

Experimental Testicular Torsion in a Rat Model: Effects of Treatment with *Pausinystalia macroceras* on Testis Functions

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Abstract

Background: Testicular torsion is a medical emergency with catastrophic sequelae that deserves the same treatment considerations and concerted efforts in research as any other complicated medical condition. The aim of this study was to investigate the effect of *Pausinystalia macroceras* (PM) bark extract on sperm quality and serum testosterone levels in testicular torsion in a rat model.

Methods: Sixty-five (65) mature male Wistar rats apportioned randomly into four experimental groups of A to C; were further divided into four subgroups according to duration of torsion. Group D were the normal regular rats. Each group/subgroup comprised five rats. Testis maintained in the torted position (T) for 1, 2, 3 and 4 hr in Group A (subgroups: $A_{T1}+PM$, $A_{T2}+PM$, $A_{T3}+PM$, and $A_{T4}+PM$). Group B (subgroups: B_1+PM , B_2+PM , B_3+PM , B_4+PM) were sham-operated animals, which did not undergo torsion and served as the sham control group. Group C subgroups: C_{T1} , C_{T2} , C_{T3} and C_{T4} were torted as in A. All animals (except groups C and D) were treated by PM extract (0.1 g/kg b.w. per day) for 56 days. Group D rats were fed distilled water. Serum testosterone concentrations and sperm quality (motility and count) were measured. Analyses of variance with Scheffe's post-hoc test were carried out on the data.

Results: PM extract had a positive effect (significant; $p<0.5$) on the sperm count and motility in rats with testicular torsion compared to those not receiving the extract. There was also an increase in serum testosterone levels in the former groups.

Conclusion: Treatment of rats following testicular torsion result to the enhancement of sperm production in comparison with untreated rats.

Keywords: *Pausinystalia macroceras*, Rats, Sperm quality, Torsion-detortion.

To cite this article: Ikebuaso AD, Yama OE, Duru FIO, Oyebadejo SA. Experimental Testicular Torsion in a Rat Model: Effects of Treatment with *Pausinystalia Macroceras* on Testis Functions. *J Reprod Infertil.* 2012;13(4):218-224.

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Received: Apr. 24, 2012

Accepted: Aug. 18, 2012

Introduction

Testicular torsion is twisting of the spermatic cord usually confined to the mesentery that joins the testis to the epididymis (1). Torsion initially obstructs venous return. The subsequent equalization of venous blood return and arterial blood pressure compromises the arterial flow resulting in testicular ischemia. It is a urological emergency referred to as acute scrotum, because early diagnosis and surgical intervention determine prognosis of spermatogenesis. Misdi-

agnosis and inappropriate treatment lead to male infertility (2). In the absence of female factor infertility, efforts to improve sperm quality is seen as a method of increasing the fertilizing ability of the spermatozoa and hence, fertility. *In vitro* studies have shown that sperm motility is enhanced by calcium (3), creatine phosphate and pentoxifylline (4). In a recent study, researchers reported that the aqueous extract of the stem bark of *Pausinystalia macroceras* (PM) is capable of enhancing

testicular function in Wistar rats (5). It has been used for over five decades as an aphrodisiac and to treat male impotence in North America and Europe (6). However, there is no reference in the literature to the effects of PM on the damage caused by testicular ischemia-reperfusion injury either in rat or man.

PM is a native of the coastal forests of West Africa from South-East Nigeria (7) to Gabon (8). Predominantly found within the forest, PM was classified by Letouzey in 1985 (9) as Atlantic Biagran evergreens in these geographical locations.

This research investigates the enhancement of sperm production by PM bark extract in male Wistar rats following ischemia-reperfusion injury of the testes.

Methods

Preparing bark extract of Pausynistalia macroceras:

The bark extracts of PM were purchased from the local market in Lagos, Nigeria. It was authenticated by a taxonomist in the Forest Herbarium (Botany Department) of University of Ibadan. There the voucher specimen was deposited (ascension number FHI 108875). It was dried in an oven (between 30–36 °C) for a week, grounded and weighed; 120 g of PM powder was Soxhlet extracted with distilled water to give the extract mean yield of 29.63% (w/w), which was later stored at –4 °C until ready for use.

