Histopathological changes of male reproductive organs treated with oral administration of indomethacin in male rat

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Abstract

The present study was undertaken to make information about the toxicopathological effects of different doses of indomethacin on male rats' genital organs. The experiment was done on sixty male rats aged (8 weeks) and weighing (170-230 gm), they were divided equally into 4 groups: Group1: Rats served as control (C) and received distilled water for 2 months. Group 2: Rats received by gavage indomethacin diluted in distilled water at 1 mg/kg/B.W/day for 2 months. Group 3: Rats received by gavage indomethacin diluted in distilled water at 2 mg /kg /B.W/day for 2 months. Group4: Rats received by gavage indomethacin diluted in distilled water at 3 mg /kg /B.W/day for 2 months. At the end of experiment scarification (every 30 and 60 days) of the rats has been done. Specimens from testes and epididymis were excised for histopathological examination. The results showed that the pathological changes were dose and time dependent. It was concluded from the study that prolonged use of indomethacin is harmful to the genital organs.

التغيرات المرضية النسجية التكاثرية المتسببة عن التجريع الفموي بعقار الاندوميثاسين في ذكور الجرذان

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الخلاصة

هدفت الدراسة الحالية إلى معرفة التأثيرات المرضية السمية لعقار الاندوميثاسين على الأعضاء التناسلية لذكور الجرذان الناجمة عن التجريع الفموي لجرعات مختلفة من العقار. أجريت التجربة على 60 ذكر من الجرذان (عمر 8 أسابيع) ووزن (170−230 غم). حيث قسمت إلى اربع مجاميع متساوية وكما يأتي: المجموعة الأولى: أعطيت ماء مقطر لمدة شهرين وعدت مجموعة سيطرة. المجموعة الثانية: جرعت عقار الاندوميثاسين وبتركيز 1ملغم/ كغم من وزن الجسم/ يوم ولمدة شهرين المجموعة الثالثة: جرعت عقار الاندوميثاسين وبتركيز 2 ملغم/ كغم من وزن الجسم/ يوم ولمدة شهرين. المجموعة الرابعة: جرعت عقار الاندوميثاسين وبتركيز 3 ملغم/ كغم من وزن الجسم/ يوم ولمدة شهرين. تم اخذ عينات من كل من الخصية والبربخ لغرض إجراء التشريح المرضي النسجي بعد مرور 30 و60 يوم من التجربة. اظهر الفحص المجهري للخصية والبربخ حصول آفات مرضية ازدادت شدتها مع زيادة الجرعة المعطاة والمدة الزمنية للتجريع. نستنتج من الدراسة ان استخدام عقار الاندوميثاسين لفترة زمنية طويلة يؤدي إلى حصول تأثيرات مرضية سمية تؤدي إلى تلف في أعضاء التكاثر الذكرية.

Introduction

Indomethacin is a non steroidal anti-inflammatory drug (NSAID), posse's analgesic and antipyretic activity (1) by non selectivity inhibiting both cyclooxygenase (COX1) and (COX2) (2). NSAIDs inhibit synthesis of prostaglandins from arachidonic acid by the COX enzymes (3). Cox-1 constituently expressed and is required for physiological process such as maintenance of gastro-intestinal mucosa and vascular hemoestasis, whereas COX-2 is an inducible enzyme that has been linked to inflammatory reactions and cytokine release (4). Although it is effective in pain control, indomethacin

associated with many side effects like gastric and peptic ulcers, nausea, headache, dizziness, depression and psychosis (5). There are some reports assign that indomethacin induces decrease in testicular weight, sperm count, and motility (6). It also leads to mitochondrial oxidative stress accompanied with the generation of intra-mitochondrial reactive oxygen species (ROS), leading to imbalance between oxidants and antioxidants status in living system (7). ROS and lipid peroxidation play a role in testicular pathogenesis induced by NSAIDs like indomethacin drug (8).Therefore, the aim of present study was to make knowledge on the toxicopathological effect of indomethacin in male genital organs.

