

Palliative Effect of *Pausinystallia macroceras* on Testicular Ischemic Reperfusion Injury in Wistar Rats: a Histological Study

Efecto Paliativo de *Pausinystallia macroceras* sobre la Lesión Testicular por Isquemia-Reperusión en Ratas Wistar: un Estudio Histológico

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SUMMARY: Testicular torsion is a disorder involving the scrotum that results in a compromise of its blood supply. The aim was to investigate the effect of *Pausinystallia macroceras* (PM) on testicular histology following torsion-detorsion at different time intervals ranging from 1 to 4 hours 65 mature male Wistar rats allotted randomly into seven groups (A to G; E& F further divided into 4-subgroups). Each group/subgroup comprised 5 rats. Testis maintained in the torsted position (T) for 1, 2, 3 and 4 hours in Groups A (AT1+PM), B (BT2+PM), C (CT3+PM) and D (DT4+PM). Group E subgroups (E1+PM, E2+PM, E3+PM, E4+PM -) were sham operated, without torsion served as the sham control. Group F subgroups (F1T1, F2T2, F3T3 and F4T4) were torsted as in A. All animals (except groups F & G) treated with PM extract (0.1 g/kg.b.w/day) for 56 days. Group G rats (normal control). Testes processed for histological studies. In AT1+PM showed preserved seminiferous tubules. BT2+PM, revealed varying number of necrosed and apoptotic seminiferous tubules. Group CT3+PM rats were similar to BT2+PM although with a slightly higher proportion of seminiferous tubules had undergone necrosis. In DT4+PM, sections showed few viable spermatozoa within the seminiferous tubules. When compared to the torsted group F; showed extensive areas of seminiferous tubular necrosis (F3T3) as well as damage to the interstitium; while in F4T4 there were no viable testicular tissues seen. In conclusion, PM significantly prevented the cellular changes and cell death observed especially in group AT1+PM and BT2+PM.

KEY WORDS: *Pausinystallia macroceras*; Torsion-Detorsion; Wistar rats; Testes.

INTRODUCTION

Testicular torsion (TT) is the winding of the spermatic cord which is usually confined to the mesentery that joins the testis to the epididymis (Rains & Mann, 1988). It is a urological emergency referred to as acute scrotum, requiring early diagnosis and surgical intervention to prevent testicular damage. Misdiagnosis and inappropriate treatment lead to male infertility (Williamson, 1997). Each year, testicular torsion affects one in 4,000 males younger than 25 years in USA. TT occurs usually in the absence of any precipitating event (Nöske *et al.*, 1998). Only 4 to 8 percent of cases are a result of trauma (Seng & Moissinac, 2000). Other factors predisposing a patient to TT include an increase in testicular volume often associated with puberty, testicular tumour, testicles with horizontal lie, a history of cryptorchidism and a spermatic cord with a long intrascrotal portion (Arce *et al.*, 2002). Torsion

initially obstructs venous return; subsequent equalization of venous blood return and arterial blood pressures compromise arterial flow resulting in testicular ischemia.

The management usually involves reperfusion of ischemic tissues; but these leads to a sequence of events that further injure the tissue (Ozkan *et al.*, 2004). The injuries produced by reperfusion can be more severe than that induced by ischemia (Ozokutan *et al.*, 2000). The pathophysiology involves stimulating an intracellular signaling cascade in the endothelial cells of the ischemic vessels. This results in neutrophil recruitment, an increase in intracellular reactive oxygen species (ROS), and eventual germ cell specific apoptosis (Turner & Miller, 1997; Lysiak *et al.*, 2001, 2003).

