INVESTIGATION OF THE EFFECT OF DICLOFENAC SODIUM AND THYMOQUINONE EXPOSURE IN PREGNANCY ON THE NUMBER OF RAT SPERMATOGONIUM CELLS BY STEREOLOGICAL METHODS

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ABSTRACT

Diclofenac sodium (DS) is a nonsteroidal antiinflammatory (NSAID) drug that has analgesic, antipyretic and anti-inflammatory effect. Thymoquinone, the active constituent of Nigella sativa, has bright yellow crystal. The aim of this study was to investigate the effects of diclofenac sodium and thymoquinone applied in pregnancy on the number of spermatogonium cells by stereological methods. Pregnant rats were separated into Control, Saline (SF), Diclofenac Sodium (DS), Thymoquinone (TQ) and Diclofenac Sodium + Thymoquinone (DS + TQ) groups. DS and TQ was given between days 5and 15 of gestation. At the end of the postnatal tenth week, rats were perfused under anesthesia and the right testes were removed. it was embedded in the paraffin. Sections were examined with light microscope. Stereologically, the physical disector method for total cell number of spermatogonium, and the Cavalieri principle for total testis volume were applied. As a result, diclofenac sodium and thymoquinone not caused a significant change on total cell number of spermatogonium in ten week old rats ($p > 0.05$).

Keywords: Diclofenac sodium, Rat, Spermatogonium, Stereology, Thymoquinone

INTRODUCTION

The effect of nonsteroidal antiinflammatory drugs(NSAID) t is weaker than steroid antiinflammatory drugs. NSAID are preferred in many painful diseases such as headache, myalgia, arthralgia, dental pain. Because of it is not caused narcotic conditions such as drug dependence, numbness and confusion. NSAID inhibite the cyclooxygenase (COX-1 and COX-2) enzymes that catalyze the formation of prostaglandins (PG) in tissues (Dökmeci, 2000; Kayaalp, 2005).

Nigella sativa (NS) is a herbaceous plant species of the family Ranunculaceae, native to southern and eastern Europe and eastern Mediterranean countries (Gad et all., 1963; Al-Gaby, 1998; Khan, 1999). Much research has been done on the materials obtained from the seeds and seeds of the nigella sativa . It has been widely used in the treatment of asthma, headache, colds, jaundice, gas remover, diuretic, many rheumatic and inflammatory diseases among the people in the Far East and many Asian countries in the past (Houghton et all., 1995; Badary et all., 1998; Worthen et all., 1998; Badary, 1999; Burits ve Bucar, 2000; Morsi, 2000; Al-Ghamdi, 2001; El-Abhar, 2003).

MATERIALS AND METHODS

In the study, 20 adult Albino Wistar female rats weighing 200-300 g were obtained from the laboratory and application center of Van Yüzüncü Yıl University and 5 male rats were used for mating. For mating, each cage was placed with 4 female and 1 male rat. The animals were fed standard food, tap water and *ad libitum* in a 12 hour light and dark environment. As a result of daily vaginal plaque control, the day when the vaginal plague was seen was accepted as the zero day of the pregnancy.

Pregnant rats were divided into 5 groups as Control, Saline, Diclofenac sodium, Thymoquinone and Diclofenac Sodium+Thymoquinone. There was no administration to the control group. Between 5-15 days of gestation; serum physiological group was given daily 1ml/kg Saline/day (ip), diclofenac sodium group 5 mg / kg DS (IM), thymoquinone group 5mg/kg TQ dissolved in drinking water in the, Diclofenac sodium + thymoquinone group 6.1mg/kg DS (IM) and 5mg/kg dissolved in drinking water.

