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EFFECT OF CIGARETTE SMOKES DENSITY ON HISTOPHATOLOGIS TESTIS OF RATS

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ABSTRACT

The aim of this study was to know the effect of cigarette smokes to the diameter of seminiferous tubulus testis and the number of Leydig cell of rats exposed to the cigarettes smoke. Some constituents of cigarette smoke was nitrogen, amoniac, CO*,CO_2, NO* , NO_2*, H_2S, Tar, nicotin and metal would influence reproductive system. Twenty four adult male rats were divided to four groups i.e : one group for control (C) and three groups for treatment by cigarette smoke of 38 mV cigarette smokes (T1), 63 mV cigarette smokes (T2), and 181 mV cigarette smokes (T3). The rats in the each treatment group were exposed to the cigarette smoke for 30 days (15 minutes every day). The rats in the control group were kept in the fresh room air. The result of this treatment showed that the diameter of seminiferous tubulus testis of rats between control group and treatment groups were significantly different. ($p < 0.01$), but there was no significant difference in the diameter of seminiferous tubulus testis rats between treatment groups by cigarettes smoke.The mean average diameter of seminiferous tubulus of control group 54.348 ± 16.60 µm; 40.63 ± 5.44 µm T1 group; 37.44 ± 6.02 µm for T2 group and 33.57 ± 6.70 µm for T3 group. The mean number of Leydig cell were 33.57 ± 4.43 cells for control group (C); 24.5 ± 2.95 cells for T1 group; 22 ± 8.63 cells for T2 group and 19 ± 1.19 cells for T3 group. The number of Leydig cell for control group and treatment groups were respectively different. ($P<0.01$), but there was no significant difference of the number of Leydig cell between treatment groups. The result of this study indicates that cigarette smokes influenced the histological structure of rats testis.

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INTRODUCTION

The effect of smoking and its complication on health of human are social problem in all countries. Smoke of cigarette implied some free radical substances and particals such asnitrogen, amoniac, CO*,CO_2, NO* , NO_2^*, H_2S, Tar, nicotin and metal which have negative effect on reproductive system of human and animals (Halliwell B and Gutteridge,2007).Cigarette smoke was proinflamantory , irritant and activate alveolar macrophages to produce O_2^*, H_2O_2 and possibly NO*. When the free radical amount higher without in equilibrium of increases antioxydan in the tissure cause defective metabolism and cell or organ function in the body (Muthuvel et.al, 2006). Nicotin will be absorbed respiratory truck and mucous quickly. Nicotin will be transfered to liver, kidney and lung, and will be transformed to kotinin by cytochrom P450 (Gaffari et.al, 2009). In this study the different number of cigarette, period of exposure cigarette smoke have been evaluated, and reduction of diameter of seminiferous tubulus and the number of Leydig cell on the interstitial tissue of the testis rats had been calculated.

MATERIALS AND METHODS

The total of 24 male of rats which the mean age of 8 weeks and body weight 150-200 grams divided into four groups

.i.e:control group (C), treatment group (T1) exposure 38 mV of cigarette smoke ; Treatment group (T2) exposure 63 mV smoke from cigarette smoke, and treatment group (T3) exposure 181 mV smoke from cigarette smoke. The cigarette for this study we had choosen kretek cigarette. For treatment the smoke to the rats, we designed a special apparatus for keeping the rats to smoke , a glass box in cube shape with size of 40 X 40 X 50 cm to seperate the rats from the environment. Each smoking procedure went on 15 minutes consist of 10 minutes smoke exposure and 5 minutes for rest. Each week, the group of rats were exposed to smoke for six days, each day by 38 mV cigarettes smoke density for T1 group; 68 mV cigarets smokes density for T2 group and 181 mV cigarettes sokes density for T3 group. As long as the study periode, six rats in the control group were maintained in a similar place and exposed to the room air.

After 30 days, the rats in the four groups were anesthetized by chloroform, and then removed the testis from scrotum and fixed in 10% formaline. The testis from each rat was sliced and stained by haematoxyline-eosin. Histopathological examination was done by inspected slide microscopic of the testis samples of each group.

