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Studies on cigarette smoke induced oxidative DNA damage and reduced spermatogenesis in rats

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Abstract

In the present work, the effect of exposure to cigarette smoke on male fertility in rats, as characterized by changes in the relative weight of sex organs, epididymal sperm count, activity of marker enzymes and DNA damage was evaluated. Exposure of rats to cigarette smoke caused a gradual decrease in total body weight gain and relative weight of the epididymis and seminal vesicles by 30 and 40% respectively. Epididymal sperm count was reduced significantly by 25% ($P \leq 0.05$) after 2 weeks and by 41% ($P \leq 0.001$) after 4 weeks of exposure. Exposure to cigarette smoke had reduced the activity of sorbitol dehydrogenase by 18% ($P \leq 0.05$) and increased the activity of lactate dehydrogenase by 28% ($P \leq 0.05$). The changes in both key enzymes were significant, which reflected the inhibitory effect of cigarette smoke on spermatogenesis and sperm maturation. The toxic effect of exposure could be explained partially due to induction of DNA damage and oxidative stress as shown by the significant increase in serum 8-hydroxy-2`-deoxyguanosine from 22.83 to 37.33 ng ml^{-1} blood.

Key words

Cigarette smoke, DNA damage, Infertility, Spermatogenesis, Sperm count

Introduction

Cigarette smoking (passive smoking) is well known to be associated with decrease in pregnancy rate (Zinamin *et al.,* 2000), by affecting female and male fertility, by decreasing sperm count and motility (Sofikitis *et al.,* 1995; Arabi and Moshtaghi, 2005) and by decreasing sperm density (Zavos and Zarmakoupis-Zavos, 1999; Kunzle *et al.,* 2003). It leads to secretary dysfunction of the Leydig cells and deficiency in sperm maturation and spermatogenesis, a major cause of dyspermia (Parazzini *et al.,*1993; Yamamoto *et al.,*1998). In humans, cigarette smoke affects seminal quality (Martini *et al.,* 2004) in addition to its effect on sperm aneuploidy (Shi *et al.,* 2001; Robbins *et al.,* 2005)

More recent literature focus on the adverse effects of cigarette smoke on male fertility of non-smokers who were exposed for a long period to cigarette smoke (passive smoking). Reduction in sperm density, motility and possible adverse effect on morphology have been demonstrated (Hughes and Brennan 1996; Vine, 1996; Hull *et al.,* 2000). It induces apoptosis in rat testis (Rajpurkar *et al.,* 2002). Pretreatment with antioxidants like vitamin E reduced the degenerative effect of cigarette smoke on testicular tissues (Hanadi *et al.,* 2011). Honey, which has an antioxidant activity, has been found to induce impaired sexual behavior and fertility in male rats (Mohamed et al 2013).

The objective of the present research was to test the effect of cigarette smoke on epididymal sperm count and activity of key enzymes, like sorbitol dehydrogenase and lactate dehydrogenase in spermatogenesis and maturation, and to measure oxidative stress by measuring the concentration of 8-OH-dG in blood samples.

Materials and Methods

Experimental design: Sprague Dawley rats used, for the study, were 4 weeks old males (80-120 g each), divided into three groups. Group A were not exposed to cigarette smoke and served as control. Group B rats were injected sub-cutaneously with 5 mg kg^{-1} testosterone (1 mg day^{-1} for 2 weeks). Group C the rats were exposed to cigarette smoke @ 20 cigarettes per day for a period of 2, 14, 28 days. Animals treated with cigarette smoke were kept in special glass box cages of 30 x 40 x 60 cm, with a hood over the cage to evacuate the extra smoke. The cage had inlets for ventilation, food and water intake in addition to special inlet for the smoking apparatus. The whole body of the rats were exposed to cigarette smoke for 3 hr daily (9-10 a.m., 12-1p.m. and 3-4 p.m.) for 4 weeks using a special smoking apparatus (GRIFFIN Cigarette tar measurement kit-YTH-520-M). Smoking rate 2 cm min^{-1} was (2cm of the cigarette was burnt in one min, 20 cigarettes were burnt during 3 hr daily as mentioned above) similar to that performed by human smokers, smoking 20 cigarettes per day.

All the experimental animals were maintained under normal conditions of humidity, circadian cycle, temperature and with free access to food and water. All rats received commercial standard chow (18% protein; Global 2018, Harlan Teklad, Wisconsin, USA). At the end of the experiment, the rats were anesthetized with ether and the testis, epididymis, seminal vesicles, penis, spleen, heart, brain and kidneys were removed for estimation of absolute and relative weight. Similarly, estimation of organs relative weight, epididymal sperm count and enzymatic measurements were performed on treated and non-treated animals. Experiments were performed following the guidelines of animal care of the National Institute of Health, and experiments were approved by the Ethical Committee, Faculty of Medicine.

