis**

The rats were dosed for five (5) weeks and they were humanely sacrificed at the end of the 5th week. The male genitalia were surgically exposed to harvest the cauda epididymis and the left testicle. Semen samples from the cauda epididymis were analyzed for motility, sperm concentration, Live-Dead ratio and count. The cauda epididymis was incised and a sample of the semen was put on glass slide and a drop of normal saline was added. The glass slide was covered with cover slip and viewed under microscope to check for the motility.

Smear of the same glass slide used for motility test was used for Live-dead ratio and Morphology. The glass slide was stained with Eosin-Nigrosine and viewed under Light microscope. Spermatozoa morphology determined include headless tail, tailless head, rudimentary tail, curved tail, bent tail, curved mid-piece, bent mid-piece and looped tail. The cauda epididymis was crushed in 5ml Normal Saline. A drop of the cauda epididymis homogenate was placed on haemocytometer and viewed under light microscope for Sperm concentration.

**2.5 Statistical Analysis**

Data from this study were presented as mean±Standard Error of Mean (SEM). Differences between the mean values were determined at p<0.05 using one-way ANOVA and Duncan post-hoc test on GraphPad Prism 5.0.

**3. RESULTS**

**3.1 Sperm Morphology**

There was a non-significant increase in the number of spermatozoa with normal head without tail in all the groups. The percentage abnormality in rats group 5 was highest (5.0±0.55%). A non-significant increase was observed in the number of spermatozoa with normal tail without head but the rats in Group 3 had a lesser number (4.0±0.55%) when...
There was non-significant reduction in sperm motility was observed in the volume of spermatozoa for each of the treatment groups compared to the control group. A non-significant increase was observed in the volume of spermatozoa with normal head with bend tail compared to rats of group 3 with least increase (5.16±0.02) compared to control group. Apparently, its statistically unchanged. A significant reduction in sperm cell count in rats of the treatment groups was observed compared to the rats of the control group with the highest sperm count (142.8±4.10).

4. DISCUSSION

This study evaluated the toxicological effect of methanol extract administration of L. breviflora Roberty and Xanthosoma sagittifolium Schott on male reproductive system of Wistar rats using total sperm cell count, morphology, motility and live/dead sperm cell ratio. Male fertility can be assessed by these andrological parameters as indices of ability of a sperm cell to fertilize a fertile ovum, with >50% abnormal sperm cells, <50% motility and <20X10^9/ml cells considered critical percentages in human males [21,22].

The sperm cell morphological abnormalities are classified as primary or secondary abnormalities and both types were observed in rats orally administration of L. breviflora Roberty and X. sagittifolium Schott in this study [23]. The only primary sperm abnormality was rudimentary tail, while normal head without tail, normal tail without head, bent tail, curved tail, curved mid-piece, bent mid-piece, and looped were secondary abnormalities observed. Primary abnormalities are usually associated with the spermatogenesis, while secondary abnormalities are usually due to changes taking place during maturation and storage of spermatozoa in the epididymis [18].

A non-significant increase in rudimentary tail sperm abnormality was observed in rats treated with the extracts relative to the control rats, while those treated with Lisinopril and hydrochlorothiazide had similar total number of abnormal sperm cells. Aberrations in the process of spermatogenesis cause primary sperm abnormality with rudimentary tailed cells usually immotile, and unable to fertilize mature ovum [24].
Table 1. Percentage occurrence of different sperm abnormalities observed in the rats of the control and test groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Tail-less head (% Headless tail (%</th>
<th>Rudimentary tail (%</th>
<th>Bent tail (%</th>
<th>Curved tail (%</th>
<th>Curved mid-piece (%</th>
<th>Bent mid-piece (%</th>
<th>Looped tail (%</th>
<th>Total sperm count</th>
<th>%Sperm Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>4.2±0.37</td>
<td>4.4±0.51</td>
<td>1.4±0.24</td>
<td>8.0±0.55</td>
<td>8.0±0.32</td>
<td>9.0±0.45</td>
<td>1.6±0.40</td>
<td>412±1.22</td>
<td>10.83</td>
</tr>
<tr>
<td>Hypert</td>
<td>4.2±0.58</td>
<td>4.4±0.51</td>
<td>1.6±0.40</td>
<td>9.2±0.20</td>
<td>8.8±0.37</td>
<td>9.6±0.40</td>
<td>1.6±0.40</td>
<td>406±2.91</td>
<td>12.02</td>
</tr>
<tr>
<td>Lisinop</td>
<td>4.2±0.58</td>
<td>4.0±0.55</td>
<td>1.4±0.24</td>
<td>10.0±0.32</td>
<td>10.8±0.37</td>
<td>10.2±0.20</td>
<td>1.8±0.37</td>
<td>405±2.77</td>
<td>13.02</td>
</tr>
<tr>
<td>Hydrochl</td>
<td>4.4±0.51</td>
<td>4.4±0.51</td>
<td>1.4±0.24</td>
<td>10.4±0.24</td>
<td>10.8±0.37</td>
<td>10.2±0.20</td>
<td>2.6±0.50</td>
<td>407±2.55</td>
<td>13.32</td>
</tr>
<tr>
<td>Lb100</td>
<td>5.0±0.55</td>
<td>4.6±0.51</td>
<td>1.8±0.37</td>
<td>10.0±0.45</td>
<td>10.4±0.87</td>
<td>10.4±0.93</td>
<td>2.2±0.37</td>
<td>403±2.00</td>
<td>13.77</td>
</tr>
<tr>
<td>Lb200</td>
<td>4.6±0.51</td>
<td>4.5±0.51</td>
<td>2.0±0.45</td>
<td>9.8±0.86</td>
<td>11.2±1.07</td>
<td>11.6±0.81</td>
<td>2.2±0.37</td>
<td>406±2.92</td>
<td>13.84</td>
</tr>
<tr>
<td>Xs100</td>
<td>4.6±0.51</td>
<td>4.6±0.60</td>
<td>2.0±0.45</td>
<td>10.2±0.37</td>
<td>10.6±0.68</td>
<td>10.2±0.37</td>
<td>2.0±0.45</td>
<td>406±2.92</td>
<td>13.50</td>
</tr>
<tr>
<td>Xs200</td>
<td>4.6±0.51</td>
<td>4.8±0.37</td>
<td>2.0±0.32</td>
<td>10.0±0.45</td>
<td>11.0±0.55</td>
<td>11.4±0.68</td>
<td>11.2±0.20</td>
<td>406±2.92</td>
<td>14.10</td>
</tr>
</tbody>
</table>

