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

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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 ≤ 0.05) after 2 weeks and by 41% (P ≤ 0.001) after 4 weeks of exposure. Exposure to cigarette smoke had reduced the activity of sorbitol dehydrogenase by 18% (P ≤ 0.05) and increased the activity of lactate dehydrogenase by 28% (P ≤ 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 blood.

Key words: Cigarette smoke, DNA damage, Infertility, Spermatogenesis, Sperm count

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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; Kunze et al., 2003). It leads to secretory 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).

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Animal weight (g)</th>
<th>Epididymal sperm count / gr. Epid.</th>
<th>% Change</th>
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</tr>
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<tr>
<td></td>
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<td>Control</td>
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<tr>
<td>Exposure to Cig. Smoke</td>
<td>2 days</td>
<td>98</td>
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<td>14 days</td>
<td>160</td>
<td>3.02 × 10⁸</td>
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<td></td>
<td>28 days</td>
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<td>Testosterone</td>
<td>5mg kg⁻¹</td>
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Table 2: Effect of exposure to cigarette smoke on sorbitol dehydrogenase and lactate dehydrogenase activity in rats

<table>
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<th>Enzymes</th>
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Data are mean ± SEM for the numbers indicated in brackets

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 ≤ 0.05), 26% (P ≤ 0.05) and 36% (P ≤ 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 ≤ 0.005) and 40% ((P ≤ 0.01), respectively. No significant changes were observed on the

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Rats injected intraperitoneally with male sex hormone (testosterone 5mg kg\(^{-1}\)) showed 119% increase in epididymal sperm count from control values of \(3113.90 \times 10^6\) to \(6815.22 \times 10^6\) \((P \leq 0.001)\). 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 \(3113.95 \times 10^6\) to \(1899.72 \times 10^6\) 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
the activity of lactate dehydrogenase by 28 %, from 284.644 to 365.313 (P ≤ 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).

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-deoxyguanosine (8-OH-dG) and was also correlated to seminal colinine (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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References


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