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RESEARCH PAPER

THE EFFECT OF L-ARGININE ON CONTRALATERAL TESTES FOLLOWING 720° TORSION – DETORSION OF THE IPSILATERAL TESTES.

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ABSTRACT

This study was designed to evaluate the effect of torsion-detorsion on ipsilateral testes at 720° and at time intervals, on the histology of contralateral testes. 20 male rats (130-200g) were involved in the study and allowed to acclimatize for three weeks. They were fed with commercial livestock feeds and tap water ad-libitum, and grouped into seven groups (A-G) of three rats each. In groups A-F, unilateral testicular torsion lasting for 15, 30, and 45 minutes was created. Alternate groups (A, C, and E) were treated intraperitoneally with 150mg/kg/body weight of L-arginine for 1 hour after detorsion, while the remaining groups (B,D,F) were untreated with L-arginine but still allowed to undergo 1 hour detorsion. Group G served as the control. The results showed that group A presented normal seminiferous tubules with intact sertoli cells, and germ cells at various stages of development. The untreated group B presented atrophic seminiferous tubules with loss of germ cells and no luminal spermatozoon, while group D presented normal features except for interstitial hemorrhage. Normal features were observed for groups E (treated) and F (untreated). Thus, L-arginine tends to reduce oxidative stress in the contralateral testes thereby preventing interstitial oedema and preserving the spermatogenic series.

Keywords: Torsion-detorsion, Ipsilateral, Contralateral, L-arginine, Ischemia, Reperfusion.

INTRODUCTION

Rotation of the spermatic cord and the testicle about its axis is known as testicular torsion. If treated within a few hours, the testicle can usually be saved. But waiting longer can cause permanent damage and may result in infertility. When blood flow has been cut off for more than 12hours, a testicle may become so badly damaged, that it has to be removed (Kehinde et al., 2005, Heindel, 1990). One of the most commonly selected animal for studies in experimentally induced testicular torsion (Dokucu et al., 2000), orchietomy (Ashby and Leferre, 2001) and cryptorchidism (Vigueras et al., 2004), is the rat. The main pathophysiology of testicular torsion is ischemia-reperfusion injury of the testis caused by the twisted spermatic cord and its release (Anim et al., 2005; Blank et al., 1993), and which is most likely mediated by oxygen free radicals (Anim et al., 2005).

Testicular torsion factors include (1) age – testicular torsion, most common in young men and adolescent boys (2) previous testicular torsion men and adolescent boys known from studies in rat that the permanent loss of spermatogenesis seen after repair of 1- hour, 720° torsion is not due to failure of average blood flow values to return to normal (Turner and Brown, 1993). On the other hand, the pattern of testicular microvascular perfusion does remain altered in the hours and days after torsion repair (Lysiak et al., 2000).

For many years, there has been clinical concern about the contralateral testis in cases of unilateral testicular torsion, which Riyad et al., 2009 in the research titled “A clinical and experimental Review of Testicular Torsion” was supported by early laboratory studies. Careful examination of severe damage to seminiferous epithelium after repairs has never shown a significant contralateral effect on spermatogenesis (Turner, 1985) or even a number of other parameters (Turner and Brown 1985). Experimental studies on testicular histology and fertility in laboratory animals, also suggest a bilateral effect with unilateral torsion (Vigueras et al., 2004). Nagler and de-Vere (1982) demonstrated that unilateral torsion caused contralateral histological damaged.

The extract mechanism of contralateral testicular damage still remains unclear; immunological responses, subclinical attacks of contralateral testicular torsion, underlying congenital defects, release of acrosomal enzymes and alterations in the blood flow have all been proposed in earlier studies (Merimsky et al., 1984; Williamson and Thomas, 1984; Tanyel et al., 1989, Anderson and Williamson 1990), but none has been universally accepted.

