Original research article

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EFFECT OF NIMESULIDE ON SPERMATOGENESIS IN ALBINO RATS — A HISTOLOGICAL STUDY

Dr.Ram Singh Verma[1*], Dr. J.N.Puri[2], Dr.I.P.Jain[3], Dr.R.K. Srivastava[4], Dr.N.A.Ansari[5], Dr.S.P.Singh[6]

1. Department of pharmacology, Government Medical College, Kannauj, India, 2,3,5,6. Department of pharmacology, G S V M Medical College, Kanpur, India, 4. Department of Anatomy, G S V M Medical College, Kanpur, India

***Email id of corresponding author-** ramsinghverma222@gmail.com

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ABSTRACT:

Objective: The aim of the study is to find effect of nimesulide on sperrnogenesis by studying the histological changes in the testis of rats. **Material & Methods:** It was an animal model experimental study conducted in department of pharmacology ,Government Medical College, Kannauj, for this work 60 male albino- rats were taken and divided into two groups (A,B)containing, 30 rats each. The rats of group A served as a control group; and group B served as a Nimesulide group Five rats of each group will be sacrificed on day 15, 30 45 60,75 and 90 respectively under ether anesthesia for observation of testis and epididymis. **Results :** Treatment with nimesulide did not significantly affect body weight, and testis weights, but there were significant differences in testicular architecture and degenerative changes. **Conclusion :** Nimesulide administration causes suppression of spermatogenesis. Prolonged administration of nimesulide leads to necrosis of germinal epithelium and infiltration with lymphocytes and fibrosis at places Thus prolonged administration of nimesulide causes irreversible damage to germinal epithelium of testis.

Keywords: Cox 2 Inhibitors, Spermatotoxic, NSAIDS.

INTRODUCTION:

Non steroidal anti-inflamrnatory drugs NSAID are one of the most commonly used drugs(1) They are used as analgesic, antiyretic, and as antiarthritic atniinflammatory agents (2) .NSAIDS (Felson et at. 1992) differ chemically but share the same mechanism of action i.e. inhibition of prostaglandins biosynthesis via inhibition of enzyme cyclo-oxygenase COX.(3,4) There is ample evidence to suggest that prostaglandins may play an important role in spermatogenesis. **(5,6)**

There is also evidence suggesting that certain. NSAIDS may inhibit spermatogenesis if given for long duration **(8)** So it was thought worthwhile to conduct this Study to find out the potential of certain commonly used NSAIDS on sperrnogenesis by studying the histological changes in the testis of rats.

MATERIAL AND METHODS

This experimental study was carried out in the laboratory of the Department of Pharmacology,

Government Medical College, Kannauj, for this work male albino- rats were taken from animal house of the Pharmacology, Department of Medical College, Kanpur.

Experimental procedure: The weight of the male albino-rats varied from 130 to 180 gms and all the rats were maintained under uniform laboratory condition throughout the experimental period. The total number of 150 male albino-rats were divided into two groups (A,B)containing, 30 rats each. The rats of group A served as a control group; and group B served as a Nimesulide group.

GROU P A : (Controlled): Will be labeled as controlled group and fed distilled water

GROUP B(Nimesulide) will be fed Nimesulide in a dose of 35mg/kg/day

Calculation did as following for administration of drugs as under: 1 Tab Nimesulide 100 mg dissolved in l0 ml distilled water.-l ml =10mg

Five rats of each group will be sacrificed on day 15, 30 45 60,75 and 90 respectively under ether anesthesia. Testis and epididymis will be taken out and preserved n 10% formal saline will be processed for paraffin.5 micron thick section will be cut out and stained with hematoxylin and Eosine stain and Tetra Chrome stain. Slide will be examined under the microscope for the histological changes induced by the drug if any.

Diet of animal: The albino rats were maintained on regular diet of Bengal gram (25 gms) and Wheat (25gms).Under uniform laboratory condition throughout the experimental period.

RESULTS

In present study we have used rat model to evaluate the effect of Nimesulide on spermatogenesis. We closely observed the behaviour of animals during the study period with special reference to their general behaviour and food habits Then body weight was assessed every fortnightly After sacrificing the rats, testes were taken at and its morphology and weight was observed.

In terms of Animal Behavior, no differences in the behavior of rats of control group and the rats administered with Nimesulide, was noted.