Animals: Sixty-five male wistar rats, weighing 120–160 g, were divided randomly into four experimental (A-C) and a normative group D. The rats were acquired from the Animal House of the Faculty of Basic Medical Sciences University of Lagos and authenticated by a taxonomist at the Zoology Department of the same University. Rats in groups Band C were further divided collectively into eight subgroups. Each respective group and subgroup comprised five rats. The animals were housed in wire mesh cages in a cross-ventilated room (temperature 24±2.0 °C, with 12 hr light and 12 hr dark cycle). The animals were permitted unlimited access to rat chow (Livestock Feeds Plc. Ikeja, Lagos, Nigeria) and water ad libitum. The animals were allowed to acclimatize for one week before the commencement of the experiment.

Surgical procedure and experimental protocol: The degree of ischemia depends on the degree of rotation of the spermatic cord which can range from a 180° to more than 720° torsion. Ischemia can occur four hours after torsion and it is almost certain

after 24 hr (10). We performed modified Davenport method for the surgical procedure (10). Briefly; the animals were anaesthetized using intra-abdominal injection of 7 mg/kg body weight ketamine hydrochloride. A mid-scrotal incision was performed to gain access to both testes torsion was induced by twisting the testes 720° in counterclockwise direction. Testis was maintained in the torted position (T) for 1, 2, 3 and 4 hr in subgroups A_{T1}+PM, A_{T2}+PM, A_{T3}+PM, and A_{T4}+PM (Group A). Rats in Group B subgroups: B₁+PM, B₂+PM, B₃+PM, B₄+PM were sham operated, without inducing torsion served as the sham control. Group C subgroups: C_{T1}, C_{T2}, C_{T3} and C_{T4} were maintained in similar torted positions as in A above. The torted positions were achieved by suturing the testicular capsule to the scrotal wall. Following detorsion, the wounds were closed with chromic 2.0. All the animals in the groups A and B (Subgroups: A_{T1}+PM, A_{T2}+PM, A_{T3}+PM, A_{T4}+PM and B₁+PM, B₂+PM, B₃+PM, B₄+PM) were treated with PM extract at a dose of 0.1 g/kg body weight daily (11) for 56 days which is the time taken to complete a spermatogenic cycle in rats (12). No extract were administered to rats in subgroups C_{T1}, C_{T2}, C_{T3} and C_{T4}; they were used to compare the effects of the extract on the torted testes. Finally Group D rats served as the normal control (neither sham operated nor torted). They were fed distilled water for 56 days.

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (13) and were approved by the Departmental Committee of the College of Medicine University of Lagos Nigeria on the Use and Care of Animals in conformity with international acceptable standards.

Sperm counts and motility analysis: Several small cuts were made in the cauda epididymis which was then placed in a sterile universal specimen bottle, containing 1 ml of normal saline to allow motile sperm to swim up from the epididymis. Five µl of epididymal fluid was delivered onto a glass slide covered with a 22×22 mm cover slip (14) and examined under the light microscope at a magnification of ×400. The microscopic field was scanned systematically and each spermatozoon encountered was assessed. Motility was determined by counting the number of immotile spermatozoa and subtracting from the total count mul-

multiplied by $\times 100\%$. The motility was simply classified as either motile or non motile. The procedure was repeated and the average of the two readings was calculated.

Sperm count was determined using the Neubauer improved haemocytometer. A dilution ratio of 1:20 from each well-mixed sample was prepared by diluting 50 μl of epididymal spermatozoa suspended in physiological saline with 950 μl diluent. The diluent was prepared by adding 50 g of sodium carbonate and 10 ml of 35% (v/v) formalin to distilled water and making up the final solution to a volume of 1000 ml (14, 15). Both chambers of the haemocytometer were scored and the average count calculated, provided that the difference between the two counts did not exceed 1/20 of their sum (*ie.*, less than 10% difference). When the two counts were not within 10% difference, they were discarded, the sample dilution re-mixed and another haemocytometer prepared and counted. To minimize errors, three counts were conducted on each epididymis. The average of all the six counts (3 from each side) from a single rat was taken and this constituted one observation for the sperm count.