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In male journalistic medicine, herbal products are gaining popularity especially in the treatment of male sexual disorders notable amongst these is the plant *Pausynistalia macroceras* (*PM*). In 2007, Jacks *et al.* had reported its testicular enhancing properties (Jacks *et al.*, 2007). It is a plant found predominantly within the extensive forest classified by Letousey (1985) as Atlantic Biagran evergreens (dominating the coastal regions of Southeast Nigeria, Cameroon, Equatorial Guinea, Gabon and Congo-Brazzaville). It has been exploited for a plethora of ailments, but primarily used as an aphrodisiac and in the treatment of male impotence (Tyler, 1993). Recent study has showed that the aqueous extract of the stem bark of *PM* is capable of enhancing testicular function in Wister rats (Jacks *et al.*). However repute for its strong antioxidant potential (Jacks *et al.*), there is no literature reference on the possible palliative effect to damage from testicular ischemic-reperfusion injury either in rat or man. This work seeks to investigate the histological effect of *PM* on the testes of Wister rats following an ischemic-reperfusion injury.

MATERIAL AND METHOD

Animals. Sixty five male Wister rats, weighing 120–160 g, divided randomly into seven groups (A to G) were used for the study. They were acquired from the Animal house of the Faculty of Basic medical Sciences University of Lagos and authenticated by a taxonomist (Malaka, 2005) at the Zoology Department of the same University. Rats in groups E and F were further divided collectively into 8 subgroups. Each respective group and subgroup comprised 5 rats. The animals were housed in wire mesh cages in a cross-ventilated room (temperature $24 \pm 2.00\text{C}$, 12 h light and 12 h 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.

Preparation of back extract of *Pausynistalia macroceras*. The back extracts of *PM* were purchased from the local market in Lagos Nigeria. It was authenticated by a taxonomist in the Forest herbarium (Botany Department) University of Ibadan. There the voucher specimen was deposited (ascension number FHI 108875). It was dried in an oven (between 30–360C) 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 stored at -40C until ready for use.

Determination of the LD50 of back extract of *Pausynistalia macroceras*. This was done using the fixed-

dose procedure described by Walum Erik in 1998 (Walum, 1998). Briefly; the extract was given at one of three fixed doses at a time to 5 males S-D. At the dose of 460 g kg⁻¹ there were clear sign of toxicity with mortality of 50% of the rats. A dose 0.1 g kg⁻¹ body weight of back extract of *PM* was administered orally using a metal canula. Administration was by gastric gavages, done between 13.00–16.00 hours daily.

Experimental protocol and surgical procedure. The degree of ischemia depends on the degree of rotation of the spermatic cord which can range from 1800 to more than 7200 torsion. Ischemia can occur 4 hours after torsion and is almost certain after 24 hours (Davenport, 1996). The surgical procedure was by modified Davenport method. Briefly; the animals were anaesthetized using intra-abdominal injection of 7 mg kg⁻¹ body weight ketamine hydrochloride. A midscrotal incision was performed to gain access to both testes torsion was induced by twisting the testes 7200 in counterclockwise direction. Testis was maintained in the torted position (T) for 1, 2, 3 and 4 hours in Groups A (AT1+*PM*), B (BT2+*PM*), C (CT3+*PM*) and D (DT4+*PM*). Rats in Group E subgroups (E1+*PM*, E2+*PM*, E3+*PM*, E4+*PM*) were sham operated, without inducing torsion served as the sham control. Animals in Group F subgroups (F1T1, F2T2, F3T3 and F4T4) were maintained in similar torted positions as in A to D 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 Groups/subgroups: AT1+*PM*, BT2+*PM*, CT3+*PM*, DT4+*PM*, E1+*PM*, E2+*PM*, E3+*PM* and E4+*PM* -were treated with *PM* extract at a dose of 0.1 g/kg body weight daily for 56 days which is the time taken to complete a spermatogenic cycle in rats (Jegou *et al.*, 2002). No extract was administered to rats in subgroups F1T1, F2T2, F3T3 and F4T4; they were used to compare the effects of the extract on the torted testes. Finally Group G rats served as the normal control (neither sham operated nor torted). They were fed distilled water for 56 day.