Estimation sperm count : Sperms were collected from the epididymis of each rat by flushing with the same volume (10 ml) of suspension medium as described by Abdul-Ghani *et al.* (2008). Numbers of sperms were counted in four chambers (used for counting of white blood cells) of the hemocytometer slide. The sperms number was expressed per ml of suspension.

Estimation of DNA damage : The procedure described in detail by Cattley and Glover. (1993) and Chiou *et al.* (2003). DNA Damage ELISA Kit was used for detection and quantization of 8-hydroxy-2'-deoxyguanosine in serum samples of control and treated animals. It's a fast and sensitive competitive immunoassay, and 8-hydroxy-2'-deoxyguanosine is a biomarker of oxidative DNA damage and oxidative stress.

Estimation of enzymatic: Testicular lactate dehydrogenase and sorbitol dehydrogenase activities were measured using the Kits purchased from Sigma Chemical Co. as described in detail by Jana *et al.,* 2006. Enzymatic activity was expressed as µg substrate converted min^{-1} mg^{-1} protein. Protein concentration was determined spectrophotometrically at 750 nm by the method of Lowry *et al.* (1951).

Statistical Analysis : All the values are presented as Mean ± SEM for the number of experiments indicated in brackets and the data were statistically analyzed using Students t-test.

Results and Discussion

A gradual decrease in total body weight gain was observed following exposure to cigarette smoke (Fig.1). The reduction was significant after 1 to 4 weeks of exposure. The changes in relative weight of different organs were related to male sex organs as shown in Fig. 2. Intra-peritoneal injection of testosterone (5 mg kg^{-1}) for 2 weeks was found to produce significant increase on the relative weight of epididymis, penis and seminal vesicles by 22 % ($P \leq 0.05$), 26 % ($P \leq 0.05$) and 36 % ($P \leq 0.05$) respectively, while exposure of rats to cigarette smoke for 4 weeks reduced the relative weight of epididymis and seminal vesicles by 30 % ($P \leq 0.005$) and 40 % (($P \leq 0.01$), respectively. No significant changes were observed on the

Table 1 : Effect of exposure to cigarette smoke on epididymal sperm count in rats

Pre-treatment		Animal weight (g)	Epididymal sperm count / gr. Epid.		% Change	P
			Control	Treated		
Exposure to Cig. Smoke	2 days	98	0	0	0	NS
Exposure to Cig. Smoke	14 days	160	3.02×10^6	2.27×10^6	"!25	d" 0.05
Exposure to Cig. Smoke	28 days	212	3233.95×10^6	1899.72×10^6	"!41	d" 0.001
Testosterone	5mg kg^{-1}	280	3113.90×10^6	6815.22×10^6	'!119	d" 0.001

Table 2 : Effect of exposure to cigarette smoke on sorbitol dehydrogenase and lactate dehydrogenase activity in rats

Enzymes	Enzyme activity(µ mol substrate converted per min mg^{-1} $protein^{-1}$)		% Change	P
	Control	Treated		
Sorbitol dehydrogenase	1.067 ± 0.064 (7)	0.879 ± 0.032 (9)	"!18	d" 0.05
Lactate dehydrogenase	284.644 ± 10,105 (5)	365.313 ± 16.717 (5)	'!28	d"0.05

Data are mean ± SEM for the numbers indicated in brackets

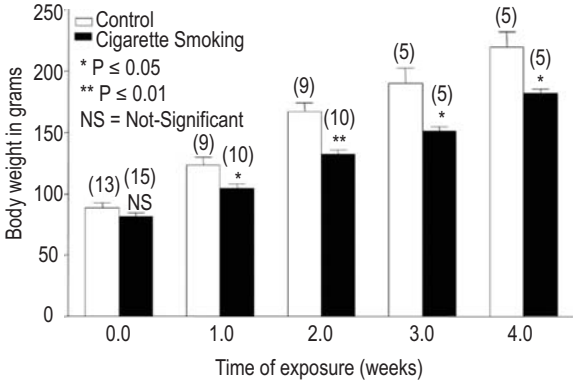

Fig. 1 : Changes in body weight following exposure to cigarette smoke. Rats exposed to cigarette smoke and controls were weighed at different time points, number in brackets indicate number of rats in each group

relative weight of other organs such as spleen, heart, brain or kidneys. Previous reports had shown that exposure to cigarette smoke resulted in significant decrease in body weight of animals (Li *et al.,* 2003; Chandra *et al.,* 2010 and Hanadi *et al.,* 2011). This decrease was reduced when animals were pretreated with vitamin E which has an antioxidant activity (Hanadi *et al.,* 2011).