7 male rats from each group were perfused under the deep anesthesia (ketamine-xylazine) by opening the thorax region for 10 weeks after birth. After perfusion, the right scrotum was removed from the incision made in the scrotal region and fixed in the Bouin solution. After the fixation, half of the

fraction were used. It was embedded in paraffin after being passed through alcohol and xylene series. Consecutive sections with a thickness of 4µm were taken in the microtome. The first pair was randomly selected and the other pair was systematically taken every 80th step. Thus, 10-14 cross-sectional pairs were taken from each tissue block. Tissue pairs were stained with hematoxylin-eosin stain and then examined under light microscope. For the total volume of tissue, the Cavalieri's Principle, for the cell number the *physical disector* counting method was applied.

In the systematic random sampling of cross-sections, field sampling with 50µm x 50µm step size in the horizontal and vertical positions was performed in the first field in the field restricted to an unbiased counting frame with 1064,782 μm^2 area, and cells without the second section were counted (Figure 1). The dotted area measurement scale was used to calculate the total volume (Figure 2).

The following formula was used for cell counting; $N = N_v \cdot V_{ref}$

N : Total particle number, N_v : Numerical density of particle, V_{ref} : Total volume of the structure.

$V_{ref} = \sum P \cdot a(p) \cdot t$

$\sum P$: Total number of dots corresponding to the structure, a(p): Area covered by a point, t: Cross section thickness

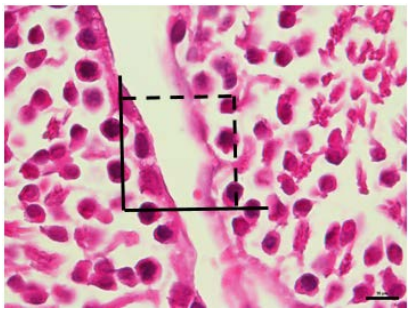

Figure 1. Unbiased counting frame

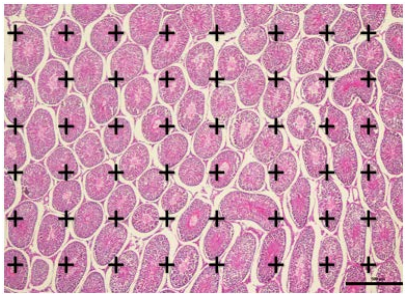

Figure 2. The dotted area measurement scale

RESULTS

There was no significant difference in total volume (Table 1 and Figure 3) and but total spermatogonium cell number (Table 2 and Figure 4) compared to the control group ($p > 0.05$)

Table 1. Total volumes of groups (mm^3) descriptive statistics and comparison results

	Groups	Median	Mean	St.Dev.	Min.	Max.	*p*.
TOTAL VOLUME (mm^3)	CONTROL	813	875.86	279.32	593	1237	.478
	SF	909	926.14	220.02	647	1252	
	DS	1117	1061.17	179.81	815	1237	
	TQ	870	901.57	86.73	828	1037	
	DS+TQ	928	885.14	143.93	692	1102	

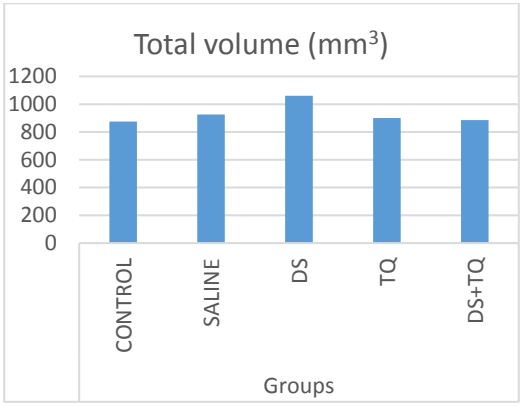

Figure 3. Total volume of the groups.

Table 2. Descriptive statistics and comparison results of total spermatogonium numbers of the groups.

