ALTERATION IN SEMEN CHARACTERISTICS AND TESTICULAR HISTOLOGY OF MALE WISTAR RATS FOLLOWING EXPOSURE TO CIGARETTE SMOKE

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ABSTRACT

This study was aimed at determining the effects of cigarette smoke and carbon monoxide exposure on the testes and semen characteristics of rats. Twelve rats were divided equally into 3 groups: A, B, and C, and were exposed to fresh atmospheric air, cotton wool smoke and cigarette smoke, respectively. Each rat in Group C was exposed to smoke passively from one stick of completely burnt cigarette, containing 0.738 g of tobacco, using a customised smoking chamber; and Group B was exposed to smoke from burnt cotton wool of the same weight. After 35 days of exposure, the animals were sacrificed and the testes processed for histology, while the semen quality was assessed using specimen from the caudal epididymis. In both treated groups B and C, there was a significant reduction in the percentage of morphologically normal spermatozoa, compared with the Control animals, while only slight variations existed in other semen parameters. The histological sections showed slight reduction in the diameter of seminiferous tubules, with decrease in the population of mature sperm cells in the lumina, and widened interstitial spaces. Both carbon monoxide and cigarette smoke pose comparable amount of danger to testicular function and male fertility.

Keywords: cigarette smoke, carbon monoxide, testes, sperm, rats

INTRODUCTION

It is increasingly clear that cigarette smoking, actively or passively, is detrimental to human health. Various studies have successfully linked the use of tobacco products to many clinical conditions, including cardiovascular diseases, metabolic diseases, respiratory conditions, and cancers, to mention but few. Secondhand, or passive, smoke is a term used for both sidestream smoke, from the burning end of a cigarette, and mainstream smoke, what is exhaled from a smoker. There are higher levels of some toxins in sidestream smoke, though it dilutes quickly as it passes through the air (Cooper and Moley, 2008; National Cancer Institute, 2006). Both active and passive smoking have the potential to harm almost every organ in the body, and are associated with the leading causes of mortality (CDC, 2006).

Cigarette smoke is toxic to testicular structure and functions, causing varying degrees of hypospermatogenesis in the seminiferous tubules (Olatunji-Bello et al, 2008). In heavy smokers, it causes teratozoospermia, whereas in light smokers, it leads to asthenozoospermia (Gaur et al, 2007).

In animal studies, cigarette smoke adversely affects the developmental processes of sperm cells (Ahmadnia et al, 2007). Degeneration and dissociation of the spermatogenic cells, thickening and irregularities of the basal lamina, with specific effects on the primary spermatocytes and sustentacular cells, were noted by Guven et al (1999), and one of the resultant effects of these is a significant reduction in sperm concentration (Smith and Ogunfeibo, 2004).

Studies have also demonstrated that exposure of a woman to cigarette smoke during pregnancy predisposes her male offspring to abnormal semen parameters (Richthoff et al, 2008; Jensen et al, 2005).

Cigarette smoke has a broad spectrum of oxidant-ionising radiation capable of generating free radicals in tissues of the body (Borek, 2001); this leads to significant changes in oxidative status (Ozyurt et al, 2006; Saleh et al, 2002), and lowers the level of endogenous antioxidants in the body (El-Zayadi, 2004).

Carbon monoxide (CO), an odourless gas produced from the incomplete burning of combustible materials, is one of the components of tobacco smoke, making up 2-6% of the smoke; this level increases many folds in smokers (Mirza et al, 2005). CO is also readily absorbed into the bloodstream, and the hypoxemia it induces could be contributory to its effects on spermatogenesis (Ahmadnia et al, 2007). The potential mechanisms for the harmful effects of smoking, therefore, include oxidative stress, inflammation, and ischemic processes (Swan and Lessov-Schlaggar, 2007). The current study investigated the effects of cigarette smoke exposure in animal models on the micro-architecture of the testes and sperm characteristics.

MATERIALS AND METHODS

Experimental Animals

A total of twelve adult male Wistar rats were used. They were housed in the Animal House of the Anatomy Department, University of Ilorin. They were fed on rat pellets and water *ad libitum* and allowed to acclimatise for 7 days. The animals which weighed on the average 160 g, were randomly divided into 3 groups of 4 animals per group.