Testosterone assay: Blood obtained from left ventricular puncture was assayed for serum testosterone. The samples were assayed in batches from a standardized curve using the Enzyme Linked Immunosorbent Assay (ELISA) method (16). The microwell kits used were from Syntro Bioresearch Inc., California USA. Using 10 μl of the standard solution, the samples and controls were dispensed into coated wells. One-hundred microliter testosterone conjugate reagent was added followed by 50 μl of anti-testosterone reagent. The contents of the microwell were thoroughly mixed and then incubated for 90 min at room temperature. The mixture was washed in distilled water and further incubated for 20 min. The reaction was stopped with 100 μl of 1 N hydrochloric acid. Absorbance was measured with an automatic spectrophotometer at 450 nm. A standard curve was obtained by plotting the concentration of the standard solution versus its absorbance and testosterone concentration was determined from the standard curve.

Volume mensuration: The testes and seminal vesicle volumes were estimated by water displacement method. This was similar to that described by Acott (17). The volumes of the two organs (right and left) from each rat were computed and the average value obtained and the parameter re-

garded as one observation, the values expressed as g/100 g body weight.

Statistical analysis: Results were expressed as mean \pm standard deviation. Analysis was carried out using analysis of variance (ANOVA) with Scheffe's post hoc test. The level of significance was considered at $p < 0.05$.

Results

The mean sperm count and motility in the treated groups $A_{T1}+PM$, $A_{T2}+PM$, $A_{T3}+PM$, $A_{T4}+PM$ were: 80, 60, 45, $25 \times 10^6/ml$ and 56 ± 4.0 , 45 ± 3.0 , 30 ± 3.0 , $10 \pm 4.0\%$, respectively. These values were significantly higher ($p < 0.05$) compared to 60, 35, 10, $0 \times 10^6/ml$ and 28 ± 4.0 , 25 ± 4.0 , 20 ± 4.0 , $0.0 \pm 0.0\%$ of sperm count and motility in groups C_{T1} , C_{T2} , C_{T3} , C_{T4} (torted untreated group), respectively. It is pertinent to note that no viable sperm cells were seen under the light microscope in group C_{T4} , during the counting process. On the other hand, the mean sperm quality in groups B_1+PM , B_2+PM , B_3+PM , B_4+PM (the sham-operated group without inducing torsion and treated with the extract) were similar to group D (normal rats, not operated, fed with distilled water only) (Table 1).

The serum testosterone in groups $A_{T1}+PM$, $A_{T2}+PM$, $A_{T3}+PM$ and $A_{T4}+PM$ were: 2.86 ± 1.5 , 2.81 ± 1.55 , 2.78 ± 1.25 , and 2.40 ± 1.5 ng/dl, respectively. These values were significantly higher ($p < 0.05$) than the values 2.66 ± 1.20 , 2.60 ± 1.05 , 2.57 ± 1.20 and 2.00 ± 1.22 ng/dl for groups C_{T1} , C_{T2} , C_{T3} and C_{T4} , respectively. There were no significant differences between the values obtained in the rats in group D and the sham-operated group B (Table 1).

Finally, it is worthy to note that the extract was able to slightly increase ($p > 0.05$) both the testes volumes (TV) and seminal vesicles (SV) in the torted models treated with the extract ($A_{T1}+PM$, $A_{T2}+PM$, $A_{T3}+PM$, and $A_{T4}+PM$) compared to C_{T1} , C_{T2} , C_{T3} and C_{T4} of the untreated torted groups. In the treated torted groups, values of TV and SV were: 0.80, 0.76, 0.74, 0.70 and 1.20, 1.00, 1.00, 0.95 cm^3 . In the untreated groups torted, TV and SV values were: 0.75, 0.73, 0.70, 0.65 and 1.00, 0.90, 0.90, 0.85 cm^3 , respectively (Table 2).