	Groups	Median	Mean	St. Dev.	Min.	Max.	p.
SPERMATO-GONIUM	CONTROL	17369400	18725263.29	3493847.09	15015858	24454712	.148
	SF	16687396	19025263.57	4901544.85	13745525	24669436	
	DS	18139663	17808428.83	2264851.98	13930674	20277770	
	TQ	21533055	24111110.14	6056530.87	18771638	37318268	
	DS+TQ	17779922	17585826.00	2649177.32	12992146	21192562	

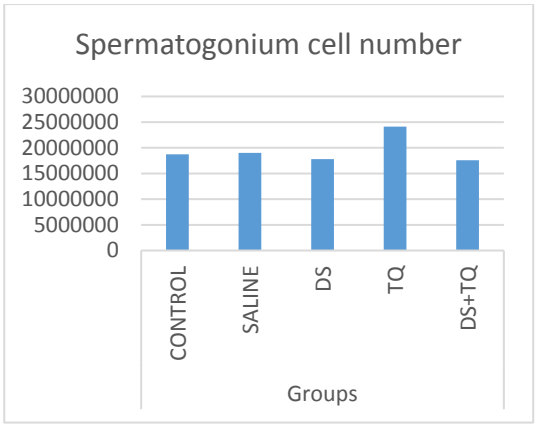

Figure 4. Spermatogonium cell number of the groups

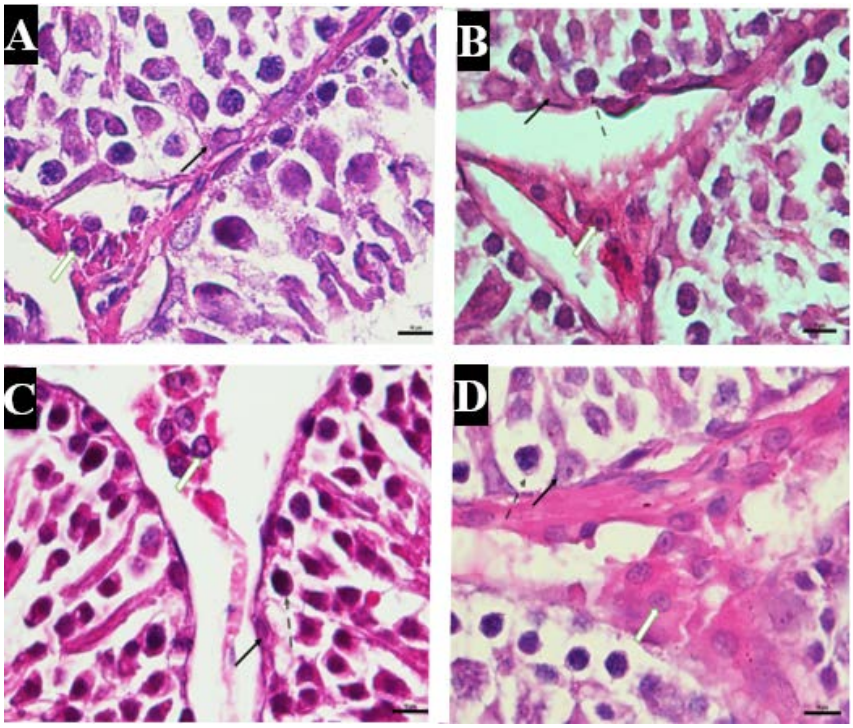

Figure 5. The microscopic images of testis. A: Control, B: DS, C: TQ, D: DS+TQ

DISCUSSION

DS damage to different tissue types. It is estimated that this property results from the bioactivation effect of the formation of reactive oxygen species such as O_2, HO and H_2O_2 (Mohan ve Sharma, 2011).

In our Literaur search, it was determined that studies on the effects of the DS on the testes in pregnancy were very few. However, there are many scientific studies related to the effects on the nervous system and other tissues (Ragbetli et all., 2007; Canan et all., 2008; Özyurt et all., 2011).

A study of histopathologic studies showed that DS (14 mg / kg) increased abnormal sperm cell count, decreased in Sertoli cell counts and left the spermatogenic serial cells (Mohan ve Sharma, 2011). It was observed that DS (9 and 18 mg/kg) caused significant decrease in the spermatogonium cell count by the sterolologically (Aslan et all., 2016)

In our study, it was observed that the DS (6.1 mg/kg) and TQ (5 mg/kg)) did not cause a significant differences on spermatogonium cells in rats.

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