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A Study on the Gonado Protective Role of Folinic Acid Against Cyclophosphamide Induced Cytotoxicity in Rat

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Abstract

Context: *The inability to bear a child is a tragedy for many couples, bringing a sense of loss, failure and exclusion. Infertility is a global problem. Anticancer therapy with cyclophosphamide in a male leads to azoospermia. The present study was designed to observe the protective role of folinic acid against sterility in an animal model.*

Study Design: *Experimental study.*

Place and Period of study: *Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University, Dhaka from July 1998 to June 1999.*

Materials and method: *The Gonadoprotective activity of folinic acid against Cyclophosphamide induced damage was studied in Long Evans Norwegian strain adult male rats. Rats were pretreated with Folinic acid (6 mg dissolved in 500 ml of 5% dextrose in aqua) orally daily for 28 days. During the Folinic acid treatment, Cyclophosphamide (50 mg/kg body weight / day) was administered intraperitoneally at an interval of 24 hours from day 15 to day 28. Cytotoxic damage was assessed by estimation of body weight, testicular weight and volume and the histological findings.*

Result : *Pre-treatment with folinic acid plus cyclophosphamide produced significant increase in the number of seminiferous tubules, spermatozoa containing tubules and mean diameter of seminiferous tubules ($p<0.001$).*

Conclusion: Folinic acid provides significant protective role against Cyclophosphamide induced gonadal damage in Long Evans male rats.

Key words : Cyclophosphamide, Folinic acid

Introduction:

Infertility is a global problem. Infertility or sterility is an absolute state of inability to conceive. Among all cases of infertility in developed countries, about 8 percent can be traced to male factors, 37 percent can be due to female factors, and 35 percent can be due to factors in both the male and female partners. In about 5 percent of couples, the cause of infertility cannot be traced to specific factors in either partner[1,2,3,4,5]. About 90% of male infertility is caused by hypogonadism resulting in impaired spermatogenesis; and 80 to 90 percent of these men have isolated deficiency of sperm production with normal androgen production of unclear etiology, i.e, idiopathic oligospermia or azoospermia[6]. The morphological abnormalities include mostly of appearance of immature cells like spermatids and spermatocytes –these are the cells of previous lineage of spermatogenesis prior to spermatozoa[7]. During anticancer therapy with cyclophosphamide on male, similar to the feature of immature ejaculate syndrome appears[8,9,10]. Treatment with cyclophosphamide leads to azoospermia and disappearance of germinal epithelium with preservation of sertoli cells[9]. So, cyclophosphamide acts as gonado toxin in human and laboratory

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animal[10]. Folinic acid therapy in 65 sterile males showed significant increase in spermatozoa number, motility, and decrease in round cell amount[11]. With these considerations in mind, the present study was designed to observe the protective role of folinic acid against sterility in an animal model.

Materials and Method:

The study was carried out on eighteen healthy adult male rats of long Evans Norwegian strain of average weight 210 gm. body weight in the Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh for 24 days. Animals were housed in standard condition and allowed to food and water ad libitum. They were divided into three groups and treated according to Table-I.

At the end of 28 days, the rats were weighed and sacrificed. Their testes were collected for measuring weight, volume and histological examination. Blood was collected for serum testosterone measurement by Radio Immuno Assay in Nuclear Medicine Institute, BSMMU. The testis was fixed in 10% formol solution, washed in tap water, dehydrated in alcohol, cleaned in xylene and embedded in melted paraffin. Serial sections of 5 micron thickness of testicular tissue were made and stained with Haematoxylin and Eosin (H&E). Mean values and standard errors were calculated for the number of seminiferous tubules, tubular diameters, spermatozoa containing tubules per microscopic field.

For statistical analysis, Student's ' t ' test was used to compare the results in the experimental groups.

Results :

The effects of Cyclophosphamide alone (Group-B) and pretreated Folinic acid then Cyclophosphamide (Group-C) on body weight, testicular weight, volume and histology after 28 days in rats are shown in Table II and III. Serum testosterone level in all groups are shown in Table IV.