Tissue preparation for histological analysis. All rats were orchidectomized on day 57 after the procedure and the testes were fixed in Bouin's solution for 24 hours and then dehydrated by passing through ascending grades of alcohol (70%, 80%, 90% and absolute alcohol). After dehydration, tissues were cleared in xylene, infiltrated, and then embedded in paraffin wax. Each testis was sectioned along the long axis in 5 μm thickness. Two sections from each rat were blocked in paraffin. Two sections of each block (total 4 sections for each testis) were stained with Haematoxylin and Eosin according to routine procedures for light microscopy.

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 (American Physiological Society & World Medical Association General Assembly, 2002) and were approved by the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

RESULTS

Histological results. The histological sections in AT1+PM showed preserved seminiferous tubules which contain large number of germ cells at all levels of maturation. The cell

organization was not distorted and normal polarity was maintained (Fig. 1). In group BT2+PM, the sections revealed varying number of necrosed and apoptotic seminiferous tubules, amid focal damage to the basement membrane. However 30–40% contained viable intraluminal germ cells which were disorganized with loss of polarity (Fig. 2). The histology of the testes in group CT3+PM rats were similar to BT2+PM although a higher proportion of seminiferous tubules had undergone necrosis (Fig. 3).

In DT4+PM, sections showed few viable spermatozoa within the seminiferous tubules. Although a larger percentage 70–75% of the seminiferous tubules had undergone necrosis and apoptosis from moderate to severe degrees (Fig. 4). The sections of sham operated rats in subgroups E1+PM, E2+PM, E3+PM, E4+PM did not differ

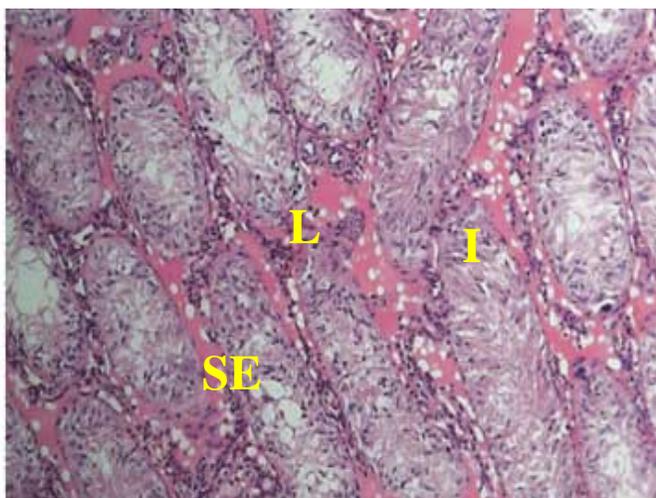
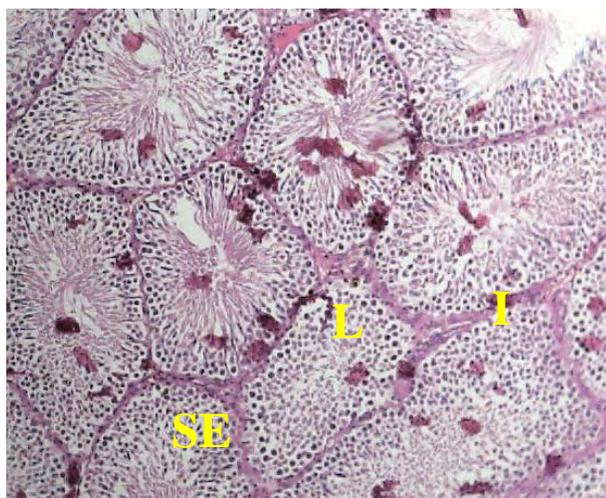


Fig. 1. Photomicrograph of testes from rats in Groups: (a) AT1+PM and (b) F1T1 Stains: Haematoxylin & Eosin. Mag. x 100; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium

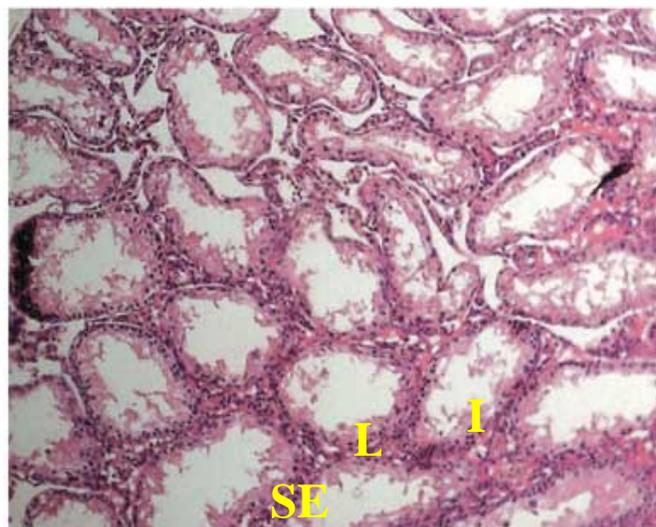
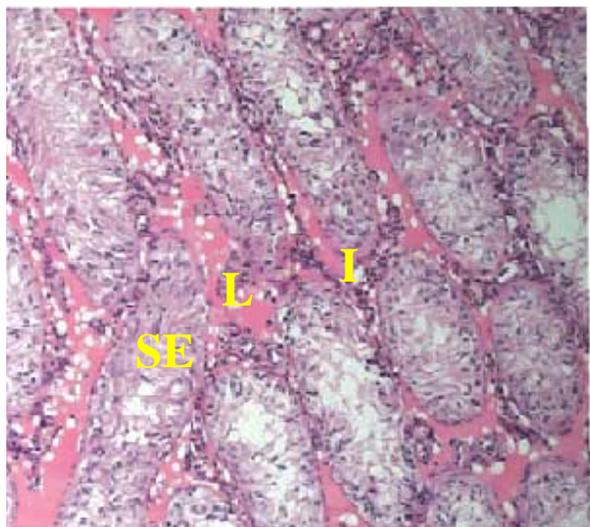


Fig. 2. Photomicrograph of testes from rats in Groups: (a) BT2+PM and (b) F2T2 Stains: Haematoxylin & Eosin. Mag. x 100; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium

from the control group (Figure 5). Group F (Figs. 1–4): F1T1 showed cellular damage with disorganization, significant reduction in the number of germ cells and focal necrosis as well as damage to the basement membrane and apoptosis. These aforementioned cellular injuries are also observed in

F2T2 but to a greater degree. F3T3 showed extensive areas of seminiferous tubular necrosis as well as damage to the interstitium, vacuolation, isolated collection of apoptotic cells and significant inflammatory processes. While in F4T4 there were no viable testicular tissues observed.

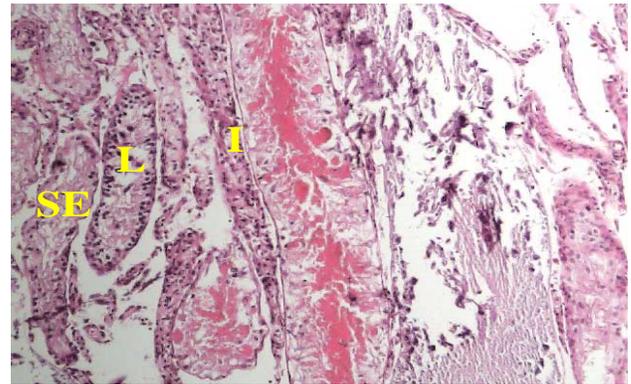
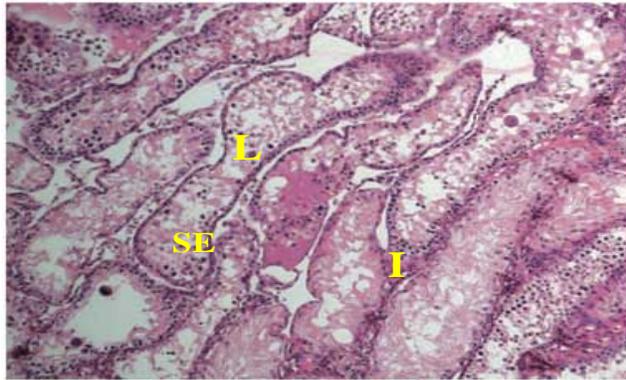