Materials and Method

- Experimental animals: Sixty male rats were used for the present study. The animals were housed in metal cages in the animal house in College of Vet. Medicine University of Baghdad and were fed on standard rat pellets, with water provided *ad libitum*, they were allowed to acclimatize for 10-14 days at room temperature.
- Chemicals: The indomethacin reconstituted in distilled water prior to daily administration.
- Dose calculation: The dose of indomethacin was calculated according to Alam (9).
- Experimental protocol: Sixty male rats aged (8 weeks) and weight (60-80 gm) was divided equally into 4 groups: Group1: Rats served as control (C) and received distilled water for 2 months. Group2: Rats received by gavages indomethacin diluted in distilled water at 1 mg/kg/B.W/day for 2 months. Group3: Rats received by gavage indomethacin diluted in distilled water at 2 mg /kg /B.W/day for 2 months. Group 4: Rats received by gavage indomethacin diluted in distilled water at 3 mg/kg/ B.W/day for 2 months.
- Histopathological study: At the end of each experiment scarification (every 30 and 60 days) of rats have been done. Five animals of each group were scarified by intramuscular injection of high dose of Ketamin hydrochloride. Testes and epididymis were dissected out fixed in 10% formaldehyde solution the specimens were sectioned (5μm thickness) and stained with hematoxylin and eosin according to Luna (10).

Results and Discussion

- Microscopic examinations: Control group: There were no significant microscopic lesions in the control groups along the period of experiment. G2: Testes: Therapeutic dose (1 mg/kg B.W) G_2 At 30 days period: Rats showed disorganized germinal epithelium with degenerative changes of the majority of the seminiferous tubules, these changes were characterized by vacuolation of spermatogenic cells and sertoli cells with interstitial odema that was represented by faint eosinopilic fluid (Fig. 1), At 60 days period: This period was characterized by widening of interstitial spaces due to increase in the amount of odematous fluid as compared with the previous period. In addition to the formation of intraluminal multinucleated spermatid giant cells (Fig. 2). G3: The majority of seminiferous tubules were shrunken with different shapes and apparent diminished layers of germinal epithelium, with wide lumina and wide interstitial spaces (Fig. 3). Other histopathological changes were characterized by fibrous thickening of tunica albuginea with vacuolation of spermatogenic cells. (Fig. 4), At 60 days period: testicular tissue showed marked decrease in germinal epithelium cell numbers, partial necrosis in some tubules with absences of nuclei especially in primary spermatocytes and germinal epithelium detachment from basement membrane with pyknosis of spermatogenic cells (Fig. 5). In addition to hyalinization of many seminiferous tubules (Fig. 6). G4: In addition to histopathological changes seen in the previous doses. The testicular tissue of rats showed coagulative necrosis and

depletion of germinal epithelium with hyalinization of luminal contents were greater in number than at 2 fold (Fig. 7). At 60 days period: In addition to the previous period, the main histopathological appearance was characterized by the presence large number of tubules containing only spermatogonia, which were scanty in number, other tissue sections showed that the remaining of seminiferous tubules are with irregular basement membrane with complete absence of spermatogenic cells (Fig. 8).

- Epididymis: G2 at 30 days period: The histopathological examinations revealed mild edema and slight interstitial of mononuclear cells infiltration, at 60 days period: Increase in amount of edematous fluid with moderate interstitial infiltration of mononuclear cells and proliferation of granulation tissue (Fig. 9). G3 at 30, 60 day's period: The cauda epididymis showed oligospermia and vacuolation of few germinal epithelium cells (Fig. 10). G4 at 30 days period: Tissue section exhibited that caput epididymis undergo sloughing of some germinal epithelium cells with intra-luminal cellular exudates (Fig.11). At 60 days period: At these periods the majority of the epididymis ducts had no spermatozoa in their Lumina (Fig. 12).

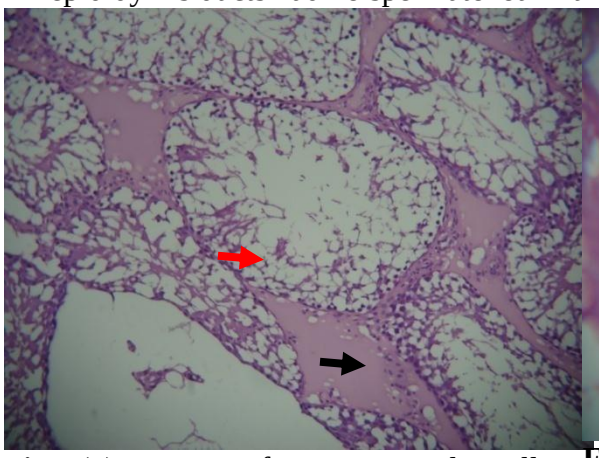

Fig. (1) Testes of rat received orally 1mg/kg B.W. of indomethacin for 30 days showing vacuolation of spermatogenic cells and sertoli cells (→) with interstitial edema that was represented by faint eosinopilic fluid (→) (H& E 10X).

