Original research article

International Journal of Medical Science and Education | pISSN- 2348 4438 | eISSN-2349- 3208

A HISTOLOGICAL STUDY ON THE EFFECT OF DICLOFENAC SODIUM (DECLOPHEN) AND DICLOFENAC POTESSIUM ADMINISTRATION ON SPERMATOGENESIS CELLS OF ALBINO RATS

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Received: 11/07/2015 ***Revised: 10/09/2015*** ***Accepted: 26/09/2015***

ABSTRACT:

Background: To aim of the study is to find effect of Diclofenac sodium and diclofenac potassium administration on spermatogenesis cells of albino rats 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 90 male albino- rats were taken and divided into three groups (A,B,C)containing, 30 rats each. The rats of group A served as a control group; and group B served as a Diclofenac sodium and Group C Diclofenac K .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 Diclofenac sodium and potessium did not significantly affect body weight, and testis weights, but there were significant differences in spermatogenesis and well correlated with duration of drug administration. **Conclusion:** Diclofenac potassium administration in albino rats also causes suppression of spermatogenesis. This suppression of spermatogenesis is directly proportional to the duration of drug administration and is reversible in nature; Suppression of spermatogenesis is more marked than diclofenac sodium group.

Keywords: Cox 2 Inhibitors, Spermatotoxic, Spermatids, NSAIDS

INTRODUCTION

Anti-inflammatory analgesic and antipyretic drugs are exogenous group of compounds, often chemically unrelated, but share certain therapeutic actions and side effect. The prototype is aspirin; hence these compounds are often referred to as aspirin - like drugs: they also are frequently called non-steroidal anti-inflammatory drugs (NSAIDs).**(1)**

NSAIDs had been known to inhibit a wide variety of reactions Vane et al, 1995 demonstrated that low concentrations of aspirin and indomethacin inhibited the enzymatic production of prostaglandins. Participated in the pathogenesis of inflammation and fever. **(2)** NSAIDS inhibit the biosynthesis and release of prostaglandins in all cells **(2,3,4).**

The first enzyme in the prostaglandin synthetic pathway is prostaglandin endoperoxide synthase, or fatty acid cyclooxygenase. This enzyme converts arachiadonic acid to the unstable intermediates and PGG2 and PGH2. It is now appreciated that there are two forms of cyclooxygenase, termed cyclooxygenase -1 (COX-1) and cyclooxygenase -2 (COX-2).

COX-1 is a constitutive isoform found in blood vessels, stomach and kidney, while COX-2 is induced in setting of inflammation by cytokines and inflammatory mediators. Aspirin and NSAIDs inhibit the cyclooxygenase enzyme and postaglandin production; they do not inhibit lipoxyenase pathways and hence do not suppress leukotriene formation.Although there is good evidence that therapeutic doses of aspirin and NSAIDs reduce prostaglandin biosynthesis in human beings and that of there is a reasonably good rank order correlation between the potency of drugs as inhibitors of cyclooxygenase and their anti-inflammatory activity. **(5)**

Furthermore, it has been reported that the extensive use of diclofenac increases the risk of acute myocardial infarction and several cases of severe local reactions associated with intramuscular injection of diclofenac have been reported. **(6)** Diclofenac was found to generate protein adducts in the livers of treated mice as well as in rat hepatocytes via protein acylation by the drug glucuronide. **(7)** In vitro experiments with cultured rat hepatocytes have shown, however, that the covalent binding of diclofenac is neither the only nor the major cause of acute cytotoxicity.**(8)**

The effect of Diclofenac Na and K on reproductive males cannot be ignored and it is highly desirable to search its harmful effects. Hence, this study is aimed at determining the possible negative effects of Diclofenac Na and K on spermatogenesis cells of albino 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 90 male albino-rats were divided into three groups (A, B and C) containing, 30 rats each. The rats of group A served as a control group; and group B served as a Diclofenac Na group and GROUP C as a (Diclofenac K) group.

GROUP A (Controlled): Will be labelled as controlled group and fed distilled water

GROUP B (Diclofenac Na) :will be fed Diclofenac sodium in a dose of 7 gm/kg/day.

GROUP C (Diclofenac K): will be fed Diclofenac Potassium in a dose of 7 mg/kg/day.

Calculation did as following for administration of drugs as under: 1 Tab. Diclofenac Na 100 mg dissolved in 100 ml of distilled water → 1ml= 1mg.2. Tab Dictofenac K 100 mg dissolved in 100 ml of distilled water→1ml = 1 mg.

