Changes in sperm morphology and characteristics of experimentally-induced hypertensive Wistar rats treated with *Lagenaria breviflora* Roberty or *Xanthosoma sagittifolium* Exell

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 twice weekly and daily inclusion of 1% sodium chloride in drinking water. Group 2 was hypertensive but untreated rats. Two hypertensive groups were administered with Lisinopril (5mg/70kg) or Hydrochlorothiazide (12.5mg/70kg). Two hypertensive groups were assigned to each extract and these rats were administered with the extracts at doses of 100 or 200mg/kg body weight. The rats were treated per os for 5weeks and sacrificed at the end of this period. The testes were harvested and semen samples were obtained from the left cauda epididymis. Semen analysis were carried out to determine sperm morphology and characteristics.

Results: Result showed 1 primary and 7 secondary sperm abnormality types were observed with a non-significant ($p>0.05$) increase in total abnormal sperm cells. Live/dead ratio and sperm volume were unchanged but, sperm motility and count were significantly ($p<0.05$) reduced.

Conclusion: It was inferred from the study that hypertension in itself induced infertility and also treatment of the medical condition with the extracts of *L. breviflora* or *X. sagittifolium* did not reverse the infertility. Therefore, caution should be exercised when treating hypertension with these medicinal plants, particularly in male animals used for breeding.

Keywords: Lagenaria breviflora Roberty*, Xanthosoma sagittifolium* Schott*, Antihypertensive, Andrology*

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* corm, 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. MATERIAL 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* fruits were washed, cut into pieces, weighed and dried using a hot air oven set at 25^{o}C for 72 hours. The tubers of *X. sagittifolium* was peeled, cut into pieces and air dried for one week. The dried plant materials were then soaked in methanol for 96 hours. The extract was then filtered and concentrated using a rotary evaporator.

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

(25mg/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 5mg/70kg or Hydrochlorothiazide 12.5mg/70kg body weight. Groups 5 and 6 were also hypertensive rats treated with *L. breviflora* Roberty methanol extract at 100mg/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 5^{th} 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.

3. RESULTS AND DISCUSSION

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 compared to others. There was a non significant increase in spermatozoa with rudimentary tail, though the increase was observed in group 2 (1.6±0.40%), group 5 (1.8±0.37%), group 6 (2.0±0.45%), group 7 (2.0±0.45%) and group 8 (2.0±0.45%) compare to those in group 1 (control group) 0r 3 or 4 with same number of abnormality (1.4±0.24%). A non-significant increase was observed in the number of spermatozoa with normal head with bend tail compared to the control group with lesser number (8.0±0.55%) (Table 1). There was a non-significant increase in the number of spermatozoa with normal head with curved tail compared to the control group with lesser number (8.0±0.55%) of abnormality. The rats in the control group had lesser number of spermatozoa with curved mid-piece abnormality (8.0±0.32%) but was slightly higher in hypertensive group 2 (8.8±0.37%), in treatment group 3 and 4 (10.8±0.37%) with same number or 5 (10.4±0.87%) and 7 (10.6±0.68%) when compared with treatment group 6 (11.6±0.81%) and 8 (11.4±0.68%) with highest abnormality (Table 1).

The occurrence of bent mid-piece spermatozoa was slightly lesser in the control group (9.0±0.45%) compared to others, while the highest percentage abnormality was observed in group 8 (11.2±0.20). A non-significant increase in the occurrence of looped tail spermatozoa was observed. Rats in group 2 had same population of looped tail spermatozoa with the control group (1.6±0.40). Treatment rats in group 4 (2.6±0.50) had the highest population of sperm abnormality with looped tail. A significant reduction was observed in the total sperm count of all the Hypertensive rats when compared with the control group (412±1.22%). Rats in group 6, 7 and 8 had same number of total sperm count (406±2.92%) while rats of group 5 had the least number of total sperm count (403±2.00%). There was a significant increase in percentage sperm abnormality of all treatment rats. Rats of group 2 (10.02%) has a lesser percentage sperm abnormality when compared with the control group (10.83%) (Table 1).