L-arginine which is a form of Arginine, one of the twenty amino acids that constitute protein, is more compatible to the human body than the D-arginine form. It is synthesized in mammals from glutamine via Pyrroline 5- carboxylase synthase and proline oxidase in the multi-step metabolic conversion (Wu et al., 1997). Arginine is a precursor for the synthesis of Nitric Oxide (NO) and endogenous gaseous messenger molecules involved in a variety of endothelium dependent physiological effects in cardiovascular system (Wu and Meininger, 2000). Arginine is required for normal spermatogenesis.

Over 50years ago, researchers found that feeding an arginine-deficient diet to adult men for nine days, decreased sperm counts by approximately 90% and increased the percentage of non-motile sperm approximately ten fold (Holt and Albanese, 1944). Arginine supplementation has been beneficial in HIV infection and AIDs, diabetes and insulin- resistance syndrome, gastrointestinal conditions preoperative nutrition and so on (Thorne, 2005).

Since L-arginine has been found helpful in situation characterized by oxidative stress, this research is to test its usefulness on contralateral testes in testicular torsion. Specifically, this study evaluates the effect of L-arginine on the histology of contralateral testes following 720° torsion-detorsion of ipsilateral testes at different interval ranging from 15minutes to 45minutes.

MATERIALS AND METHODS

Experimental Animals: A total of twenty male wistar rats, weighing 130 – 200g were used for this experiment. The animals were procured from Isaac’s animal farm in Ipetu Local Government Area of Osun State, Nigeria. They were kept in the animal house of Anatomy Department, College of Medicine, University of Lagos, Nigeria, at an average temperature of 28 ± 2°C, with 12hr light/12hr dark cycle and were fed with commercial livestock feeds and tap-water *ad-libitum*, for three weeks. The animals were randomly divided into seven groups (A-G) of three rats each. Group G served as the control. All surgical procedures were performed under Ketamine anesthesia.

Table 1: Showing experimental procedure for each group

Group	Torsion Time	L-arginine administration	Detorsion/reperfusion time
A	15 minutes	150mg/kg body weight	1 hour
B	15 minutes	-	1 hour
C	30 minutes	150mg/kg body weight	1 hour
D	30minutes	-	1 hour
E	45 minutes	150mg/kg body weight	1 hour
F	45 minutes	-	1 hour
G	-	-	-

Unilateral testicular torsion: Unilateral testicular torsion was created by rotating the left testicles in the clockwise direction along their longitudinal axis 720° from the initial position and newly placed in the scrotum. Torsion was maintained in position by fixing the testis to the scrotum with an atraumatic silk suture (4/0) through the tunica albuginea (Reyes et al., 1999; Dokucu et al., 2002) for one hour before orchiectomy was performed and tissues immediately fixed in Bouin’s fluid.

Histological Analysis: The morphological criteria used for determining the extent of damage in the seminiferous tubules was as described by Chakraborty et al. (1986) and Reyes (1999).

RESULTS

The tissue sections from the treated group A presented normal seminiferous tubules lined by 4-5 cells thick with germ cells in various stages of development. The sertoli cells were also intact. But in the untreated group B, atrophic seminiferous tubules characterized with loss of germs cells and no luminal spermatozoa was observed. Group C (treated) also showed normal seminiferous tubules. While the untreated group D appears normal except for the observed interstitial hemorrhage, group E (treated) and F (untreated) presented normal seminiferous tubules.