The bodyweight of rats varied between 130-180 gms at the start of study. At the end of the 3 months the study was finally terminated, the weight of the rats varied between 150 to 220 gms. There was no significant difference of weight between the both groups. Thus there was general increase of body weight rats during the study.

Gross examination of testis

The colour of testis was not affected by the drug administration. It was pale brown in colour and the size of the testis was reduced in B groups. The average weight of the testis in the control group was 1025 mg. In the Nimesulide group average weight was range between 740 – 760 mg.

The average weight of the testis has been shown in table No 1

Experimental group	Average Weight
A. Control group	1025 mg
B. Nimesulide group	740 -760 mg

Histological study: The histological study was done under low power, high power and oil emersion Olympus trinacular research microscope.

Control group - Each testis was enclosed by a dense fibrous capsule, the tunica albugenia, underneath which there was loose layer of connective tissue, rich in blood vessel the tunica vasculosa. The Seminiferous tubules were closely packed. Each tubules was surrounded by fibro-elastic connective tissue and flattened epitheloid cells known as myoid cells, Internal to it was thin homogenous basement membrane (fig. No 1).

Internal to basement membrane, spermatogenic cells of increasing maturity form connective band of germinal epithelium and circumscribed a discrete lumen. The lumen was fully occupied by the tails of the sperms (fig. No. 1). The seminifèrous epithelium consist of two layers of cells.

I. Spermatogenic cells: spermatogenic cell were arrange in orderly manner in four to eight layer. Primitive germ cell i.e. spermatogonia were closely lining the basal lamina. Light and dark types of spermatogonia were well recognized, these cells were spherical, and nucleus was also spherical and rich in chromatin material (fig. No2). 2. Sertoli Cells Throughout the period of spermatogenesis the developing cells where in the association with tale pillar like cell, the sertoli cell which were sited on the basement membrane and extend perpendicularly found the basement membrane to the lumen.(fig No. 2).

Interstitial tissue - The semmferous tubule were bounded together by loose intertubular connective tissue, which contain fibroblast, collagen fibre, blood vessel, lymphatics and a group of t Interstitial cells or leydig cells.(fig No 3).

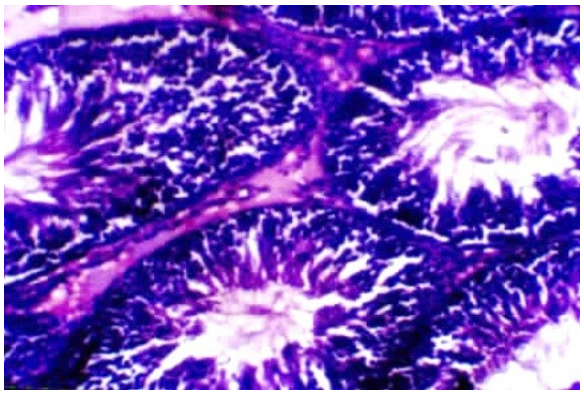

Figure 1. Control group showing active spermatogenesis

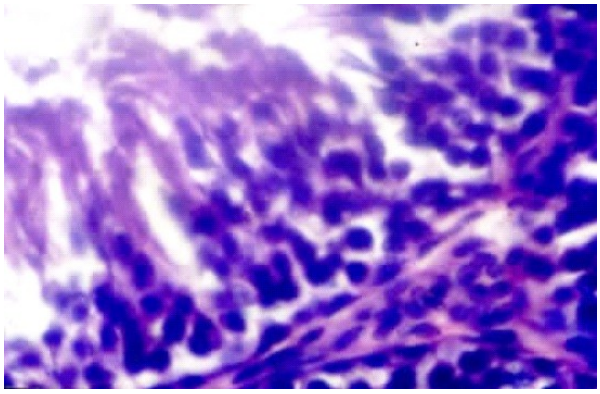

Figure 2 Control group showing seminiferous tubules basement membrane spermatogonia, spermatocyte, spermatid, sertoli cells.