Treatment of Animals

Group A animals were exposed to normal fresh atmospheric air, and served as the Control Group; Group B animals were exposed to smoke from burnt cotton wool weighing 0.738 g; while Group C animals were exposed to smoke from a stick of Rothmans® cigarette, one stick per rat. The amount of tobacco in each stick of cigarette was 0.738 g (equal weight with the cotton wool of Group B), containing 1 mg of nicotine. Smoke of cotton wool served as a source of carbon monoxide in this study. Exposure of the Wistar rats to smoke was carried out in a customised Smoking Chamber at 1900 h daily for 35 days.

Specimen Collection

The animals were sacrificed by cervical dislocation 24 hrs after the last administration. The testes and epididymis were delivered via an abdominal section. While the testes were fixed in 10% formal saline solution, and appropriately processed for histological section using the Haematoxylin and Eosin staining techniques (Banchrof et al., 1996), the semen for seminal analysis was obtained from the caudal epididymis.

Semen Analysis: Sperm Cell Concentration

The right epididymis was minced with anatomic scizzors in 5 ml of normal saline, placed in a rocker for 10 min and allowed to incubate at room temperature for 2 min. Thereafter the supernatant was diluted at 1:100 with a solution containing 5 g sodium bicarbonate and 1 ml formalin (35%). The total number of spermatozoa was counted using the new improved Neuber's counting chamber (haemocytometer), expressed as number of sperm cells in millions/ml (Yokoi and Mayi, 2005).

Sperm Motility

To determine the motility, the fluid from the caudal epididymis was diluted with Tris buffer solution (Sonmez *et al*, 2005) to 0.5 ml. An aliquot of this solution was observed under the light microscope. The mean motility estimation was reported as the final motility score for each sample, expressed as percentage (%).

Sperm Morphology

The original dilution for motility, 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada), was used. Abnormal features were observed and categorized as tail defects, neck and middle piece defects, and head defects; and the findings were expressed as percentage (%) of morphologically normal sperm (Saalu *et al*, 2010).

Life and Death Ratio

Live and dead sperms were distinguished by adding one drop of Eosin Y stain to one drop of semen at room temperature, for 1-2 min, and smearing the mixture on a microscopic slide (Siegel, 1993)

Statistical analysis of data

The SPSS 15.0 for Windows Evaluation Version was use. Data obtained were expressed as Means ± SEM, and analysed using the Student's t-test; the level of significance was taken at p values < 0.05.

RESULTS

Physical Observation

The rats were aggressive during their exposure to the smoke, but calm after administration. There were cases of falling of furs, and decrease in weight especially during the first two weeks of exposure. There was no obvious change in the morphology of the testes of the exposed animals compared with the Control.

Semen Analysis

Results of semen analysis showed increased sperm count in the treatment groups ($p>0.05$) especially the group exposed to cotton wool smoke (Table 2), when compared with the Control Group. Significant reduction in percentage of morphologically normal spermatozoa ($p<0.05$) was noticed in the treatment groups; and animals exposed to smoke from a burnt cotton wool had the lower percentage of normal spermatozoa compared with those exposed to cigarette smoke. The differences in sperm motility from one group to another was minimal and not statistically significance ($p>0.05$).

The forward movement of the sperm cells was in the category 'C': "Fair Forward Directional Movement" in animals exposed to smoke from cotton wool (Group B) as well as cigarette (Group C), compared with the Control animals which had "Excellent Forward Directional Movement" (Category A).

Histological Observation

Normal testicular architecture was seen in transverse section of the testes of animals in Control Group; numerous spermatogenic cells were demonstrated in the seminiferous

tubules at different stages of development; the lumina were filled with many spermatozoa, and the interstitial space was shown. Animals exposed to cotton wool smoke and cigarette smoke showed reduced diameter of seminiferous tubules, less population of spermatozoa in some lumen, compared with others and the Control Group, and wider and scanty interstitial spaces.