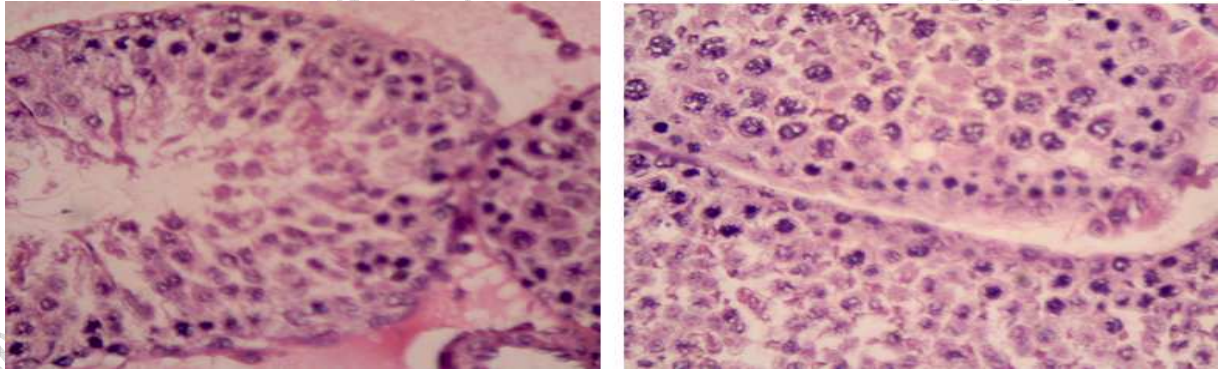


Plate 1: Group G (control) Testes (H&E X400) showing normal testicular histology.

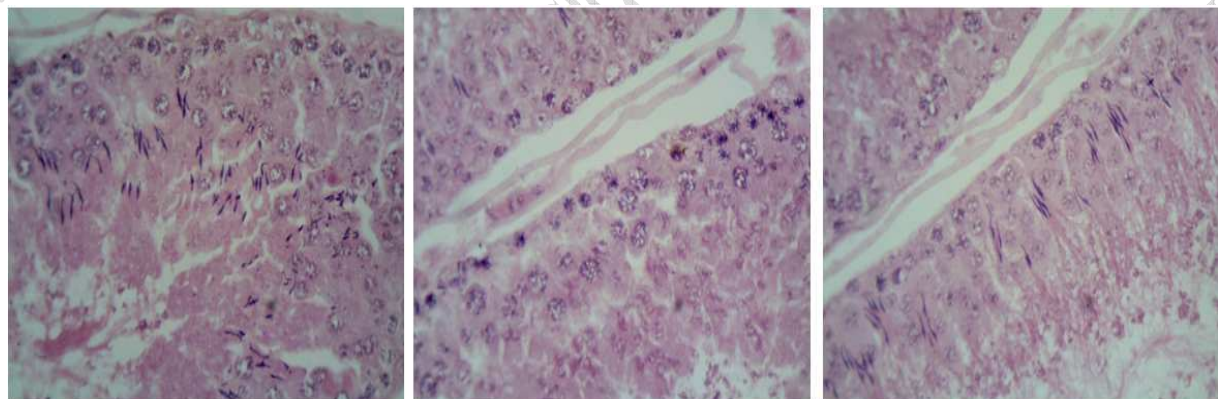


Plate 2: Group A (treated). Testes (H&E X400) showing normal seminiferous tubules lined by 4-5 cells thick with germ cells in various stages of development. The sertoli cells were also intact.

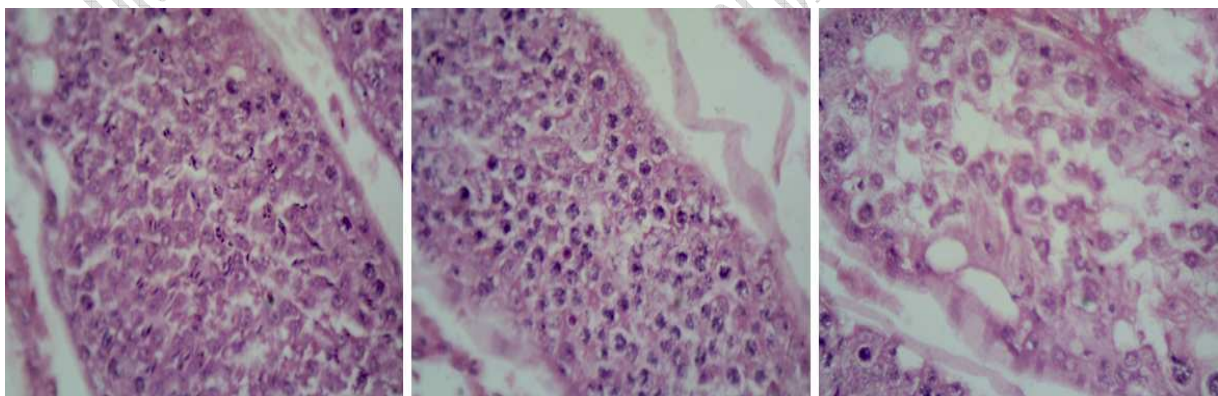


Plate 3: Group B (untreated) Testes Section (H&E x400) showing atrophic seminiferous tubules characterized with loss of germs cells and no luminal spermatozoon