Fig. 3. Photomicrograph of testes from rats in Groups: (a) CT3+PM and (b) F3T3 Stains: Haematoxylin & Eosin. Mag. x 100; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium

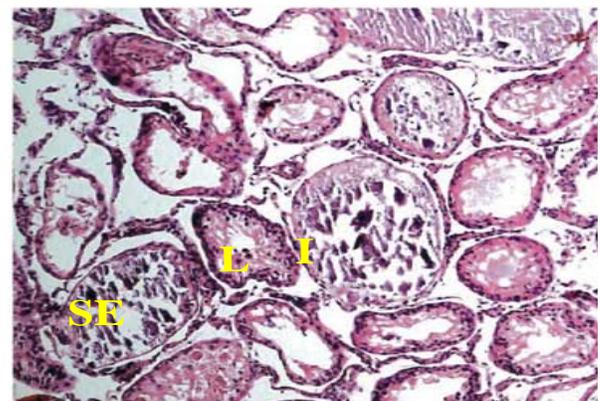
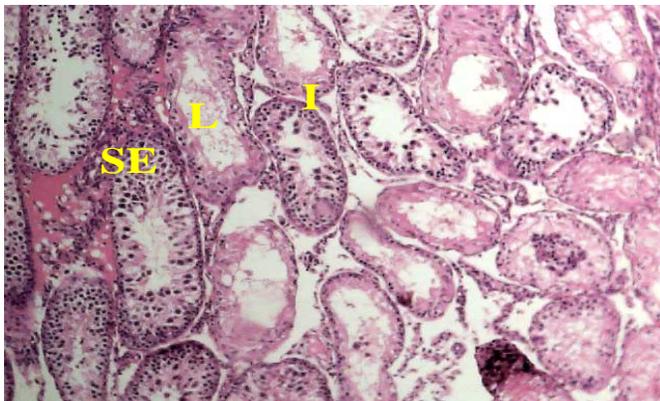


Fig. 4. Photomicrograph of testes from rats in Groups: (a) DT4+PM and (b) F4T4 Stains: Haematoxylin & Eosin. Mag. x 100; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium

The sections of control group G (Fig. 5) comprised numerous seminiferous tubules, which contained germ cells at various levels of differentiation; there is maintenance of normal germ cell polarity. The loose interstitium contained normal Leydig cells. The sertoli cells in between the spermatogonia were also observed to be normal.

DISCUSSION

Testicular responses to experimental torsion have been studied by a number of laboratories with attempts at reducing oxidative stress in the testis after an ischemic reperfusion injury (Akgür *et al.*, 1994; Uz *et al.*, 2002; Ozkan *et al.*). The ultimate goals were the determination of effective means at improving or salvaging a tormented testis. In this study,

the histology of testes of the various experimental groups have shown different features compared to that of their normal control counterpart (Group G).

On account of uniformity with available data, some of the results were not unanticipated. For instance, torsion induces ischemic hypoxia on the affected tissues. The severity/reversibility depending on the duration of impaired blood flow (McCord & Roy, 1982; Barada *et al.*, 1989; al Mufti *et al.*, 1995). The first 60–90 minutes is critical for the reperfusion injury as a result of the free oxygen radicals originating from neutrophils and parenchymal cells (Prillaman & Tunner, 1997). The tissues involved showed features of relative cell injuries depending on the duration. Therefore torsion in the first few hours, led to diverse degree of testicular injuries which range from: sloughing of the germ cells swelling, vacuolation, fatty changes ballooning of the