Discussion

In this study, caudal epididymal fluid analysis, parallel testosterone levels of the sera, volumes of testes and seminal vesicles in the torted-testicular rat models treated with PM extract were contrast

Table 1. Sperm quality (count and motility) and serum testosterone concentrations for experimental and control Wistar rats; values are expressed in Mean±SD

| Groups (n=5) | Sperm count ($\times 10^6/ml$) | Sperm motility (%) | Testosterone values (ng/dl) |
|---------------------|----------------------------------|--------------------|-----------------------------|
| A _{T1} +PM | 80.0±2.3 | 56.0±4.0* | 2.86±1.5 |
| A _{T2} +PM | 60.0±1.2* | 45.0±3.0* | 2.81±1.6 |
| A _{T3} +PM | 45.0±1.3* | 30.0±3.0* | 2.78±1.3 |
| A _{T4} +PM | 25.0±2.0* | 10.0±4.0* | 2.40±1.5 |
| B ₁ +PM | 155.0±1.3 | 70.0±3.0 | 2.89±1.3 |
| B ₂ +PM | 132.5±27.5 | 85.8±13.1 | 2.67±1.2 |
| B ₃ +PM | 91.3±27.8 | 90.3±13.7 | 2.58±1.0 |
| B ₄ +PM | 81.7±20.2 | 74.7±4.5 | 2.87±1.2 |
| C _{T1} | 60.0±2.0 | 28.0±4.0* | 2.66±1.2 |
| C _{T2} | 35.0±1.3* | 25.0±4.0* | 2.60±1.1 |
| C _{T3} | 10.0±2.0* | 20.0±4.0* | 2.57±1.2 |
| C _{T4} | 00.0±0.0* | 00.0±0.0* | 2.00±1.2 |
| D | 156.0±1.2 | 70.0±3.0 | 2.87±1.2 |

* $p < 0.05$; Testis in torted position (_T) for 1, 2, 3 and 4 hr in A_{T1}+PM, A_{T2}+PM, A_{T3}+PM, and A_{T4}+PM and treated with Pausynistalia macroceras (PM) bark extract for 56 days. Groups B₁+PM, B₂+PM, B₃+PM, B₄+PM were sham-operated, without undergoing torsion but treated with extract (sham control). Groups C_{T1}, C_{T2}, C_{T3} and C_{T4}; testes maintained in torted positions, no extract administered. Group D, the control group, fed with distilled water only

Table 2. Approximate volume of organs and their weights using volume displacement technique; values expressed in Mean±SD

| Groups (n=5) | Average Volume of Testis (cm^3) | Average Volume of Seminal Vesicle (cm^3) |
|---------------------|-------------------------------------|--|
| A _{T1} +PM | 0.8±0.1 | 1.1±0.2 |
| A _{T2} +PM | 0.8±0.2 | 1.0±0.2 |
| A _{T3} +PM | 0.7±0.1 | 1.1±0.2 |
| A _{T4} +PM | 0.7±0.0 | 1.0±0.1 |
| B ₁ +PM | 0.7±0.1 | 1.2±0.3 |
| B ₂ +PM | 1.1±0.3 | 0.8±0.1 |
| B ₃ +PM | 0.9±0.2 | 0.1±0.3 |
| B ₄ +PM | 0.9±0.3 | 1.2±0.5 |
| C _{T1} | 0.8±0.1 | 1.1±0.2 |
| C _{T2} | 0.7±0.1 | 1.0±0.0 |
| C _{T3} | 0.7±0.2 | 0.9±0.1 |
| C _{T4} | 0.7±0.1 | 0.9±0.1 |
| D | 0.9±1.0 | 1.2±0.3 |

Testis in torted positions (_T) for 1, 2, 3 and 4 hr in A_{T1}+PM, A_{T2}+PM, A_{T3}+PM, and A_{T4}+PM and treated with Pausynistalia macroceras (PM) bark extract for 56 days. Groups E₁+PM, B₁+PM, B₂+PM, B₃+PM, B₄+PM were sham-operated, without inducing torsion but treated with the extract (sham control). Groups C_{T1}, C_{T2}, C_{T3} and C_{T4}; testes maintained in torted positions, not extract treated. Group D, the control group, fed with distilled water only

ed with those that underwent torsion without the extract treatment. It is imperative to note that findings in the sham control group (operated, untorsioned group treated with PM extract) were similar to normative rats (not operated untorsioned group fed with distilled water).