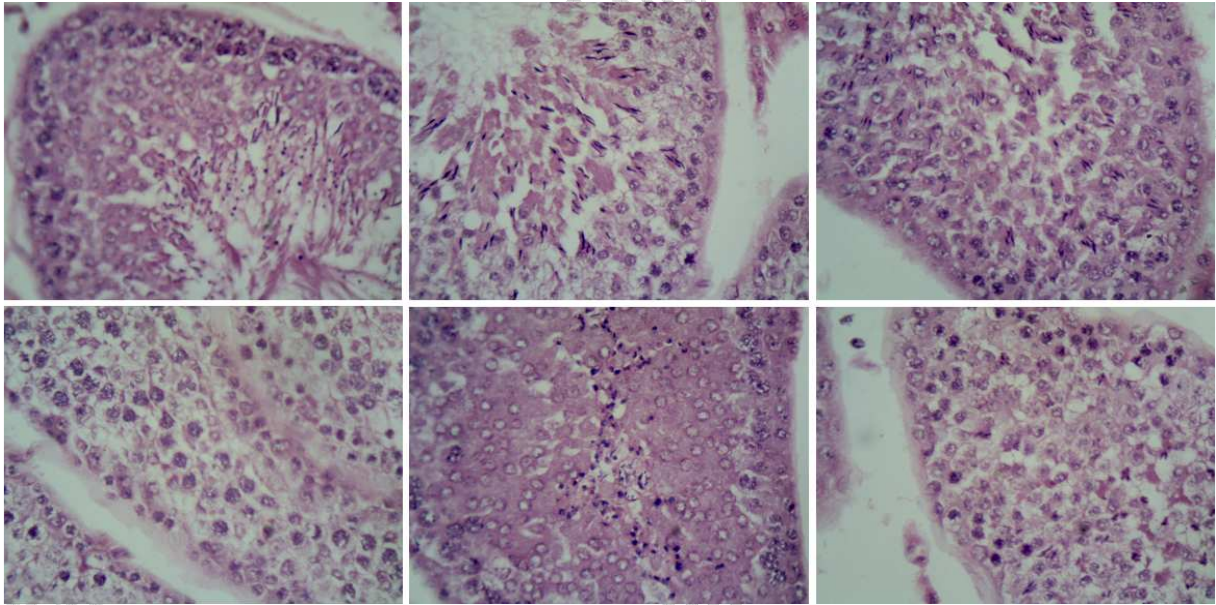


Plate 4: Group C (treated) Testes Section (H&E x100) showing normal seminiferous tubules.