Tables

Groups	Final weight	Initial weight	Difference
A: Control (fresh air)	162 ±9.12	177 ±9.09	-15
B: Cotton Wool	187 ±8.02	192 ±7.82	-5
C: Cigarette Smoke	173 ±1.11	163 ±1.18	10

Table 1: Weight of animals

Parameters	**Treatment Groups**		
	A:	**B:**	**C:**
Sperm concentration (x10^6/ml)	46.95 ±5.03	57.90 ±7.04**	52.07 ±7.90**
Sperm Morphology (% Normal)	87.77 ±1.32	32.28 ±7.03*	34.06 ±0.65*
Sperm motility (%)	84.53 ±1.56	86.71 ±1.59	84.15 ±3.91
Life and Death Ratio (%)	90.26 ±1.10	87.33 ±1.77	90.95 ±2.24
Progressivity	A	C	C

Table 2: Semen parameters (Mean ±SEM)

*p<0.05: significant statistical difference; **p>0.05: statistical difference not significant
A: Excellent Forward Directional Movement
B: Good Forward Directional Movement
C: Fair Forward Directional Movement

Figures

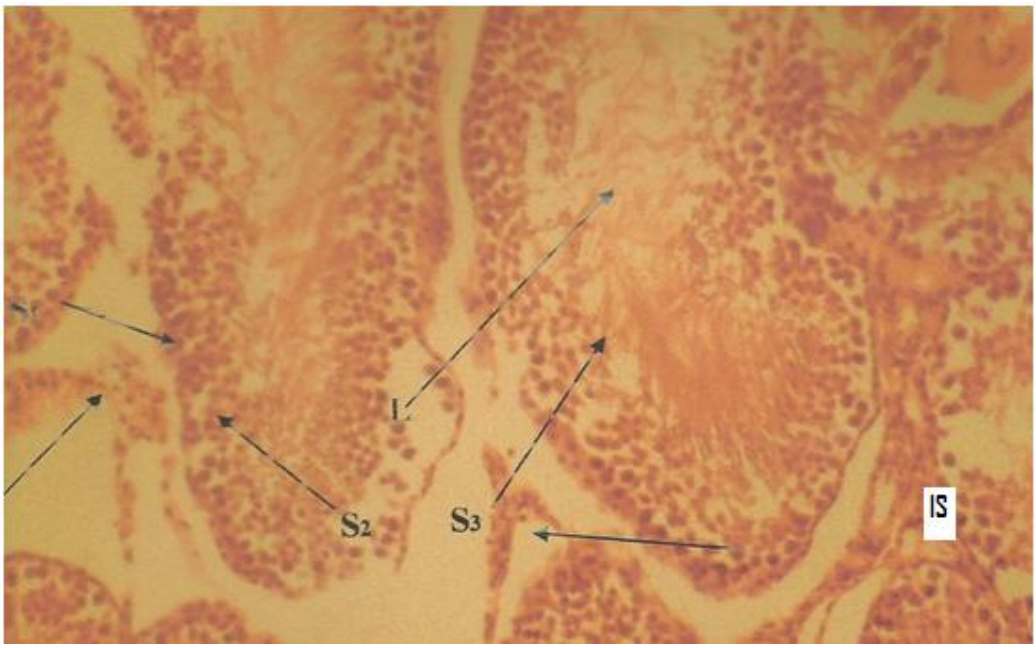

Fig1: Photomicrograph of the transverse section of the testis of Control Wistar rats showing normal microarchitecture, with seminiferous tubules having numerous spermatogenic cells (S_1:spermatogonium, S_2:spermatocyte, etc) at different stages of development; the lumina (L) are filled with many mature spermatozoa (S_3); IS: Interstitial space. H&E x100.