Histological parameters of control rat showed normal gonadal structure (Fig.-1). The decrease in gross parameters and histological parameters in rats of group B and C were statistically significant (Table II and III , Fig. 2 and Fig. 3). The change of rat body weight, testicular weight and volume, were not statistically significant between the rats of Group-B and C. But there were marked improvements in the number of seminiferous tubules, spermatozoa containing tubules per microscopic field and tubular diameter in rats of Group-C in comparison to rats of Group-B, which was statistically significant (Table II and III, Fig. 2 and Fig. 3).

Differences of Serum testosterone level, in all group of rats were statistically not significant (Table IV).

Table-I

Drug and sacrifice schedule:

Group	Number of Rats (n)	Drug(s)	Dose and Route of administration	Drug dosing schedule	Date of sacrifice
A	6	No drug	-	-	At Day 28
B	6	Cyclophosphamide	50 mg/kg/day-given Intraperitoneally	From Day 14 in every alternate day	At Day 28
C	6	Folinic Acid	6 mg / 500 ml 5% Dextrose in aqua given orally	Everyday	At Day 28

Table – II

Estimation of the weight of the testis in relation to the body weight of the rat.

Rat Groups	Rat Body Weight (Gm)			Rat Testes Weight (Gm)		Testes Volume(ml) Mean ±SEM
	Initial weight	Final weight	Change of body weight	Testes weight (Mg) Mean ± SEM	Testes weight as Gm% of final body weight	
A (n=6)	211.66±5.86	293.66±6.11	+39.49±5.93	30.9±0.7	1.04±0.01	3.11±0.04
B (n=6)	212.5±5.59	248.66±5.62	+17.01±1.63	23.5±0.7***	0.93±0.01***	2.71±0.06***
C (n=6)	211.66±4.94	249.66±4.73	+18.04±1.4	23.6±0.6NS	0.94±0.01NS	2.7±0.05NS

Group-A : normal diet for 28 days without any Drug

Group-B : normal diet for 24 days and received Cyclophosphamide in every alternate day starting from Day 15 up to day28.

Group-C : Folinic acid (6mg in 500ml of 5% dextrose in aqua) orally daily for 28 days, and then received Cyclophosphamide in every alternate day starting from day15 up to day28.

p- values :

Group A vs Group B : *** $p < 0.001$ [*** *Highly significant*]

Group B vs Group C : NS $p > 0.05$ [NS = *Not significant*]

Table III

Results of Histological parameters

Rat Groups	Number of seminiferous tubules Mean ± SEM	Spermatozoa containing seminiferous tubules Mean ± SEM	Diameter of seminiferous tubules(μ) Mean ± SEM
A (n=6)	22 ± 0.57	97.82 ± 0.97	287.43 ± 4.45
B (n=6)	13.83 ± 0.6***	3.43 ± 1.54***	166.66 ± 6.15***
C (n=6)	18.5 ±0.76***	47.36 ± 2.1***	214.97 ± 6.91***

p- values :

Group B vs Group A : *** $p < 0.001$ [*** *Highly significant*]

Group C vs Group B *** $p < 0.001$ [*** *Highly significant*]

Table IV

Estimation of serum testosterone level

Rat groups	Serum testosterone nmol/L Mean± SEM	*p*-value
A (n=6)	17.5±3.81	-
B (n=6)	14.16±3.94*NS*	Group B vs Group A : NS $p > 0.05$[NS =Not significant]
C (n=6)	15.33±4.13*NS*	Group C vs Group B : NS $p > 0.05$[NS =Not significant]

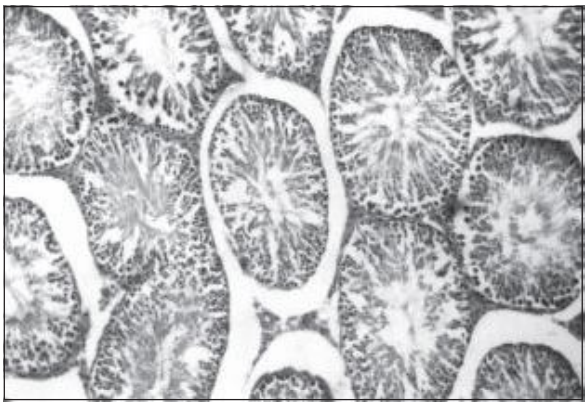

Fig.-1: *Photomicrograph showing histological structure of testis in group A- showing normal architecture with normal arrangement of germ cells in the seminiferous tubules and Leydig cells in the interstitial space (H&E stain, X400).*