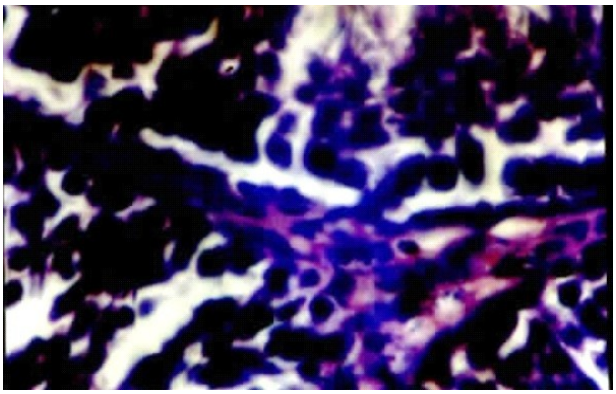

Figure 3. Control group showing leydig cells showing polygonal cells

Nimesulide Group: Severe degenerative changes seen. There were is much necrosis in germ cells that large vacuoles were seen in the area of somniferous tubules lined by germ cells. Intertubular connective tissue in substantially increased Large vacuoles are seen in intertubular areas (Fig No 8) Spermatogenesis is markedly suppressed infiltrative area is showing number of vacuoles formation (Fig No 7) Seminiferous tubules are loosely arranged. Lumen of many saminiferous tubules is almost free of spermatids (Fig No 6) Many of the sertoli cells are free. No spermatids are attached to it (Fig No 5) Basement membrane is thickened Peritubular fibrosis is seen (Fig. No. 4).

In many, tubules signs of necrosis were seen (Fig. No.9). Cells are oedematous and cellular markings are lost .Infiltration with lymphocytes and fibrosis is seen at places. Amount of suppression of spermatogenesis and necrosis were directly proportional to duration of administration of drugs.No effect on Leydig cells seen.

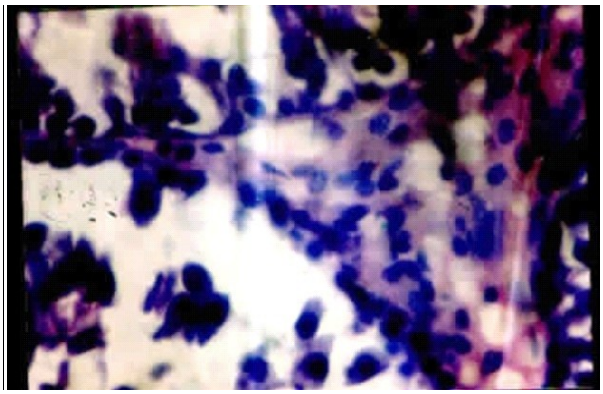

Figure 4 Nimesulide group showing thickened basement membrane and peritubular fibrosis

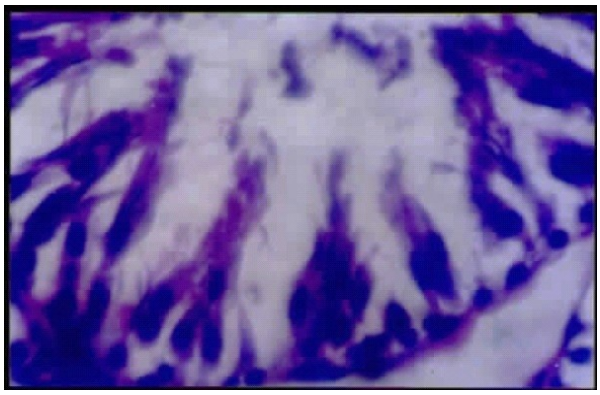

Figure 5. Nimesulide group showing no sertoli cells , no spermatid attached to it