Table 2. Sperm motility, liveability and count, and seminal volume of hypertensive Wistar rats treated with L. breviflora Roberty fruit or X. sagittifolium Schott corn

<table>
<thead>
<tr>
<th>Group</th>
<th>Motility (%)</th>
<th>Live/Dead (%)</th>
<th>Volume (cm³)</th>
<th>Count (X106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93.2±0.92</td>
<td>97.2±0.58</td>
<td>5.14±0.02</td>
<td>142.8±4.10</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>78.0±2.00*</td>
<td>96.2±0.73</td>
<td>5.18±0.02</td>
<td>112.2±6.73*</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>74.0±2.45*</td>
<td>96.8±0.73</td>
<td>5.16±0.02</td>
<td>101.8±5.08*</td>
</tr>
<tr>
<td>Hydrochloro</td>
<td>76.0±2.45*</td>
<td>96.8±0.73</td>
<td>5.14±0.02</td>
<td>104.2±2.35*</td>
</tr>
<tr>
<td>Lb100</td>
<td>74.0±2.45*</td>
<td>96.2±0.73</td>
<td>5.18±0.02</td>
<td>98.4±5.39*</td>
</tr>
<tr>
<td>Lb200</td>
<td>78.0±3.74*</td>
<td>96.8±0.73</td>
<td>5.18±0.02</td>
<td>104.0±4.56*</td>
</tr>
<tr>
<td>Xs100</td>
<td>76.0±2.45*</td>
<td>96.8±0.73</td>
<td>5.18±0.02</td>
<td>109.6±8.00*</td>
</tr>
<tr>
<td>Xs200</td>
<td>74.0±2.45*</td>
<td>96.8±0.73</td>
<td>5.18±0.02</td>
<td>93.0±4.76*</td>
</tr>
</tbody>
</table>

Hypertensive – Hypertensive not treated, Lisinopril – Lisinopril 5 mg/70 kg, Hydrochloro – Hydrochlorothiazide 12.5 mg/70 kg, Lb100/Lb200 – Lagenaria breviflora extract 100 mg/kg or 200 mg/kg, Xs100/Xs200 – Xanthosoma sagittifolium extract 100 mg/kg or 200 mg/kg
Secondary abnormality recorded a non-significantly (p>0.05) higher incidence of bent mid-piece and tail, closely followed by curved mid-piece and tail. The other secondary sperm abnormalities observed were tailless head, headless tail and looped tail. These abnormalities occur in the process of maturation of sperm cells in the seminiferous tubules [25]. The total abnormal cells were non-significantly increased with a decrease in total sperm count. It can be inferred from this study that prolonged administration of the fruit extract of L. breviflora Roeberty and corm of X. sagittifolium Schott may have deleterious effects on the both spermatogenesis and maturation. Treatment of hypertension is usually life-long in established clinical cases.

Sperm morphology as well as characteristics were adversely affected by prolonged administration of the extract, as sperm motility and count in rats in the treatment groups were significantly reduced compared to those observed in the control group, but the live/dead sperm cells ratio and volume were within the same range as those of the control rats. A previous study on methanol extract of L. breviflora in normal Wistar rats administered with the extract for 14 days showed a similar result [18]. Comparing these two studies may give some insight into likely reproductive toxicity that may be encountered in a more prolonged administration of L. breviflora.

A contrary report was documented by Farombi et al. [26] for Curcuma longa L. (family Cucurbitaceae), a close relative of X. sagittifolium Schott is believed to induce fertility which is contrary to effect of X. sagittifolium Schott [27]. Aqueous extract of stem bark of Lophira lanceolata was reported to be a fertility enhancer in male Sprague dawley rats demonstrated by increased sperm numbers without alterations in sperm motility and morphology [28]. Ekere et al. [29] also reported that methanol extract of leaves of Draceana arborea had fertility enhancing activities in male Sprague dawley rats by increase sperm numbers and increase the mean testicular weight.

5. CONCLUSION

Findings in this study show that hypertension itself induced infertility in male Wistar rats due to reduced blood flow to the seminiferous tubules in hypertensive patient which induce degeneration of the seminiferous tubules causing significant reduction in total sperm count. The oral administration of the extracts of the fruit of L. breviflora Roeberty and corm of X. sagittifolium Schott respectively causes increased sperm cell abnormalities with more secondary abnormalities than the primary sperm cells abnormalities. This suggests that prolonged administration of these extract may cause a marginal and non-significant further progression of the infertility caused by hypertension. Therefore, care should be taken in the use of this medicinal plant for treatment of hypertension in both human and animals especially male animals used for breeding.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as the code of conduct of Animal Experimentation set by Animal Care and Use Research Ethics Committee (ACUREC), University of Ibadan, Nigeria. All experiments have been examined and approved by the ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

3. Phillips GB, Jing TY, Resnick LM, Barbagallo M, Laragh JH, Sealey JE. Sex hormones and hemostatic risk factors for


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