The testis tissue was clissified base on the diameter of seminiferous tubulus (Table 1 and table 2) and the number of Leydig cell of each rate (table 3 dan table 4), inspected in a microscopic field at X 400. Data analiysis were performed by the SPSS sofware. (Statistical Package for The Social Sciences, Version 11.5 .SPSS Inc, Chicago, II.USA. Analysis of Variance Test (ANOVA) and Duncan Testwere used for comparison of the diameter of seminiferoustubulus and the number of Leydig Cell.

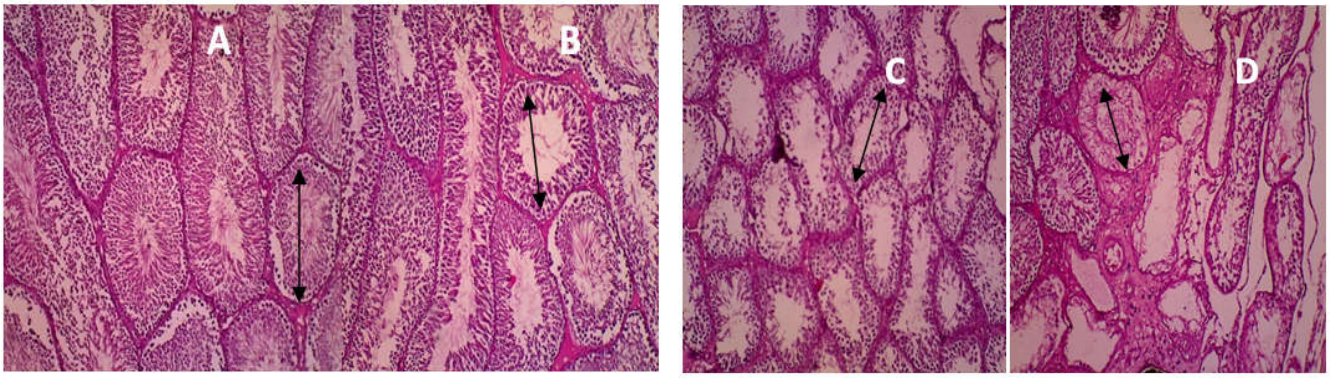

Figure 1. Diameter of Seminiferous tubules of rats testis. (A) Control Group; (B) T1 group; (C) T2 group, and (D) T3 group

Table.1. Analysis of Variance test of diameter of seminiferous tubulus between control group and all the treatment groups

	Sum of squares	df	Mean square	F	Sig
Between groups	1471.163	3	490.388	5.074	0.009
Within groups	1932.772	20	96.639		
Total	3403.934	23			

Table 2. The mean average diameter of seminiferous tubulus testis rats

Treatment	Mean ± SD
Control	54.348 ± 16.60
T1	40.630 ± 5.44
T2	37.440 ± 6.02
T3	33.57 ± 6.70