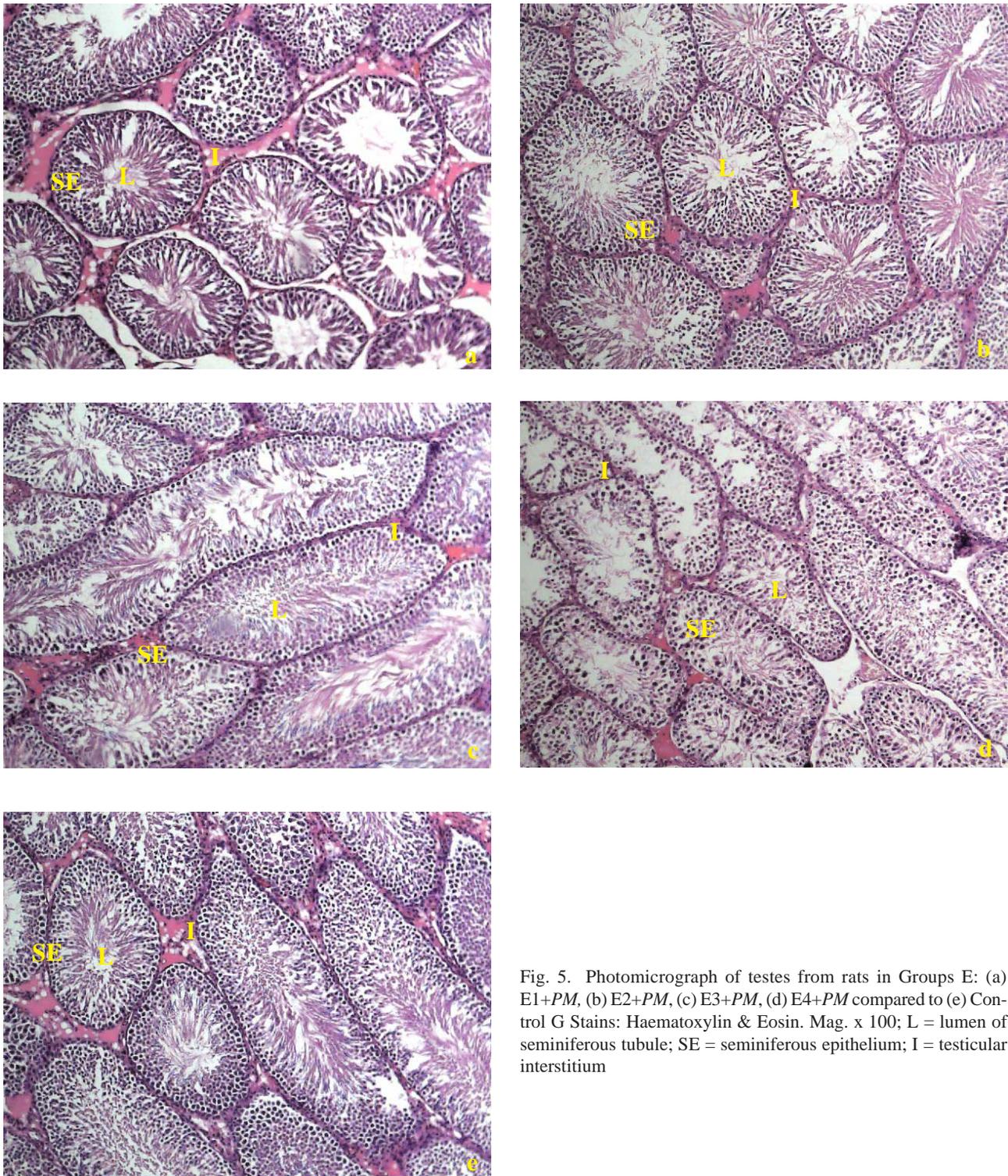


Fig. 5. Photomicrograph of testes from rats in Groups E: (a) E1+PM, (b) E2+PM, (c) E3+PM, (d) E4+PM compared to (e) Control G Stains: Haematoxylin & Eosin. Mag. x 100; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium

cells necrosis, apoptosis as well as damage to the basement membrane. These were consistent with our results in the observed photomicrographs of rats in groups F1, F2, F3 and F4 treated for varying durations. Furthermore comparing these

to histological sections in Groups: AT1+PM, BT2+PM, CT3+PM and DT4+PM, treated with PM (0.1 g/kg), a significant improvement to the damage inflicted on the testicular tissues were evident. These findings suggest that

PM had some palliative effect on testicular ischemia reperfusion injury, which may have been mediated via its antioxidative potential (Jacks *et al.*).