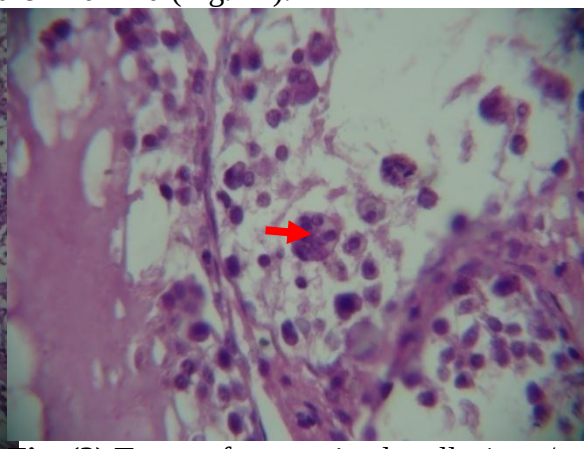

Fig. (2) Testes of rat received orally 1 mg/ kg B. W. of indomethacin for 60 days showing intraluminal multinucleated spermatid giant cells (→) (H& E 40X).

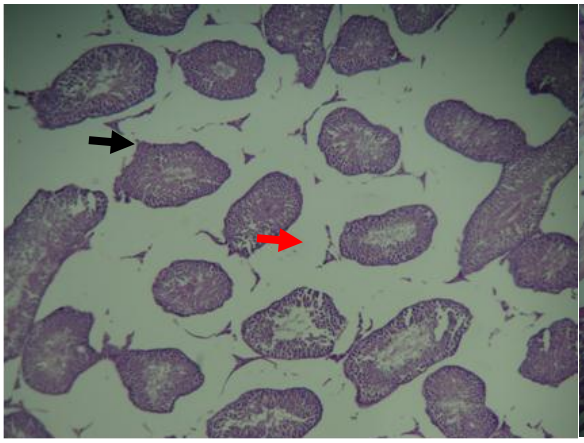

Fig. (3) Testes of rat received orally 2mg/kg B.W. of indomethacin for 30 days showing shrunken of seminiferous tubules (→) and wide interstitial spaces (→) (H&E 10 X).

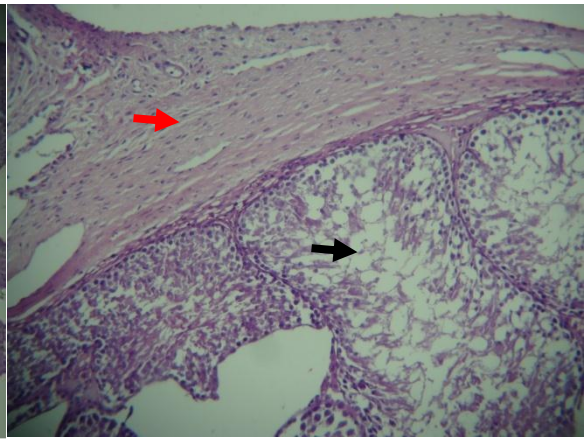

Fig. (4) Testes of rat received orally 2mg/kg B.W. of indomethacin for 30 days showing fibrous thickening of tunica albuginea (→) with vacuolation of spermatogenic cells (→) (H&E 40X).

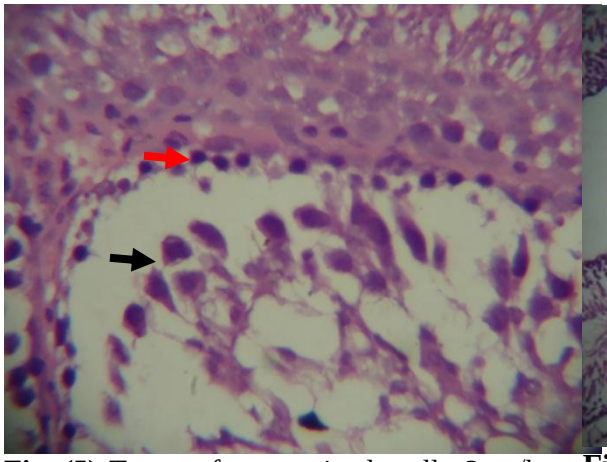

Fig. (5) Testes of rat received orally 2mg/kg B.W. of indomethacin for 60 days showing germinal epithelium detachment from basement membrane (→) with pyknosis and vacuolation of spermatogonia(→)(H&E 40X).