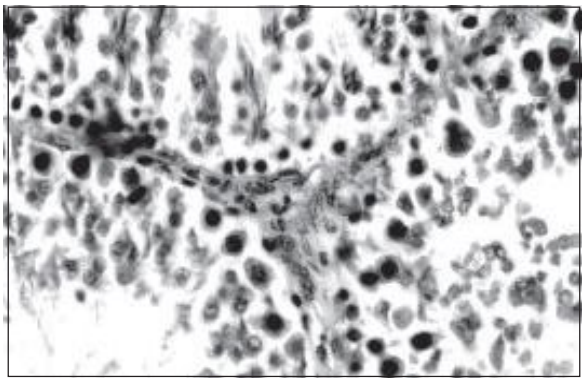

Fig.-2: *Photomicrograph showing Cyclophosphamide treated rat testis in Group-B, revealing damage of seminiferous tubules with absence of spermatozoa in the lumen (H&E stain, X100).*

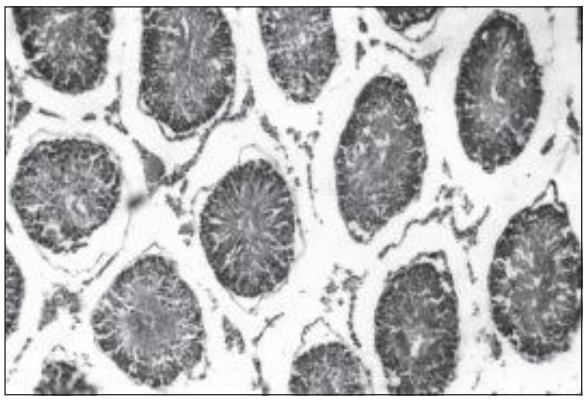

Fig.-3: *Photomicrograph showing folinic acid and cyclophosphamide treated rat testes in Group-C, revealing some restoration of seminiferous tubular structure with presence of spermatozoa in some tubules (H&E stain, X100).*

Discussion

The dose and duration of Cyclophosphamide and Folinic acid treatment in the rats were selected from previous observations[11]. In the present study, Cyclophosphamide was treated for 14 days to ensure damage of all dividing germinal cell in a cycle of seminiferous epithelium[12]. Cyclophosphamide was found to produce testicular damage in rats as evident by significant reduction of body weight, testicular weight & volume, number of seminiferous tubules, number of spermatozoa containing tubules and mean tubular diameter. The result was consistent with the findings of other investigators who conducted similar type of study[9,10]. Cyclophosphamide has the property of becoming strong electrophiles to target molecules of nucleus of dividing cells. These reaction result in the formation of covalent linkage by alkylation of various nucleofilic moieties such as phosphate, amino, sulfhydryl, carboxyl, and imidazole groups in DNA[13].

In the present study Folinic acid pre treatment showed significant gonadal protection against Cyclophosphamide. The possible role of folinic acid in preventing testicular damage could be due to effect on mammalian DNA synthesis[14,15,16,17]. As folate cofactors are essential for one carbon transfer reaction involved in de novo synthesis of the purine heterocycles. Inhibition of synthesis of thymidylic acid (2- deoxythymidine monophosphate - dTMP), an essential precursor of DNA, is also suggested[16]. Folates also prevent uracil incorporation into human DNA and thus prevent DNA breakage[17].

In the present study, Cyclophosphamide had no significant effects on steroidogenesis, as the present cytotoxic dose might had no toxic effects on Leydig cell. These finding closely agree with previous observations[9,18]. The study suggests that folinic acid has got some protective role against testicular damage of rats. Further study in human regarding its dose, duration of treatment and determination of margin of safety are suggested.

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