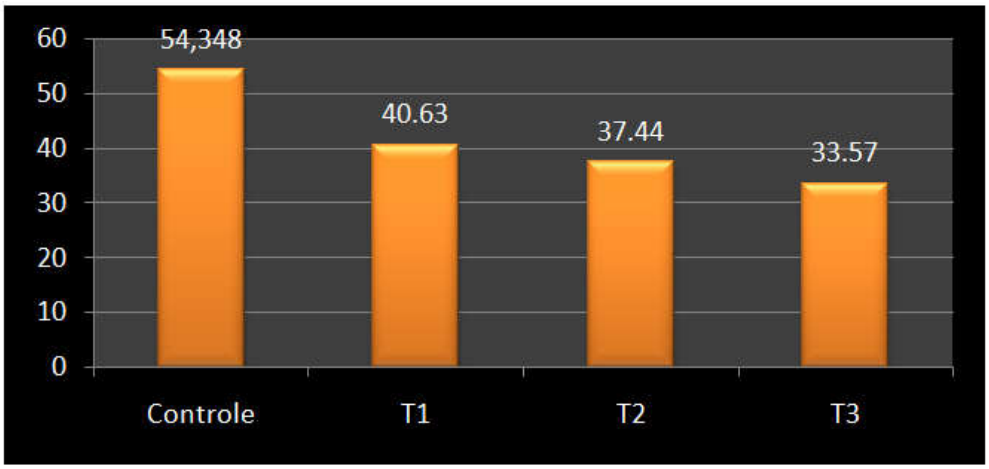

Figure 2. Diagram of the mean average diameter of seminiferous tubulus testis rats

RESULTS

The significant difference of diameters of seminiferous tubulus was detected between the control group and all the treatment groups (T1, T2, and T3) (P < 0,01) (Table .1), but there is no significant difference of diameters of seminiferous tubulus between treatment groups for rats. (Table. 3).

The mean average diameter of seminiferous tubulus testis rats of control group was 54.348 ± 16.60 µm; the P1 group was 40.630 ± 5.44 µm; the P2 group was 37.440 ± 6.02 µm; and the P3 group was 33.57 ± 6.70. µm. The significant difference of the number of Leiydig cells between control group and all the treatment groups (T1, T2, and T3) (P < 0.01) (Table 4.), but there are no significant difference of the Leydig cell

Table 3. Duncan test of diameters of seminiferous tubulus testis between control group and all treatment groups

Treatment	N	Subset for alfa = 0.05	
		1	2
T3	6	33.571	
T2	6	37.440	
T1	6	40.630	
Control Significans	6	0.253	54.348

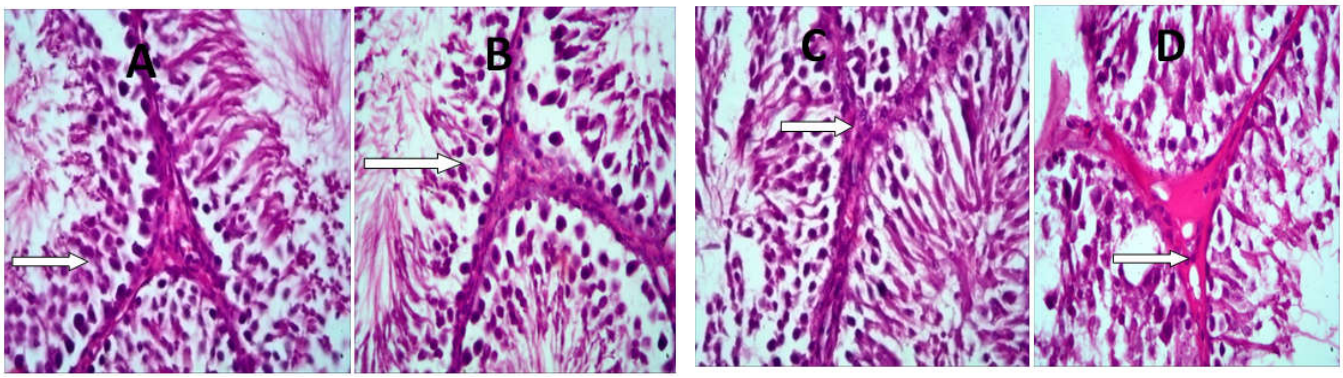

Figure. 3. Leydig cell in the interstitial tissure of rats testis. (A) Control group; (B) T1 group ; (C) ;T2 group; (D) T3 group. (⟹): Leydig Cell

Table 4. Analysis of Variance Test of number Leydig Cell between control group and all treatment groups

	Sum of squares	df	Means squares	F	Sig
Between group	1052.458	3	350.819	7.171	0.002
Within groups	978.500	20	48.925		
Total	2030.958	23			

Table 5. The mean average number of Leydig cell between control group and all treatment groups

Treatment	Mean ± SD
Control	33.60 ± 4.43
T1	24.50 ± 2.95
T2	22.00 ± 8.63
T3	19.00 ± 1.19