There are reports in the literature that PM improves the exocrine function of the testes (5). Our results seem to corroborate with this fact because PM extract had a positive effect on the sperm count and motility of the torted-testicular groups. This result supports the extracts' potential for

boosting sperm quality.

The ischemic reperfusion injury upon testicular torsion results in disruption of spermatogenesis that can be permanent with short-term suppression of testosterone secretion (18). This may render the testes aspermatogenic (19). These findings were also in tandem with our results which showed reduction in the testosterone levels, as well as sperm count and motility in the torted untreated groups.

A depleted serum testosterone concentration is indicative of Leydig cell failure (20). The observed decrease in blood testosterone levels in the sera of the torted-testicular groups is thus suggestive of Leydig cell dysfunction. These levels were almost reversed by the administration of the extract with significant results in the earlier treatment of groups with torted-testes ($A_{T1}+PM$ and $A_{T2}+PM$). In the testes maintained torted for longer periods (C_{T4}), similar treatment with PM ($A_{T4}+PM$) could not reverse Leydig cell dysfunction, leading to a decreased testosterone with no observed viable spermatozoa.

Semen is made up of sperm and seminal fluid. The latter comprises of secretions produced by the seminal vesicle and other accessory glands (21). In the seminal vesicles, major responses of increased or decreased secretory activities, manifest as weight changes of these organs (22). The endogenous increase in serum testosterone increased the secretory activity of the seminal vesicles (23). Also in rats, any increase in serum testosterone or treatment with androgens was associated with increased secretory activity of the seminal vesicles (24–26) and increased seminal vesicle weight (27). The results in the torted groups treated with the extract showed increased seminal vesicle volume and since volume varies directly to weight (28), it, therefore, means increased functional/secretory activities. This increased function could have been due solely to the effect of the extract. Researchers have earlier shown that subjects with hypofunction of the seminal vesicles have low sperm motility, which itself may cause infertility (29, 30) as reflected in our findings.

The testicular volume has been documented to correlate positively with the size of the testicular interstitium and, thus, the number of Leydig cells. These in turn correlate positively with serum testosterone concentrations, as well as testicular function (31–34). It is, therefore, dependable to imply that the increased testicular volume resulted in increased testosterone and sperm quality (count

and motility) in the PM treated torted groups as compared to the non-extract treated groups.

The sequences of highlighted events exhibited by treatment with the extract might partly explain its ability in improving spermatogenic ‘clock’ in the torted models. Other mechanisms still need to be explored which include the extract's effect on gonadotropin hormones and its antioxidant potentials, since previous studies have demonstrated loss of spermatogenesis to be due to germ cell apoptosis induced by oxidative stress (28).

Conclusion

In conclusion, the ultimate goal of managing testicular torsion in humans is discovering effective therapies for improving or salvaging a torted testis. This study showed an improvement in semen quality in the oligoasthenozoospermic torted animal model by treating with PM bark extract. Therefore, PM extract would be of potential benefit in the management of some forms of male infertility due to testicular torsion, as it is readily available and affordable in these conditions.

Acknowledgement

We wish to acknowledge Dr. M.O. Ajalah, consultant physician and pathologist at Lagos State General Hospital, in Lagos State, Nigeria for his assistance in measuring the testosterone concentrations. Also to Mr. Adeleke of the Pharmacognosy Department at Faculty of Pharmacy, University of Lagos, Nigeria for his help in the preparation of the extract (decoction) and also support of this work.

Conflict of Interest

Authors declare no conflict of interest.

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