3.2 Sperm Characteristics

A significant reduction in sperm motility was observed. The sperm motility in the rats of the control group is significantly higher (93.2±0.92%) compared to rats in each of the treatment group. There was non-significant reduction in the live: dead ratio for each rats in the treatment group, compared to the control group. A non-significant increase was observed in the volume of spermatozoa for each of the treatment groups compared to the control group. Rats in group 4 had same volume with the rats of the control group (5.14±0.02%), while rats of group 2, 5, 6, 7 and 8 had same volume (5.18±0.02) 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 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). Rats of group 8 had the least sperm count (93.0±4.76) when compared to rats in the other group (Table 2).

Table 1: Percentage occurrence of different sperm abnormalities observed in the rats of the control and test groups.

Group	Tail-less head	Headless tail	Rudimen-tary tail	Bent tail	Curved tail	Curved mid-piece	Bent mid-piece	Looped tail	Total sperm count	%Sperm Abnorma-lity
Ctrl	**4.2±0.37**	**4.4±0.51**	**1.4±0.24**	**8.0±0.55**	**8.0±0.55**	**8.0±0.32**	**9.0±0.45**	**1.6±0.40**	**412±1.22**	**10.83**
Hypert	**4.2±0.58**	**4.4±0.51**	**1.6±0.40**	**9.2±0.20**	**9.4±0.24**	**8.8±0.37**	**9.6±0.40**	**1.6±0.40**	**406±2.91**	**12.02**
Lisinop	**4.2±0.58**	**4.0±0.55**	**1.4±0.24**	**10.0±0.32**	**10.4±0.92**	**10.8±0.37**	**10.2±0.20**	**1.8±0.37**	**405±2.77**	**13.02**
Hydrochl	**4.4±0.51**	**4.4±0.51**	**1.4±0.24**	**10.4±0.24**	**10.0±0.84**	**10.8±0.37**	**10.2±0.20**	**2.6±0.50**	**407±2.55**	**13.32**
Lb100	**5.0±0.55**	**4.6±0.51**	**1.8±0.37**	**10.0±0.45**	**10.6±0.87**	**10.4±0.60**	**10.4±0.93**	**2.2±0.37**	**403±2.00**	**13.77**
Lb200	**4.6±0.51**	**4.4±0.51**	**2.0±0.45**	**9.8±0.86**	**11.2±1.07**	**11.6±0.81**	**10.4±0.87**	**2.2±0.37**	**406±2.92**	**13.84**
Xs100	**4.6±0.51**	**4.6±0.60**	**2.0±0.45**	**10.2±0.37**	**10.6±0.68**	**10.6±0.68**	**10.2±0.37**	**2.0±0.45**	**406±2.92**	**13.50**
Xs200	**4.6±0.51**	**4.8±0.37**	**2.0±0.32**	**10.0±0.45**	**11.0±0.55**	**11.4±0.68**	**11.2±0.20**	**2.2±0.37**	**406±2.92**	**14.10**

Hypert – Hypertensive not treated, Lisinop – Lisinopril 5mg/70kg, Hydrochl – Hydrochlorothiazide 12.5mg/70kg, Lb100 /Lb200 – *Lagenaria breviflora* extract 100mg/kg or 200mg/kg, Xs100/ Xs200 – *Xanthosoma sagittifolium* extract 100mg/kg or 200mg/kg

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

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

Hypertensive – Hypertensive not treated, Lisinopril – Lisinopril 5mg/70kg, Hydrochloro – Hydrochlorothiazide 12.5mg/70kg, Lb100 /Lb200 – *Lagenaria breviflora* extract 100mg/kg or 200mg/kg, Xs100/ Xs200 – *Xanthosoma sagittifolium* extract 100mg/kg or 200mg/kg

4. CONCLUSION

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 <20X10^6/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).

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* Roberty 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) showing its ability to prevent peroxidative changes in the sperm and testicular membrane, thereby enhancing sperm motility and decreasing spermatozoa abnormalities. Also, whole plants of *Colocasia esculenta* L. (family Araceae), 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.

In 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* Roberty 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.

COMPETING INTERESTS

The authors declare that there is no conflict of interest that could influence or bias our work.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

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

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