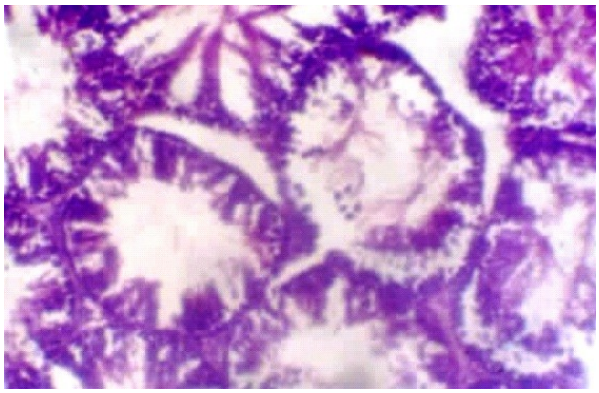

Figure 6. Nimesulide group showing marked suppression of spermatogenesis

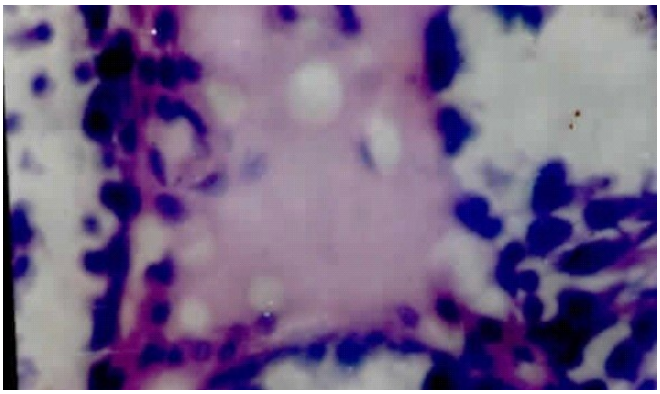

Figure 7. Nimesulide group showing marked suppression of spermatogenesis

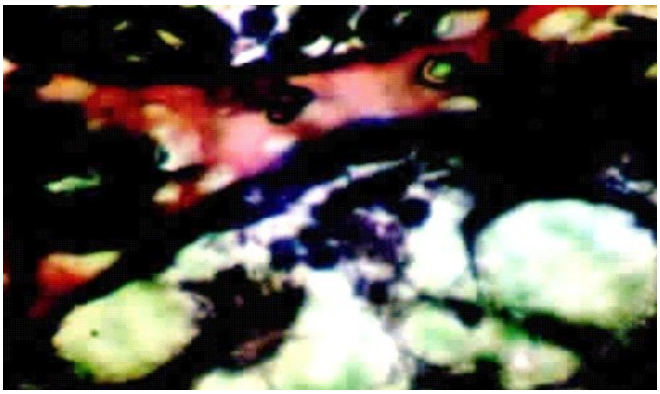

Figure 8 Nimesulide group showing large vacuoles in the area of germinal epithelium

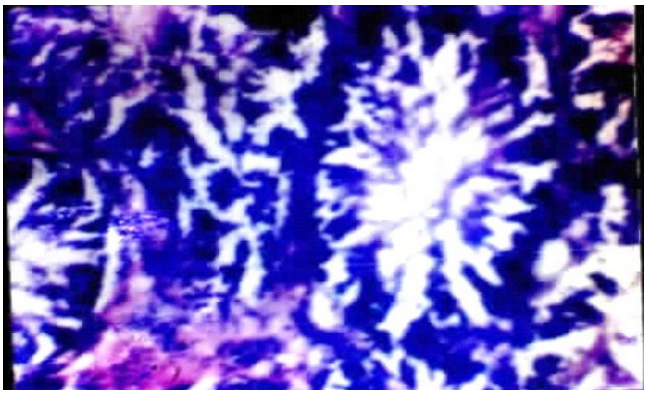

Figure 9 Nimesulide group showing sign of necrosis

DISCUSSION

Non-steroidal anti-inflammatory drugs are most commonly used analgesic, antipyretic, anti-inflammatory and anti-arthritic agents. Although these agents differ chemically but share the same mechanism of action .i.e. inhibition of prostaglandins and thromboxiane synthesis via inhibition of enzyme, cycio-oxigenase (Cox- I & Cox - II). There has been substantial progress in elucidating the mechanism of action of NSAIDS Inhibition of cyclo-oxigenase the enzyme is responsible for bio-synthesis of prostaglandins and certain related autacoids. **(8,9)** The has been ample evidence to suggest that prostaglandins may play an important role in spermatogenesis **(6)**

The colour of testis is not altered by drug administration but the size of the testis was reduced in the test group possibly because of testicular atrophy and suppression of spermatogenesis.

The average weight of the testis in controlled group was 1025 mg. The average weight were reduced in the test groups. It was 740 - 760mg in Nimesulide group. Didolkar A. K et al also found that NSAID like Aspirin given for 30 days caused decrease in the weight of testis of immature rats.(10)

Histological Examination: In nimesulide group , severe degenerative changes were seen in seminiferous tubules on prolonged administration groups (45 days onwards). There is so much necrosis in germ cells that large vacuoles are seen in the area of seminiferous tubules lines by germ cells. Inter tubular connective tissue is substantially increased, last vacuoles are seed in the inter tubular areas. Didolkar A. K et al were observed in his

experimental study that on prologe (30 day) administration of Aspirin causing decrease in the number of spermatids and increase in size of spermatocytes nuclei and finally aspirin impair the later stages of spermatogenesis. (10)