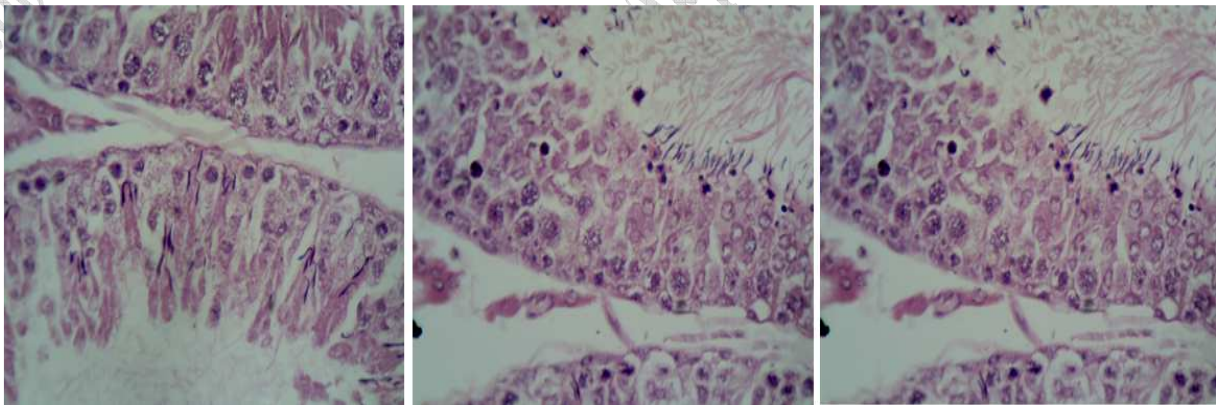


Plate 5: Group D (untreated) Testes Section (H&E x400) showing normal tissue cytoarchitecture except for the observed interstitial hemorrhage and mild atrophy of seminiferous tubules.

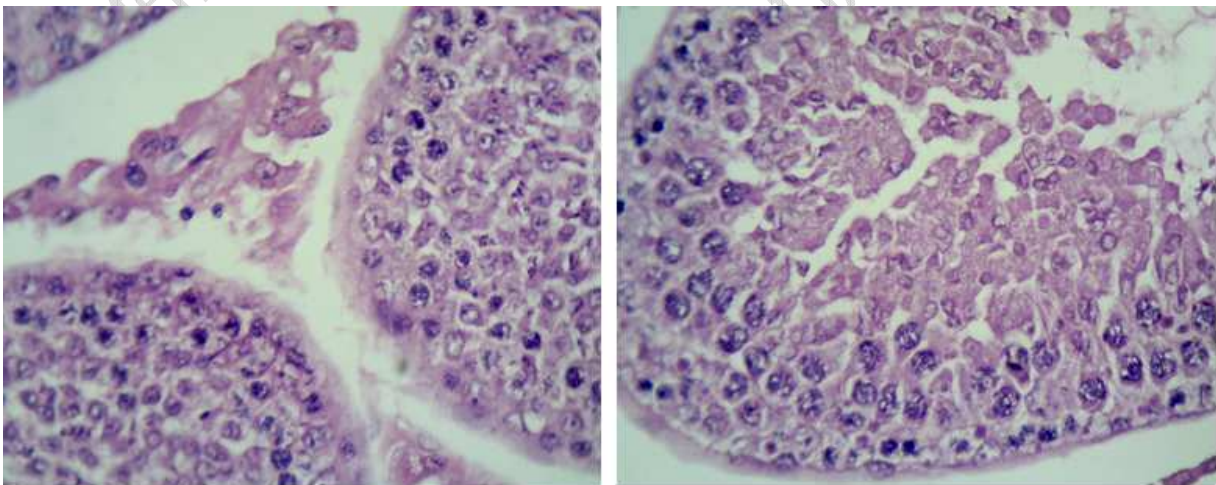


Plate 6: Group E (treated) Testes Section (H&E x100) showing normal seminiferous tubules.