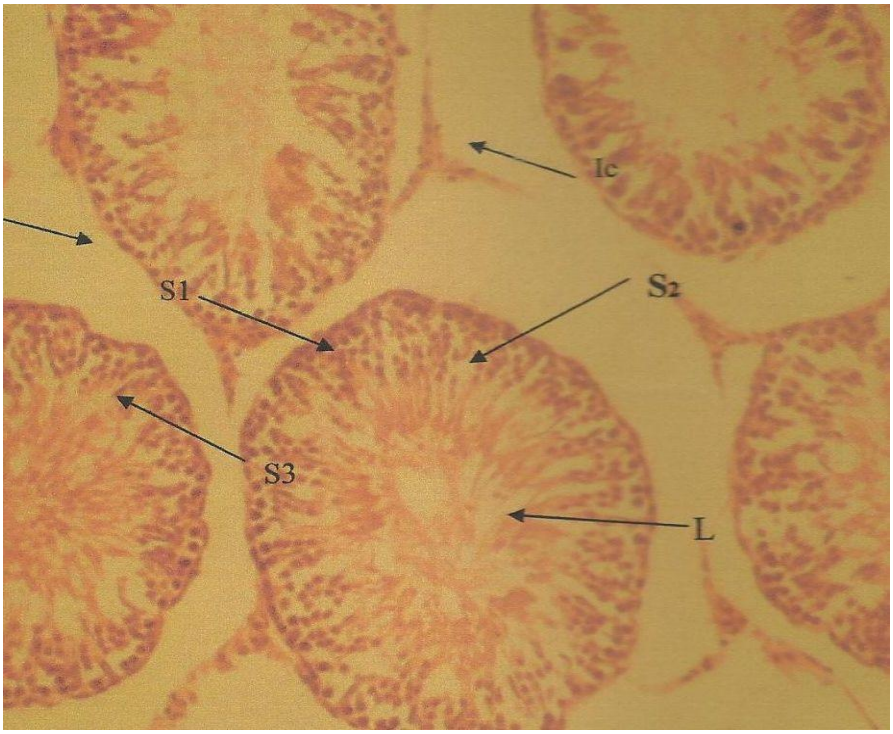

Fig2: Photomicrograph of the transverse section of the testis of animals exposed to smoke of cotton wool, showing reduced diameter of seminiferous tubules, some of whose lumens (L) are less populated with spermatozoa, compared with others and the Control Group; the Interstitium (spaces between tubules) looked wider and scanty cellular component, compared with the Control. H&E x100.