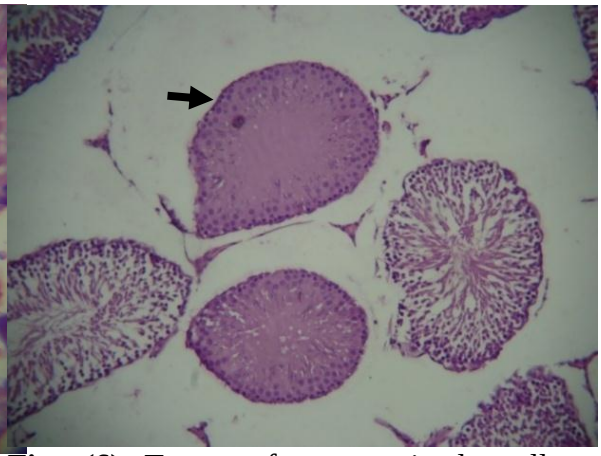

Fig. (6) Testes of rat received orally 2mg/kg B.W. of indomethacin for 60 days showing hyalinization of seminiferous tubules (→) (H&E 10X).

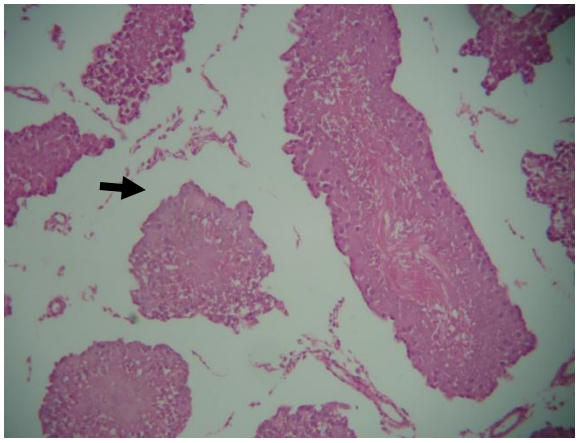

Fig. (7) Testes of rat received orally 3mg/kg B.W. of indomethacin for 30 days showing complete hyalinization of luminal seminferous tubules contents (→) (H&E 10X).

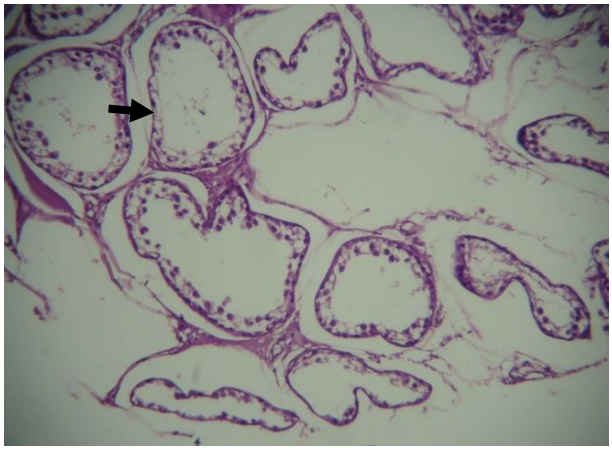

Fig. (8) Testes of rat received orally 3mg/kg B.W. of indomethacin for 60 days showing irregular basement membrane of seminferous tubules with aspermia. (→) (H&E 10X).