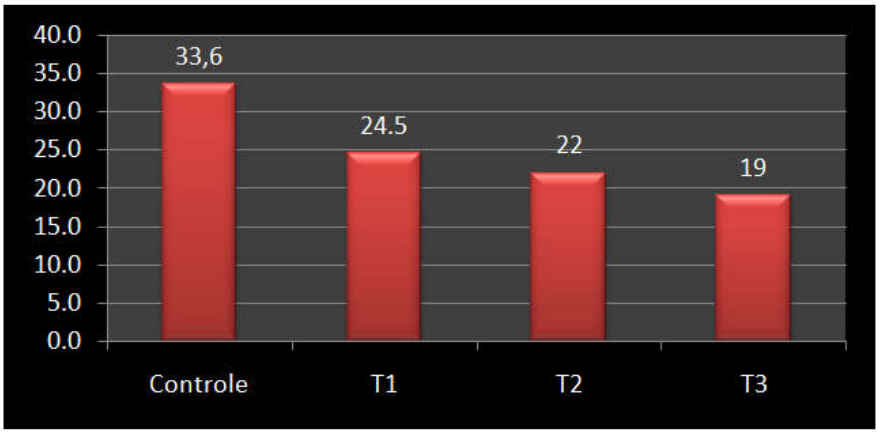

Figure 4. Diagram of mean average number of Leydig cell between control group and all treatment groups

number between treatment groups T1,T2, and T3. (Table 6.). The mean average number of Leydig cell control group is 33.6 ± 4.43; T1 group :24.5 ± 2.95; T2 group : 22 ± 8.63; and T3 : 19 ± 1.19.

Table 6. Post Hoc Test , Multiple comparasons of the number Leydig Cell between control group and all treatment groups (T1, T2, and T3)

(I) Treatment	(J) Treatment	Mean difference	Standard Error	Sig
Control	T1	14.166*	4.038	0.002
	T2	16.500*	4.038	0.001
	T3	14.833*	4.038	0.002
T1	Control	14.66*	4.038	0.002
	T2	2.333	4.038	0.570
	T3	0.666	4.038	0.871
T2	Control	16.500*	4.038	0.001
	T1	-2.333	4.038	0.579
	T3	-1.666	4.038	0.684
T3	Control	-14.833*	4.038	0.002
	T1	-0.666	4.038	0.871
	T2	1.666	4.038	0.684

DISCUSSION

In this study we used 24 male Wistar rats that divided into four groups i.e: control group (C), treatment group T1, treatment group T2, and treatment group T3.The rats in the control group were kept in the fresh room air. In this study we showed that diameter of seminiferous tubulus and the number of Leydig cells on the control group and treatment groups (T1,T2, and T3) were different significantly 99% ($p < 0,01$),but the diameter seminiferous tubulus and the number of Leydig cell on treatment groups (T1, T2, and T3) not different significantly on 99%. The degeneration for that condition may be the presence of toxic substances in cigarette smoke especially some reactive oxygen species (ROS) ,nicotine and tar. Some studies have been done on the harmful effects of cigarette smoke on the male genetal system of human and rats. In the studies of Ahmadnia et.al(2007), during 10 weeks to cigarette smoking for 15 rats showed disturbance on spermatogenesis and decreases of number Sertoli cell. Based on the studies of Sukmaningsih (2009), the effect of nicotine and tar reduced the number of spermatogonia and spermatocyte pachiten. Decreasing of the number of Leydig cell will disturb of spermatogenesis process and reduce the production of testosteron which were needed for spermatogenesis. So that, cells in epitelial tissues of seminiferus tubulus will decrease and the diameter of seminiferus tubulus become smaller.

However, the effect of igarette smoke on the reproductive system depend on some factors i.e: how many cigarette for smoking, how long time smoking to do, and what kind cigarette have been done.

Conclusion

This study showed that cigarette smoking very influence and impaired significant to the tissue of rats testis, reduced of seminiferous tubulus, and the number of Leydig cells.

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