Five rats of each group will be sacrificed on day 15, 30 45 60, 75 and 90 respectively under ether anaesthesia. 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 Diclofenac K and Diclofenac Na on spermatogenesis. We closely observed the behaviour of animals during the study period with special reference to their general behaviour and food habits. Their body weight was assessed every fortnightly after sacrificing the rats, testes were taken at and its morphology and weight was observed.

We did not notice any change in behaviour of Animal in control group and the rats administered with Diclofenac K and Diclofenac Na.

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 from 150 to 220 gms. There was no significant difference of weight among the different groups. Thus there was general increase of body weight rats during the study.

Table No 1 Showing the average weight of the testis

Experimental group	Average Weight
A. Control group	1025 mg
B. Diclofenac K group	800 -850 mg
C. Diclofenac Na	800-830 mg

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 and C groups. The average weight of the testis in the control group was 1025 mg. In the Diclofenac K group average weight was range between 800-850 mg and in Diclofenac Na group average weight was range between 800-830 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 seminiferous 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. No 2). 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 semminiferous 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).

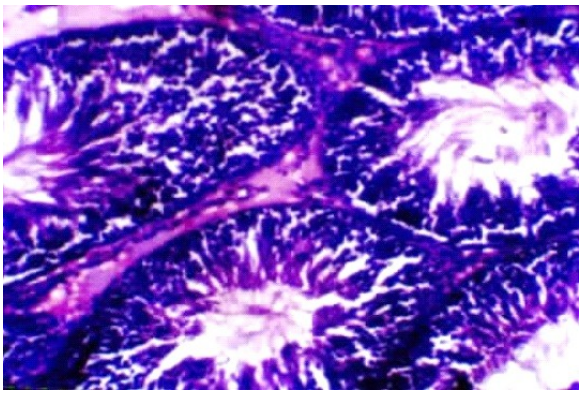

Figure 1. Control group showing active spermatogenesis

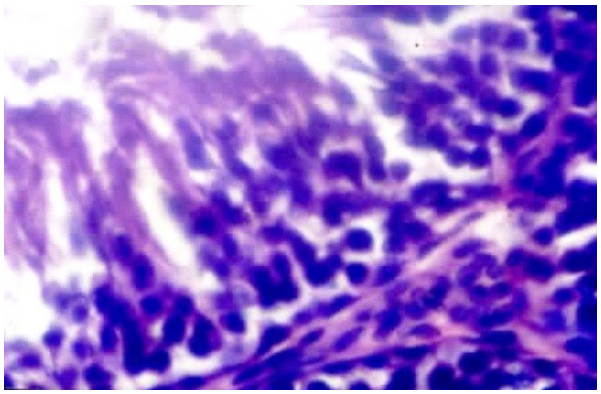

Figure 2 Control group showing seminiferous tubules basement membrane spermatogonia, spermatocyte, spermatid, sertoli cells.

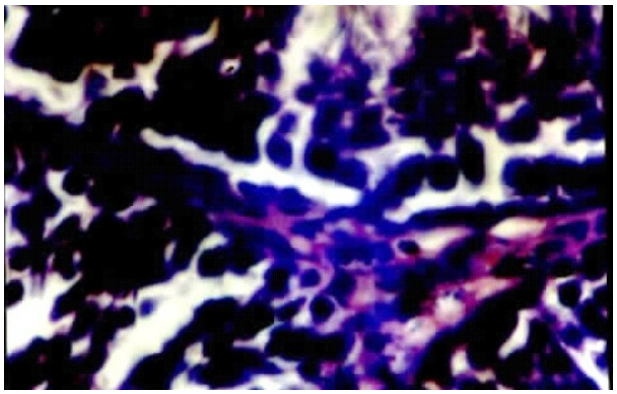

Figure 3. Control group showing leydig cells showing polygonal cells

DICLOFENAC-NA GROUP

Each testis was enclosed by dense fibrous capsule, the tunica albugenia, underneath which there was loose layer connective tissue, rich in blood vessels the tunica vasculosa. The seminiferous tubules were loosely packed (fig No 4).The basement membrane was thickened Internal to basement membrane spermatogenic cells of increasing maturity were disperse arranged, thus the typical architecture of germinal epithelium was altered (fig No 5), The lumen was having fewer tails of spermatids. The

sertoli cells were occupied by fewer spermatids. (fig No 6).

Number of layer of spermatogenic cells were reduced and remained only one to four. In many seminiferous tubules the germinal epithelium was split at the level of light and dark type sprmatogonia and it appeared as of the germinal epithelium in sloughing off .(Fig No 7)

Fewer spermatids were seen fitted into the apical recesses sertoli cells and no the lumen of many of the seminiferous tubules were almost empty (Fig No 8) The changes were directly proportionate to the duration of administration of the diclofenac sodium.