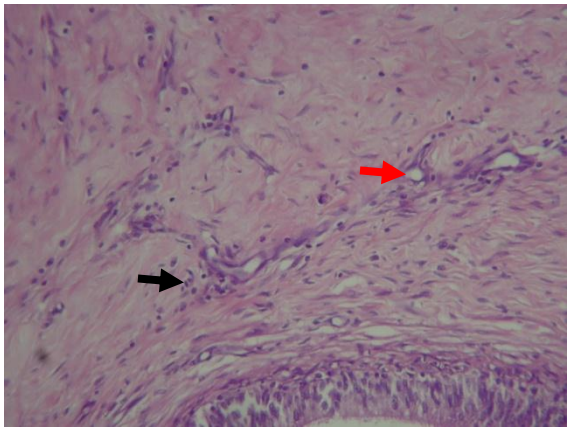

Fig. (9) Epididymis of rat received orally 1mg/kg B.W. of indomethacin for 60 days showing moderate interstitial infiltration of mononuclear cells (→) with proliferation of granulation tissue (→) (H&E 10X).

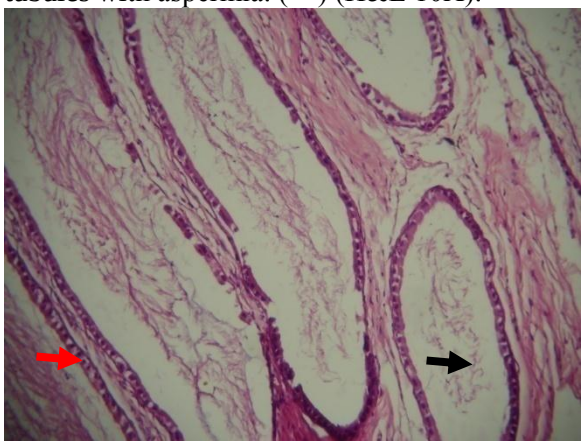

Fig. (10) Epididymis of rat received orally 2mg/kg B.W. of indomethacin for 60 days showing low sperm density (→) with vacuolation of germinal epithelium cells (→) (H&E 40X).

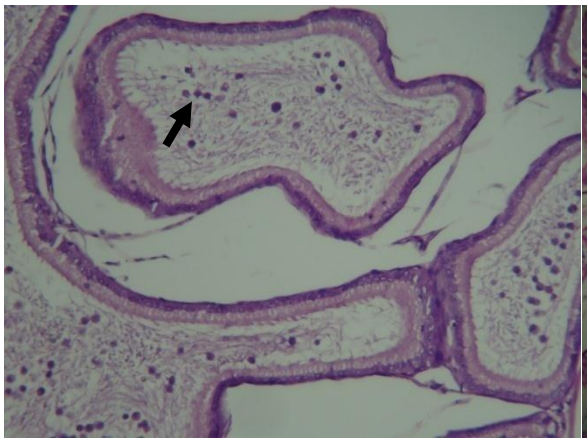

Fig. (11) Epididymis of rat received orally 3mg/kg B.W. of indomethacin for 30 days showing intraluminal cellular exudates (→) (H&E 10X).

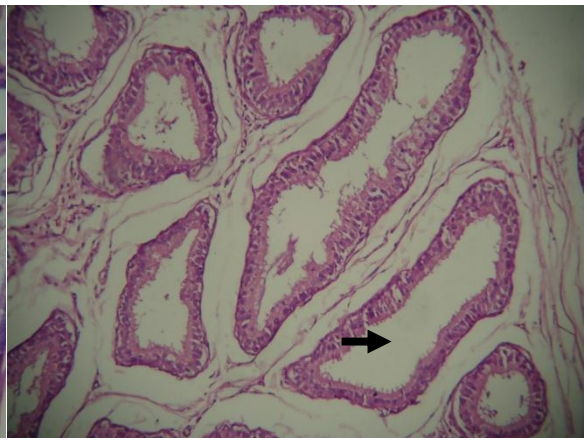

Fig. (12) Epididymis of rat received orally 3mg/kg B.W. of indomethacin for 60 days showing Asperemia (→) (H&E 10 X).