Rats injected intraperitoneally with male sex hormone (testosterone 5mg kg^{-1}) showed 119% increase in epididymal sperm count from control values of 3113.90 x 10^6 to 6815.22 x 10^6 ($P \leq 0.001$). Exposure to cigarette smoke for 2 weeks significantly decreased the sperm count by 25% ($P \leq 0.05$), while exposure for 4 weeks reduced epididymal sperm count from 3113.95 x 10^6 to 1899.72 x 10^6 a significant decrease of 41 % ($P \leq 0.001$), as shown in Table 1. Cigarette smoke is well known to reduce the development of sperms by reducing the diameter of seminiferous tubules, number of germ cells, Leydig cells and Sertoli cells (Ahmadnia *et al.,* 2007) in addition to decrease in serum testosterone levels which contribute to decrease in sperm count. Furthermore, passive smoking was found to damage spermatogenic epithelia, Leydig cells and Sertoli cells, reduced T and LH levels and blocked proliferation of spermatogenic cells (Zhang *et al.,* 2009).

In humans it's well known that prenatal exposure to maternal smoking reduces the number of germ and somatic cells in embryonic male and female gonads (Mamsen *et al.,* 2010).

Exposure to cigarette smoke for 4 weeks decreased testicular activity of sorbitol dehydrogenase enzyme from 1.067 to 0.879, a decrease of 18 % ($P \leq 0.05$). At same time it increased

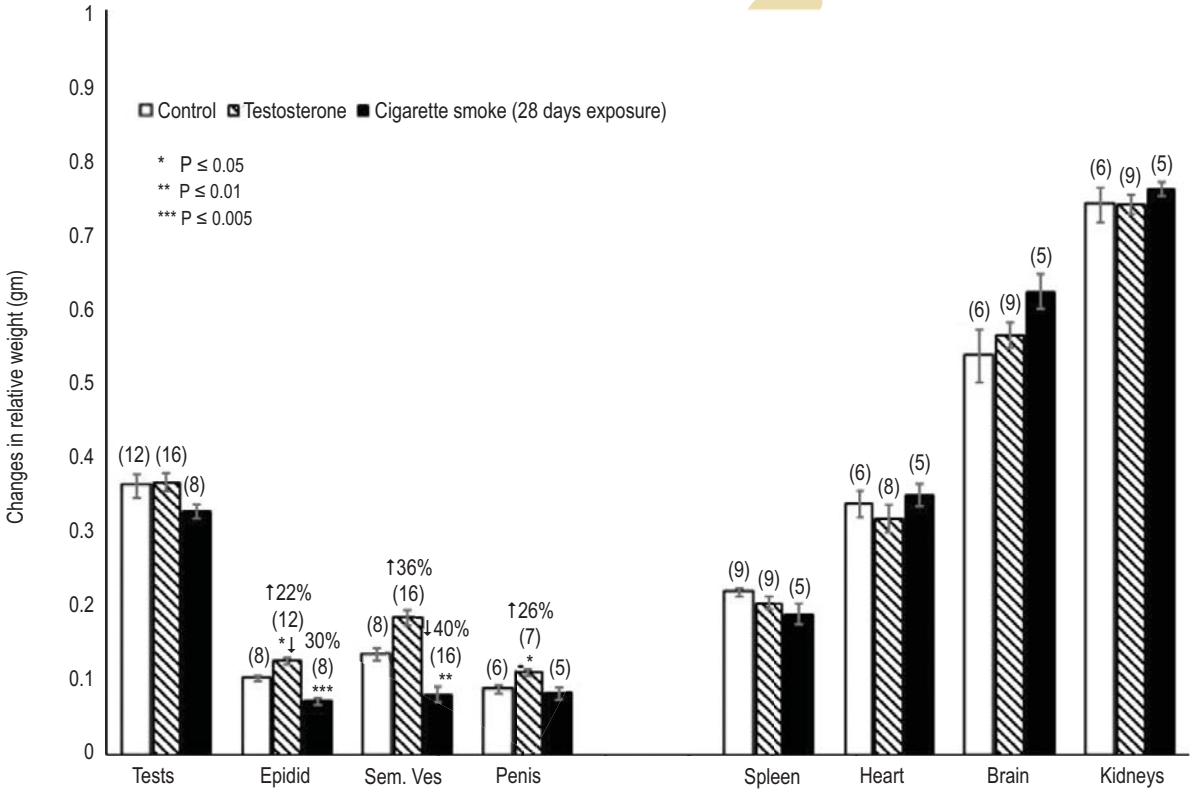

Fig. 2 : Changes in relative weight of different body organs following exposure of rats to cigarette smoke for 4 weeks (black bars) compared to control rats (white bars). Striped bars are rats treated with testosterone (5mg Kg^{-1}) = 1 mg day^{-1} for 2 weeks. Number in brackets indicates number of rats in each group

