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Role of Vitamin E on Antispermatogenic Effects of Indomethacin on Number of Sperm Containing Seminiferous Tubules of Testes in Long Evans Rats

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Abstract

Context: *Indomethacin is the most commonly and widely used nonsteroidal antiinflammatory analgesic and antipyretic drug. Despite its effectiveness as an antiinflammatory use, indomethacin causes inhibition of spermatogenesis leading to infertility. On the other hand, vitamin E enhances spermatogenesis. Therefore, the present study was designed to observe the protective role of vitamin E on indomethacin induced testicular damage.*

Objective: *To observe the effects of vitamin E on indomethacin induced testicular damage in Long Evans rats.*

Study design: *An experimental study.*

Place and period of study: *The study was carried out in the Department of Anatomy, Sir Salimullah Medical College, Dhaka.*

Materials and methods: *Eightyfour mature Long Evans male rats were divided into four groups (I, II, III and IV). The rats of group I, II and III were treated with indomethacin at different doses and duration. Group IV rats were treated with indomethacin and vitamin E at different doses for 49 days. Histologically the number of sperm containing and nonsperm containing seminiferous tubules were counted.*

Results: *There was significant reduction (P<0.001) in number of sperm containing seminiferous tubules when the rats were treated with indomethacin at low (2 mg/kg body wt/day) and high dose (10 mg/kg body wt/day) for 7, 14 and 42 days, respectively. On the other hand, rats treated with indomethacin and vitamin E for 49 days showed increase in number of sperm containing seminiferous tubules compared to the other groups (P<0.001).*

Conclusion: *It can be concluded from this study that vitamin E has potential role in the prevention of the antispermatogenic effects of indomethacin.*

Key words: *Seminiferous tubules, Indomethacin, Vitamin E*

Introduction

Indomethacin is the most commonly and widely used nonsteroidal antiinflammatory, analgesic and antipyretic drug. It was first introduced in 1963 for the treatment of rheumatoid arthritis and related disorders[1]. Patients receiving the therapeutic dose of indomethacin suffered from gastrointestinal upset, abdominal pain, peptic ulcer, with bleeding and perforation, also mental confusion, depression and psycosis[1]. Despite its effectiveness as an antiinflammatory drug, toxic effect to testes have significantly restricted its use.

Within each testis, there are almost 200 million of seminiferous tubules, and th ese structures account for 80 90% of the testicular mass[2]. The germinal epithelium of seminiferous tubules of the testis is one of the most proliferating tissue in the body capable of producing millions of spermatozoa every

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hour[3,4]. Oligospermia and azoospermia have been reported after the use of indomethacin[5]. Indomethacin causes inhibition of spermatogenesis by inhibition of prostaglandin synthesis leading to infertility in human being[6]. On the other hand, vitamin E is a fat soluble vitamin. It enhances spermatogenesis by inhibition of lipid peroxidation and lowers the incidence of abnormal sperm production[7,8]. Therefore, the present study was designed to observe the protective role of vitamin E on indomethacin induced testicular damage.

Materials and Methods

Eightyfour mature Long Evans male rats, 2.5 to 3.5 months old, weighing 200 300 gms were included in this study.

Drugs: The following drugs were used in the present study:

1) Indomethacin powder: (a) Low dose 2 mg/kg body weight/day, (b) High dose 10 mg/kg body weight/day. Indomethacin suspensions were made in distilled water and administered intragastrically[5,6].
2) Vitamin E powder, dose 100 mg/kg body weight/day. Suspensions of vitamin E were made by mixing it with a vehicle and administered intragastrically[8].
3) Vehicle: Distilled water for control group, dose 2 ml/rat/day.

Methods

Eightyfour male rats were divided into four main groups (I, II, III and IV). These main groups were again divided in subgroups a (control), b (2 mg indomethacin/kg body wt/day) and c (10 mg/kg body wt/day) on the basis of the dose of indomethacin. Each subgroup contained 7 rats. Grouping of animals and their treatment were done on the basis of duration of treatment[9].

Rats of group I, II and III were treated by indomethacin at low and high doses for 7, 14 and 42 days respectively. Group IV rats were treated with indomethacin and vitamin E for 49 days.

Principally assigning seminiferous tubules as 'spermatogenic' or 'non spermatogenic' in histological section assessed the damaging effect of testes[10]. For this, 6 micron thick paraffin sections were prepared from 4 mm thick tissue blocks and transverse to the longitudinal axis of testes fixed in 10% formol saline and were stained with hematoxyline and eosin. The number of round or oval seminiferous tubular cross sections were then estimated separately in 7 good testicular sections from 7 different rats of each group, and the percentage of round tubules that exhibited spermatogenesis was calculated. For this estimation, the tissue section on the slide was divided into four quadrants on a counting circle printed on a transparent sheet placed inside the eyepiece to superimpose in turn of the four quadrants close to where the four quadrants met (Fig.-1). The microscopic field inside the circle (under X40 objective, x 10 eyepiece) was then examined to count the tubules that were completely within the circle and were circular or oval in shape and categorize them as spermatogenic or nonspermatogenic (Fig.-1). Spermatogenesis was considered to be present if at least three spermatogonia (not sertoli cells) were seen on the tubule wall in close proximity. It was found that a proportion (6.39%) of tubule cross sections in the normal control group was completely devoid of spermatogenic elements and contained only sertoli cells. A correction for the 'background' was applied in the indomethacin treated groups and thus the proportion of tubules exhibiting spermatogenesis was calculated from N/[T (T.P)] (where N equals the number of cross sections with spermatogenic cells, T equals the total number of cross sections counted, and P equals the proportion of the total which was assumed to be devoid of cells at the time of treatment)[11].