The very sensitive cellular constituents of the testicular spermatogenic epithelium and the high rate of mitotic activity make this organ more vulnerable to environmental and occupational hazards than other body tissue (11). It has also been postulated that the fertility in human is even more sensitive, as the output of human sperms is lesser times less than other mammals in terms of the number of sperms produced per gram of tissue. So any factor could be detected concerning the laboratory animals studies as a reproductive hazard is also expected to exert detrimental effects on the human reproductive performance (12). Therefore this study had shown that when the NSAIDs improperly used could serve as sources of harm to animals since it was known that indomethacin has a greater toxic effect on rodents (13). The present study exhibited that the degenerative changes and necrosis of spermatogenic epithelium was dose and time dependent, it was more obvious after 2 months of administration and that may be attributed to the disruption of pro-oxidant/ antioxidant balance which might lead to tissue injury, Oxidative damaging and production of lipid peroxide and formation of free radicals, similar explanation were described by Hemieda (8). Nolte *et al*., (14) who suggested that vacuolation of the spermatogenic cells might result from the dilatation and vesiculation of endoplasmic reticulum and mitochondrial swelling, and the much larger vacuoles are often phagocytotic vacuoles remaining after the digestion of necrotic germ cells. Similarly, Creasy and Foster (15) explained that vacuolization of the germinal cells may be due to the dilatation of smooth endoplasmic reticulum that possibly represents cellular permeability changes. In view of the fact that sertoli cells are the supportive cells within the seminiferous tubules and provide a multitude of factors required for spermatogenesis (16). Consequently, sloughing of germs cells pointed out the setoli cells damage due to microtubules impairment (17). These changes may be attributed to indomethacin induced lipid peroxidation (LOP) and the reduction in testosterone hormone. Where in testosterone it is required for the attachment of different generations of germ cells in seminiferous tubules. Therefore low, level of intratesticular testosterone may lead to detachment of germ cells from seminiferous epithelium and may initiate germ cell apoptosis and subsequent male infertility (18). The coagulative necrosis, depletion of the germinal and hyalinization of the luminal content were seen especially in rats administrated the 3 folds dose. And that was related to the loss of those populations through apoptosis or differentiation failure. (19 and 20) suggest an explanation of these changes was the reduced expression of setoli cell growth factor Glial cell line- derived neutrophic factor (GDNF) as well as retraction of the sertoli cells cytoplasmic processes that are normally supporting germ cells that might depress the spermatogonial differentiations.Similarly explanation was described in mutant mice (21). Bagoli *et al*. (6) reported that indomethacin probably crosses blood testes barriers and causes degeneration of seminiferous tubules, necrosis and decreases spermatogenesis, as well as the ability of activated metabolites of indomethacin to cause cross-linking of DNA strands, interfering with the normal cell division in all rapidly proliferating tissues. The changes in the cellular integrity could be due to the oxidation stress developed by the indomethacin-induced generation of ROS. Furthermore, indomethcin has also been shown to reduce the testicular level

of endogenous antioxidant in rats, these antioxidant are important markers of oxidative stress (22). Most seminiferous tubules contain sloughed germinal epithelium cells in their lumina. Bin Dohaish and Melebary (23) reported that the accumulated degenerated germ cells in the lumina of seminiferous tubules may be related to the defect of sertoli cells resulting in loss of spermatogenic epithelium and may lead to testicular destruction and infertility. The present results exhibited a gradual increase in the testicular basement membrane and fibrous thickening of the tunica albuginea. This thickening might be result from increase in the amount of collagenous fibers that could result from either increase in the population of collagen by fibroblast or decrease the rate of collagen phagcytosis (24, 25) Mentioned that the tendency toward fibrosis may be one of the possible explanations for the shrinkage of seminiferous tubules. Aydos *et al*. (26) stated that any changes in the proportion of collagen fibers and myoid cells may prevent the appropriate release of spermatozoa from the germinal epithelium into the lumen. The tendency of formation of intraluminal multinucleated spermatid giant cells might be due to widening of the intracellular bridges between the adjacent spermatids resulting in subsequent fusion of two or more cells. Similarly to (27, 28) Explained that these cells might be an indication of degeneration of the spermatogenic cells. However it might result from karyokinesis without cytokinesis of spermatid (14). In the current study, irregularities in the basement membrane were also noticed and that could be secondary to tubular shrinkage in the degenerated seminiferous tubules or as a result of contraction of myoid cells. Concerning pathological alterations affecting the epididymis as a consequence of testicular injury due to indomethacin administration, the majority of the epididymal ducts had no or low number of spermatozoa in the lumina found. (29) showed Significant reduction ($p<0.001$) in number of sperm containing seminiferous tubules in treated rat with low dose (2mg/k.g/B.W/day) and high dose (10mg/k.g/B.W/day) for 7, 14, 42 days respectively which finally reflected on epididymis. It was concluded from this study that the indomethacin have a harmful effect on genital system of male rats especially in high doses.

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