It appears that nimesulide somehow interfere with the lipid metabolism may be testosterone synthesis is inhibited and hence large amount of lipids are accumulated which lead to vacuole formation. (Fig. No.8). Thus is appears that nimusulide is toxic to the germinal epithelium of testis. In an animal model study, Uqochukwu et al determined that the administration of nimesulide has an effect on the testosterone and estradiol levels.(11)

The spermatogenesis is markedly suppressed, inter tubular area is showing no. of vacuoles formation (Fig. No. 8). Seminiferous tubules are loosely arranged, lumen of many seminiferous tubules is almost free of spermatids (Fig. No, 5), Many of sertoli cells are free no spermatids are attached to it (Fig. No 6). Basement membrane is thickened peri tubular fibrosis is seen. In many tubules sign of necrosis were seen (Fig. No, 4). Cells are edematous cellular marking are lost (Fig.No. 9). Infiltration with lymphocyte and fibrosis is seen at places in 75 and 90 days group. Studies on mice demonstrated a awful effect of COX 2 inhibitors on sperm parameters .(12)

On contrary some studies did not showed a significant spermatoxic effect of nimesulide in animal model study(11,13,) probability of this result is a small duration of drug administration.

Thus it appears that prolonged administration of nimesulide as usually given in case of osteoarthritis and ankylosing spondylitis etc.,may lead to irreversible suppression of spermatogenesis and testicular atrophy.

CONCLUSION:

It was concluded that, Nimesulide administration causes suppression of spermatogenesis. Prolonged administration of nimesulide leads to necrosis of germinal epithelium and infiltration with lymphocytes and fibrosis at places Thus prolonged administration of nimesulide causes irreversible damage to germinal epithelium of testis.

REFERENCES

1. Nuki G. Nonsteroidal and anti-inflammatory agents Br. Med.J. 1983; 287: 39-43.

2. Steinmeyer J. Pharmacological basis for the therapy of pain and inflammation with non-steroidal anti-inflammatory drugs. Arthritis Res. 2000;2:379–85.

3. Vane JR. Inhibition of prostaglandin biosynthesis as a mechanism of action of aspirin-like drugs. Nat New Biol. 1971;231:232–235.

4. Meade E. A. Smith W.L. and DeWitt. D.L Differential inhibition of prostaglandin endoperoxide. synthase (cycloxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J. Biol. Chem., 1993,268:6610-6614.

5. Frungieri MB, Calandra RS, Mayerhofer A, Matzkin ME. Cyclooxygenase and prostaglandins in somatic cell populations of the testis. Reproduction.2015 ;149(4):R169-80. doi: 10.1530/REP-14-0392. Epub 2014 Dec 12.

6. Singh SK, Dominic CJ. Prostaglandin F-2 alpha induced changes in the sex organ of male laboratory mouse, Exp Clin Endocrinol 1986;88 (3): 309-15.

7. Moskovitz B, Munichor M,Levin DR. Effect of diclofenac sodium (Voltaren) on spermatogenesis of infertile oligospermic patients. Eur Urol. 1988;14(5):395-7.

8. Rao PPN, Kabir SN, Mohamed T. Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): Progress in Small Molecule Drug Development. Pharmaceuticals. 2010;3(5):1530–1549.

9. Brooks. P.M. and Day R.O., Nonsteroidal antiinflammatory drugs--differences and similarities. N Engl J Med. 1991 Jun 13;324(24):1716-25.

10. Didolkar, A. K., Patel, P. B. and Roychowdhury, D. Effect of Aspirin on Spermatogenesis in Mature and Immature Rats. International Journal of Andrology, 1980;3: 585–593. doi:10.1111/j.1365-2605.1980.tb00146.x

11. Ugochukwu AP, Ebere OO, Okwuoma A. Effects of nimesulide on testicular functions in prepubertal albino rats. J Basic Clin Physiol Pharmacol 2011;22(4):137-40.

12. J. H. Kennedy, N. Korn, and R. J.Thurston, “Prostaglandin levels in seminal plasma and sperm extracts of the domestic turkey, and the effects of cyclooxygenase inhibitors on sperm mobility,” Reproductive Biology and Endocrinology, vol. 1, article 74, 2003.

13. D. Canale, I. Scaricabarozzi, P. Giorgi, P. Turchi, M. Ducci, and G. F. Menchini−Fabris, “Use of a novel non−steroidal anti−inflammatory drug, nimesulide, in the treatment of abacterial prostatovesiculitis,” Andrologia, vol. 25, no. 3, pp. 163–166, 1993.