For statistical analysis, the data obtained from different groups of rats were analysed and comparisons made using Student's 't' test.

Results

There was significant reduction ($P<0.001$) in number of sperm containing seminiferous tubules when the rats were treated with indomethacin at low (2 mg/kg body weight/day) and high dose (10 mg/kg body weight/day) for 7, 14 and 42 days, respectively. On the other hand, rats treated with indomethacin and vitamin E for 49 days showed significant increase ($P<0.001$) in number of sperm containing seminiferous tubules compared to control and other groups (Table-I & Fig. 3,4,5).

Table-I

Effect of indomethacin/indomethacin plus vitamin E on number of sperm and nonsperm containing tubules of different groups of rats

			Number of tubules/high power field (X40)			
	Duration of treatment	Total	Sperm containing		Nonsperm containing	
Groups	(days)	Mean±SD	Mean±SD	Percent	Mean±SD	Percent
Ia	7	19.14±1.46 (18.0 22.0)	17.92±1.37 (16.85 20.59)	93.61	1.22±0.10 (1.15 1.41)	6.39
Ib	7	22.86±5.64 (14.0 31.0)	19.25±5.23 (11.11 27.02)	83.75	3.60±0.56 (2.89 4.59)	16.25
Ic	7	29.14±5.08 (24.0 36.0)	21.57±3.00 (18.47 25.70)	74.47	7.57±2.44 (5.33 10.30)	25.53
IIa	14	22.71±2.87 (19.0 27.0)	21.26±2.68 (1.79 25.27)	93.61	1.45±0.19 (1.21 1.73)	6.39
IIb	14	21.14±3.24 (16.0 25.0)	15.65±3.16 (10.98 19.41)	73.53	5.50±0.38 (5.02 6.21)	26.47
IIc	14	27.71±5.82 (20.0 36.0)	12.77±0.37 (12.28 13.30)	47.58	14.94±5.45 (7.72 22.70)	52.42
IIIa	42	18.14±0.90 (17.0 19.0)	16.99±0.84 (15.92 17.79)	93.63	1.16±0.06 (1.08 1.21)	6.37
IIIb	42	26.43±6.16 (18.0 34.0)	17.18±4.01 (10.91 21.83)	65.83	9.25±4.03 (1.28 12.17)	34.17
IIIc	42	30.00±6.29 (24.0 39.0)	1.92±0.40 (1.53 2.49)	6.39	28.08±5.89 (22.47 36.51)	93.61
IVa	49	16.00±2.08 (13.0 19.0)	14.98±1.95 (12.17 17.79)	93.62	1.02±0.13 (0.83 1.21)	6.38
IVb	49	24.86±5.34 (19.0 33.0)	21.27±5.00 (15.79 28.89)	85.26	3.59±0.34 (3.21 4.11)	14.74
IVc	49	20.86±1.35 (19.0 23.0)	16.10±1.32 (14.72 18.53)	77.13	4.76±0.55 (4.21 5.41)	22.87
Ia vs Ib	7	$>0.10^{ns}$	$>0.50^{ns}$		$<0.001^{***}$	
Ia vs Ic	7	$<0.001^{***}$	$<0.05^{*}$		$<0.001^{***}$	
Ib vs Ic	7	$<0.05^{*}$	$>0.10^{ns}$		$<0.001^{***}$	
IIa vs IIb	14	$>0.10^{ns}$	$<0.01^{**}$		$<0.001^{***}$	
IIa vs IIc	14	$>0.05^{ns}$	$<0.001^{***}$		$<0.001^{***}$	
IIb vs IIc	14	$<0.05^{*}$	$<0.05^{*}$		$<0.001^{***}$	
IIIa vs IIIb	42	$<0.01^{**}$	$>0.50^{ns}$		$<0.001^{***}$	
IIIa vs IIIc	42	$<0.001^{***}$	$<0.001^{***}$		$<0.001^{***}$	
IIIb vs IIIc	42	$>0.10^{ns}$	$<0.001^{***}$		$<0.001^{***}$	
IVa vs IVb	49	$<0.001^{***}$	$<0.01^{**}$		$<0.001^{***}$	
IVa vs IVc	49	$<0.001^{***}$	$>0.10^{ns}$		$<0.001^{***}$	
IVb vs IVc	49	$>0.05^{ns}$	$<0.05^{*}$		$<0.001^{***}$	
IIIb vs IVb		$>0.50^{ns}$	$>0.50^{ns}$		$<0.01^{**}$	
IIIc vs IVc		$>0.10^{ns}$	$<0.001^{***}$		$<0.001^{***}$	