The testicular sections in group AT1+*PM* rats showed preserved seminiferous tubules. It contained large number of cells at all level of maturity compared to Group F1 which underwent torsion to a similar degree and duration but received no extract. Groups BT2+*PM* and CT3+*PM* showed improvement in their cytoarchitectures compared to F2 and F3. Lastly in animals in DT4+*PM* showed very few seminiferous tubules (insignificant) in contrast to group F4 which did not show any viable testicular tissue.

Within the various torted groups treated with the extract, an inverse relativity in the number of viable germ cells present was observed. The seminiferous tubules in rats

in group AT1+*PM* had more viable germ cells than BT2+*PM*, CT3+*PM* and DT4+*PM* in that order. As supported by the reduction in the disorganization of germ cells, apoptosis, focal necrosis and damage to the basement membrane.

The protective effect of *PM* on testicular ischemia reperfusion injury cannot be overlooked since it has significantly prevented the cellular changes and cell death observed especially in group AT1+*PM* and BT2+*PM*. However, the effect is minimal at duration ranging from 3–4 hours.

In conclusion this work has been able to observe the effect on testicular histology of *PM* (0.1g/kg) given for a period of 56 days following torsion-detorsion at time intervals ranging from 1 hour to 4 hours. This effect was protective probably due to the antioxidant effect of the extract.

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RESUMEN: La torsión testicular es un trastorno que involucra el escroto resultando en un compromiso del suministro sanguíneo. El objetivo fue investigar el efecto de *Pausinystallia macroceras* (*PM*) en la histología testicular tras torsión-detorsión a intervalos de tiempo diferentes que van desde 1 a 4 horas en 65 ratas macho Wistar maduras, asignando aleatoriamente en siete grupos (desde A a G, mientras que E y F se dividieron en 4 subgrupos). Cada grupo/subgrupo estuvo compuesto por 5 ratas. Los testículos se mantuvieron en posición torsionada (T) durante 1, 2, 3 y 4 horas en los grupos A (AT1 + *PM*), B (BT2 + *PM*), C (CT3 + *PM*) y D (DT4 + *PM*). El grupo E, subgrupos (E1 + *PM*, E2 + *PM* + *PM* E3, E4 + *PM*) fueron operados por modelo sham sin torsión, que sirvió de control. El grupo F, subgrupos (F1T1, F2T2, F3T3 y F4T4) fueron torsionados como en A. Todos los animales (excepto los grupos F y G) fueron tratados con extracto de AM (0,1 g/kg peso corporal/día) durante 56 días. El grupo G fueron ratas control (control normal). Los testículos fueron procesados para el estudio histológico. En AT1 + *PM* se observó preservación de los túbulos seminíferos. BT2 + *PM*, reveló un número variable de túbulos seminíferos con necrosis y apoptosis. El grupo de ratas CT3 + *PM* fue similar a BT2 + *PM*, aunque un porcentaje ligeramente superior de los túbulos seminíferos mostraron necrosis. En DT4 + *PM*, los cortes mostraron pocos espermatozoides viables dentro de los túbulos seminíferos. En comparación con el grupo F torsionado mostró extensas áreas de necrosis tubular (F3T3), así como daños en el intersticio; mientras que en F4T4 no hubo tejido testicular viable. En conclusión, *PM* previno significativamente cambios celulares y la muerte celular observada, especialmente en el grupo AT1 + *PM* y BT2 + *PM*.

PALABRAS CLAVE: *Pausinystallia macroceras*; Torsión/Detorsión; Ratas Wistar; Testículos.

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