the activity of lactate dehydrogenase by 28 %, from 284.644 to 365.313 ($P \leq 0.05$) as shown in Table 2. A reduction of 18% in SDH activity indicated inhibition of secondary maturation of sex organs, while 28% increase in LDH indicated anti-spermatogenic activity (Hills and Means 1972; Kobayashi *et al.,* 2002; Abdul-Ghani *et al.,* 2008). Previous studies have reported that exposure to cigarette smoke reduced secretory function of leydig cells and epididymal sperm maturation process and sperm capacity for fertilization (Yamamoto *et al.,* 1998; Kapawa *et al.,* 2004).

DNA damage was estimated by measuring the concentration of 8-OH-dG in blood serum following 4 weeks exposure to cigarette smoke as compared to control values in the same rats. Figure 3 shows a significant increase of 65% in 8-OH-dG concentration in serum blood which indicated a significant increase in DNA damage. Oxidative stress was achieved even with short term exposure to cigarette smoke (Campos *et al.,* 2013). Honey, which has antioxidant activity increased male fertility (Abdul-Ghani et al 2008) and showed protective effect against cigarette smoke induced impaired sexual behavior and fertility in male rats (Mohamed *et al.,* 2013).

Smoking affects sperm quality by reducing sperm motility and acrosomal reaction (Arabi and Moshtaghi, 2005), and by affecting sperm morphology (Gomathi *et al.,* 1993; Trummer *et al.,* 2002). On the other hand, it showed no effect on sperm nuclear size, shape or chromatin texture (Vine *et al.,* 1997). A decrease in semen quality is correlated to the number of cigarettes smoked per day (Al-Bader *et al.,* 1999; Zhang *et al.,* 2000; Pasqualotto *et al*., 2006) and other correlation to the number of years of smoking (Chia *et al.,* 1994; Wang *et al.,* 2001), with heavy smoking sperm concentration was reduced significantly by 19 to 29 % (Ramlau-Hansen *et al.,* 2007).

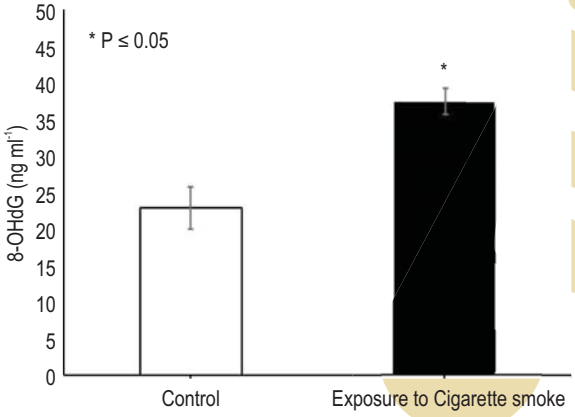

Fig. 3 : DNA damage due to exposure of rats to cigarette smoke. Concentration of 8-hydroxy-deoxy-guanosine was measured in serum samples of rats before exposure to cigarette smoke (control) and after 4 weeks exposure. Values are Mean ± SEM expressed as ng 8-OHdG/ml blood

Smoking is well known to affect spermatozoal quality and fertilizing potential by increasing cadmium levels in serum and semen of smokers, which cause testicular endothelial injury, and production of anti-sperm antibodies (Omu *et al.,* 1998). Cigarette smoke was found to be associated with an increase in the level of oxidants and a simultaneous decrease in the level of antioxidants in the rat testis (Rajpurkar *et al.,* 2000), and apoptosis may be one of the pathogenic mechanisms responsible for defective spermatogenesis (Rajpurkar *et al.,* 2002).

Whether reduction in sperm count is related to any of the toxic compounds in cigarette smoke or to generalized hypoxia is not yet clear. Oxidants in cigarette smoke are likely to damage sperm DNA which could explained by decrease in sperm quality (Shen *et al.,* 1997; Zenes *et al.,* 1999; Horak *et al.,* 2003). Sperm DNA of smokers contained significantly higher amount of 8-hydroxy-deoxy-guanosine (8-OH-dG) and was also correlated to seminal cotinine (Shen *et al.,* 1997), which could explain the risk of smoking on spermatogenesis.

Results of the present study showed that exposure to cigarette smoke (passive smoking) produced anti-spermatogenic activity, and increased DNA damage which justifies banning orders against smoking in public places.

Acknowledgments

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