Figures in parentheses indicate range. Comparison between groups done by Unpaired Student's 't' test, ns = not significant, */**/*** = significant

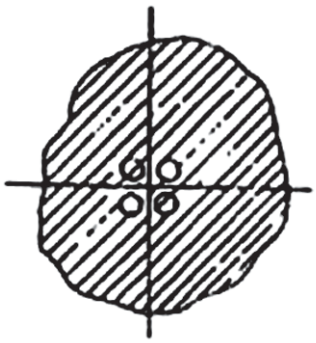

Fig.-1. *Outline of the microscopic field and of the superimposed counting circle*

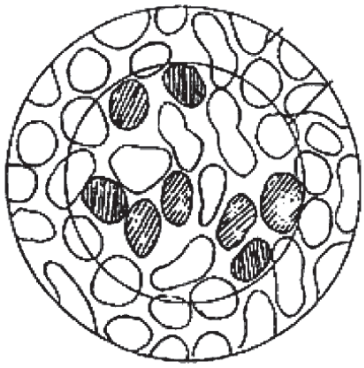

Fig.-2. *Procedure of counting spermatogenic and nonspermatogenic seminiferous tubules*

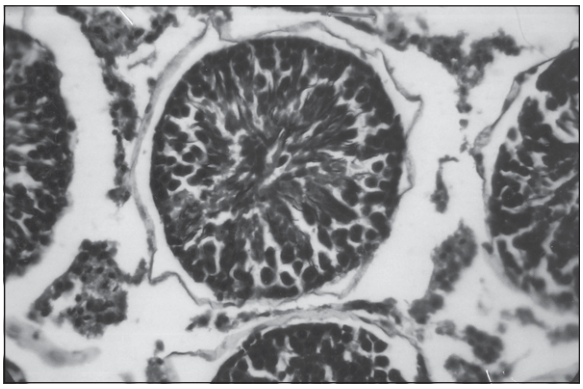

Fig.-3. *A high power (X40 objective) photomicrograph of a section of rat testis of control group showing seminiferous tubules with normal spermatogenesis [H&E stain].*

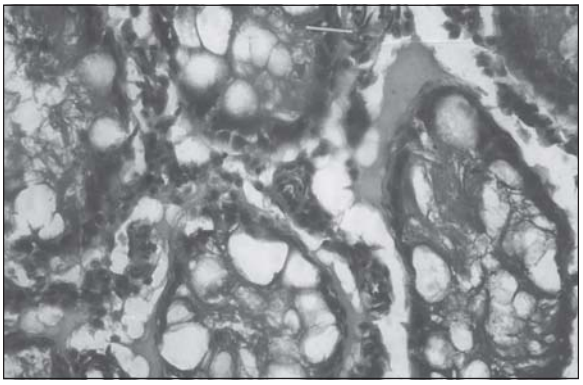

Fig.-4. *A high power (X40 objective) photomicrograph of a section of rat testis of experimental group showing seminiferous tubules devoid of germinal epithelium following 42 days treatment with indomethacin [H&E stain).*

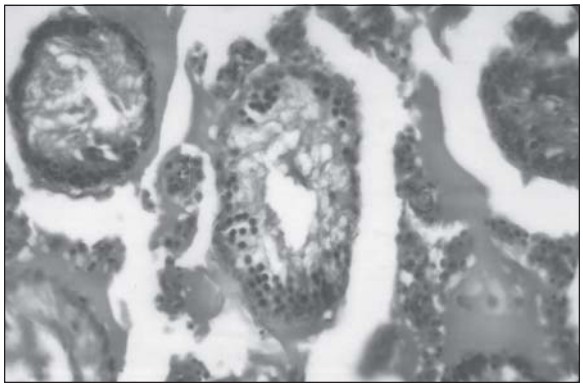

Fig. 5. *A high power (X40 objective) photomicrograph of a section of rat testis of experimental group following 49 days treatment with indomethacin plus vitamin E showing germinal epithelum preserved in most of seminiferous tubules [H&E stain].*

Discussion

The number of sperm containing seminiferous tubules of all indomethacin treated rats were reduced. The reduction in the number of sperm containing seminiferous tubules were highly significant (P<0.001) in the rats treated with indomethacin at low and high doses for longer duration (14 and 42 days).

Similar findings were also observed by Balasubramanian *et al*.[6], Kumar and Chinoy[9] and Ara[12]. The reduction in number of spermatogenic tubules may be due to the loss of germinal elements.

The number of sperm containing seminiferous tubules increased markedly and was highly significant (P<0.001) in vitamin E treated rats in

comparison to control group and also to indomethacin treated group of rats. This result was similar to the results observed by Cooper and Carpenter[7], Mishra and Acharya[8].

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