Diclofenac –K Group

Seminiferious tubules were arranged (Fig 9) signs of suppression of spermatogenesis seen in many seminiferous tubules are empty having only few spermatids (Fig No 10) Germinal cells decrease in number and loosely arranged (rig No I1a & 11 b) Many sertoli cells are seen free, not having attached spermatids. At many places signs of necrosis are also seen. The architecture of seminiferous tubules is lost the infiltration of lymphocytes is also seen (Fig. 12). Most of the cells lining the seminiferous tubules except the basal cells are sloughed out in lumen so that seminiferous tubules become empty. Architecture of the tubules is grossly lost at places (Fig No 13) The changes were directly proportionate the duration of diclofenac potassium administration.

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.(11)

Histological Examination

In ctmtrot group, the seminiferous tubules were closely packed and surrounded by a thin homogenous basement membrane internal to which were sperniatogemc cells of increasing maturity forming concentric bands of germinal epithelium and circumscribed a disrete lumen fully occupied by tails of sperms. (Fig. No. I), Spermatogenic cells were arranged in orderly manner into four to eight layers. Spermatogonia were closely lining with basal lamina, innier to that primary and secondary spermatocytes were seen. Spermatids weje usually present in groups

close to lumen. The elongated sperms head were fitted in the recesses of sertoli cells and their tails were occupying the lumen. (Fig. No. 2).

Throughout the period of spermatogenesis the developing cells were intimated associated ith tall pillar like cells the sertoli cells which were sited on the basement membrane extent perpendicularly from basal membrane to the lumen. (Fig. 2).

Seminiferous tubules were bounded together by loose intertubular connective tissue which contain fibroblast, collagen fibre blood vessels lymphatics and group of large polyhadral interstitial cells known as leydig cells. (Fig. No, 3).

Diclofenac Sodium Group:

In diclofenac sodium group showing seminiferous tubules were loosely packed (Fig. No. 4). The basement membrane was thickened. The spermatogenic cells were dispersely arranged (Fig No, 5). The lumen was having fewer tails of spermatids, the sertoti cells were occupied by fewer spermatids (Fig. No, 6). The number of layers of spermatogeaic cells were also reduced and remained only one to four. In many seminiferous tubules the germinal epithelium as split at the level of light and dark type of spermatogonia and it appeared as if the germinal epithelium is sloughing off (Fig. No. 7). Fewer sermatids were seen fitted in apical recess of sertoli cells so the lumen of seminiferous tubules were aimost empty. (Fig. No, 8)

All these findings suggest that diclofenac sodium administration in albino rats causes suppression of spermatogenesis. This suppression of spermatogenesis is directly proportional to the duration of administration of the drug. As the basal layer of the germinal epithelium is intact and there is no effect on sertoli cells and Leydig cells. It appears that this suppression of spermatogenesis is reversible.

Our findings are similar to the findings of **(12)** who found suppression of spermatogenesis in mature dogs on administration of diclofenac sodium 2mg /kg body weight subcutaneous once a day for 42 days. Our findings contrary to the findings of **(13)** who administered diclofenac sodium 100 mg daily for 30 days in infertile oligospermic patients and noted statistically significant increase in number and motility of spermatozoa in patients with higher level of seminal prostaglandins but noted no significant variations in patients with normal values of seminal prostaglandins.

Diclofenac Potassium Group:

The histological findings of seminiferous were loosely arranged (Fig. 9). Signs of suppression of spermatagenesis were seen. Many seminiferous tubules were empty having only few spermatids (Fig. 10). The germinal cells were decrease in number and loosely arranged (Fig. No. 11a & 11 b). Many sertoli cells were seen free not having attached spermatids. These findings of suppression of spermatogenesis are similar to those of diclofenac sodium group and were directly proportionate with the duration of drug administration.

At many places signs of necrosis are also seen. The architecture of seminiferous tubules is lost (Fig. No, 12), Most of the cells lining the seminiferous tubules except the basal cells are slough out in the lumen so that the seminiferous

tubules become empty. The architecture of seminiferous tubules is grossly lost at places (Fig No, 13). These findings were noted in prolonged administration group (60 days, 75 days and 90 days). Thus, it appears that the on prolonged administration of diclofenac potassium is more toxic to the germinal tissue of testis than diclofenac sodium.

CONCLUSION

It was concluded that, Diclofenac potassium administration in albino rats also causes suppression of spermatogenesis. This suppression of spermatogenesis is directly proportional to the duration of drug administration and is reversible in nature, Suppression of spermatogenesis is more marked than diclofenac sodium group .There is no effect on sertoli cells and leydig cells.

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