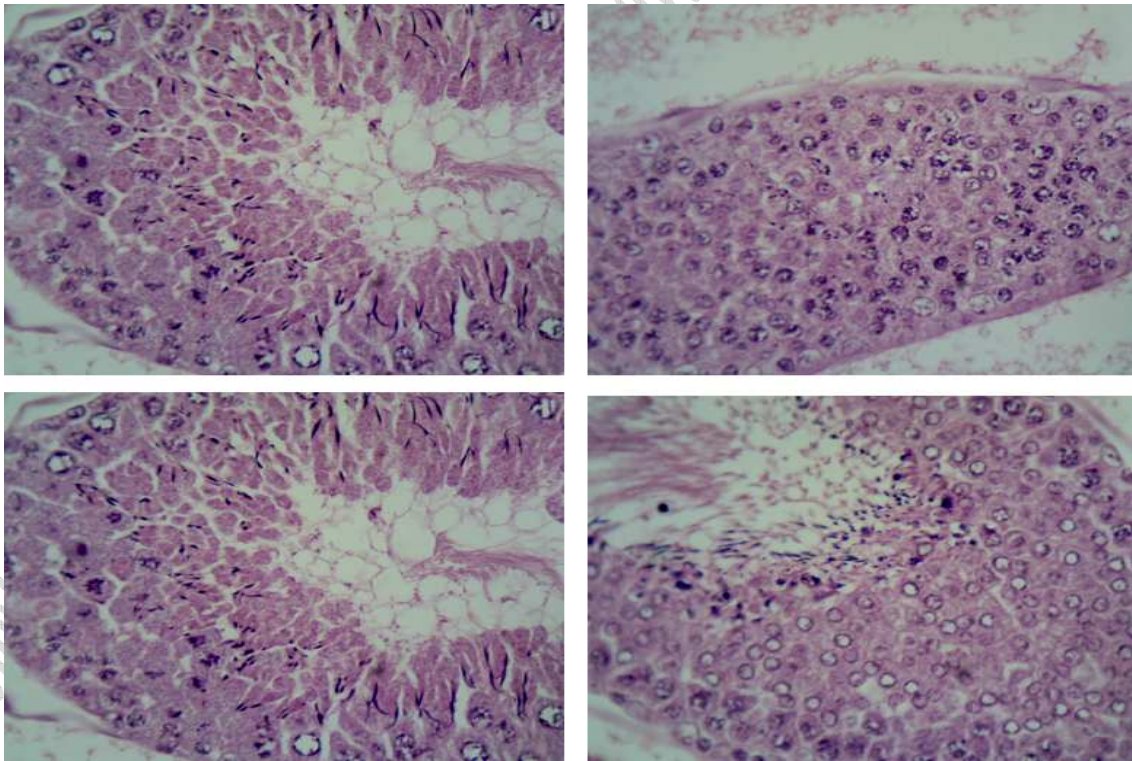


Plate 7: Group F (treated) Testes Section (H&E x100) showing normal seminiferous tubules.

DISCUSSION

Series of experimental studies on different animal species have shown that testicular torsion can produce pathological changes not only in the ipsilateral testis but also in the contralateral testis (Nagler and de Vere, 1982; Reyes et al., 1999; Turner and Brown, 1993), with a reduction in fertility (Chakraborty et al., 1986) and abnormal sperm production (Jhunjhunwala et al., 1986; Turner and Brown, 1993), as it is the case in humans (Bartsch et al., 1980, Anderson and Williamson, 1990).

Histologic sections of the contralateral testis of the treated groups all showed normal seminiferous tubules with no interstitial oedema. This may be due to the administration of L-arginine in these groups. However, the observed atrophic seminiferous tubules in groups B, as well as the interstitial oedema affirm the findings of Vigueras et al., (2004) that there is a connection between bilateral testes.

It is known that after the interruption of testicular artery and vein, blood reaching the testis through the testicular artery can only do so through the vasal artery. Only those seminiferous tubules in the immediate neighborhood of the intra-testicular part of the artery are likely to receive nourishment if the blood supply to the testis through the vasal artery is less efficient than the normal supply (Harrison, 2004).

Although both groups A and B underwent 15minutes testicular torsion, the presence of L-arginine might have preserved the spermatogenic series in A, while they were lost in B. This suggests that there is the possibility of hypoplasia of the seminiferous tubules even in the contralateral testis within 15minutes testicular torsion.

In addition, the mild to severe interstitial oedema seen in the untreated groups B, D and E, apart from the loss of germ cells in B, suggest that oxidative stress in the torsioned testis affected the contralateral testes in those groups. Thus, the interstitial cells can be said to be more sensitive to vascular changes than the germ cells.

Conclusively, the testicular interstitium is more prone to degenerative changes when testicular vascularity in the ipsilateral testis is compromised as compared to the germ cells. L-arginine tends to reduce oxidative stress in the contralateral testes thereby preventing interstitial oedema and preserving the spermatogenic series.

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AUTHOR(S) CONTRIBUTION

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