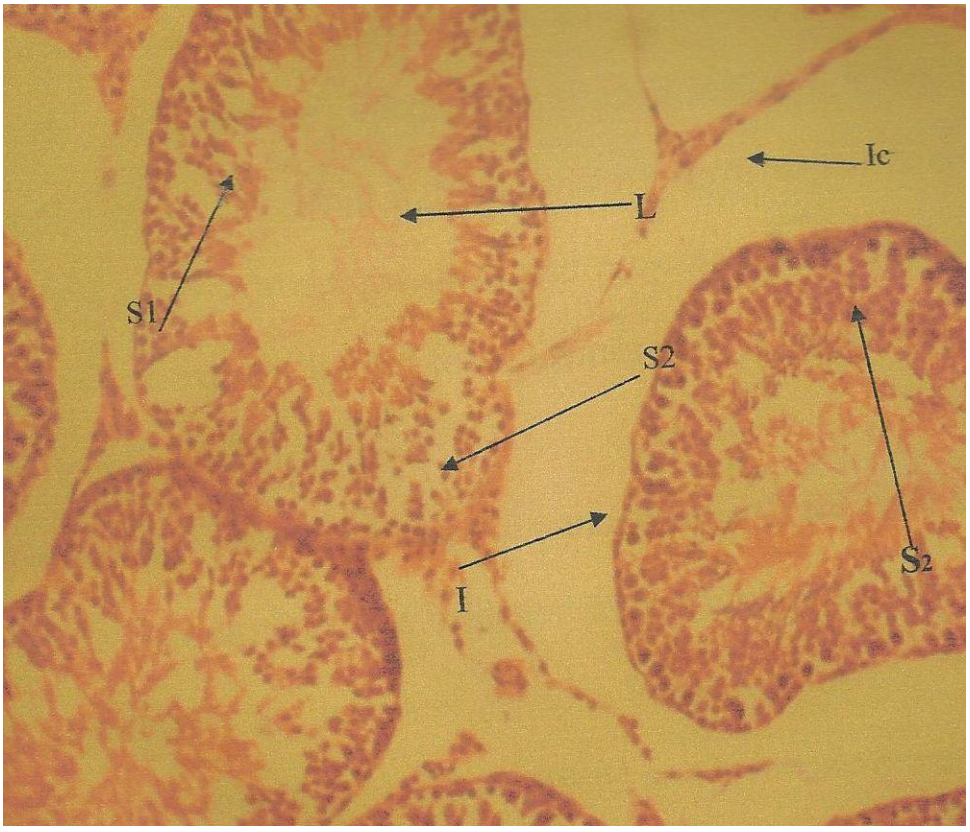

Fig3: Photomicrograph of the transverse section of the testis of animals exposed to cigarette smoke. One of the seminiferous tubules in this slide showed reduced density of spermatozoa in the lumen (L) compared with others and the Control Group; some of the tubules appeared to be reduced in diameter, while the Interstitium (I) looked wider and scanty, compared with the Control. H&E x100.

DISCUSSION

The testes are not left out in the number of tissues and organs adversely affected by cigarette smoke. Cigarette smoking affects the process of spermatogenesis, and the morphology of sperm cells appears to be a special target (Yamamoto et al, 1998; Mehrannia, 2007), although, other semen indices such as density, concentration and motility are equally affected (Hull et al., 2000; Kapawa et al, 2004).

As revealed in this study, exposure to cigarette smoke led to varying degrees of deleterious effects both on the testes and the quality of semen, with respect to spermatozoa. Studies on infertile heavy and chronic smokers revealed reduction in semen volume, sperm count, viability and forward progression when compared with infertile non-smokers; and these parameters were also much more reduced if compared with fertile non-smokers (Mehrannia, 2007). The morphology of the spermatozoa in the current study were particularly affected by the smoke, as the treated groups showed significant reduction in morphologically normal spermatozoa ($p<0.05$). The effect on sperm morphology in the current study could be due to the passive nature of the cigarette smoke exposure. Cotton wool smoke which was used as a source of carbon monoxide also led to significant reduction in the percentage of morphologically normal sperms, and hence the increase in the number of abnormally shaped spermatozoa. In the work of Hung et al (2009), semen quality and sperm functions were not affected by environmental tobacco smoke, which is a form of passive smoking.

With the use of intraperitoneal nicotine injection, Asiyah et al (2011) observed significantly low sperm motility, more dead sperms and more sperm cells with abnormal morphological features.

Carbon monoxide, the main constituent of smoke from burnt cotton wool had as much deleterious effect as cigarette smoke, which also contain carbon monoxide in addition to the many toxic substances, including nicotine. The reduction in the number of normal cells would no doubt affect male reproductive function. The “Forward Directional Movement”, or forward progression, of spermatozoa was affected equally by cigarette smoke and cotton wool smoke; as they showed only “Fair Forward Directional Movement”. The abnormal sperm cells could be in form of defects in the tail, neck, middle piece or head of the spermatozoa. Although adverse effects of smoke on sperm count, motility, and life and death ratio seemed not to be evident, as this study revealed improved sperm qualities of these parameters, other factors could be responsible. Perhaps exposure to smoke over a longer duration might cause more conspicuous adverse effects on the semen parameters.

The shrinkage seen in the histology treated animals could be a gradual effect on the activities of the seminiferous tubules, and a possible effect on spermatogenesis (Olatunji-Bello et al, 2008).

REFERENCES

1. Ahmadnia H, Ghanbari M, Moradi MR, Khaje-Dalouee M (2007): Effect of Cigarette Smoke on Spermatogenesis in Rats. Urol J. 4:159-63
2. Asiyah HA, Syazana NS, Hashida NH, Durriyyah Sharifah HA, Kamaruddin MY(2011): Effects of nicotine and Gelam honey on testis parameters and sperm qualities of juvenile rats. Scientific Research and Essays 6(26)5471-5474.
3. Banchrof JD, Steven A and Turner D (1996). Theory and practice of histopathological techniques. Fourth Ed, Churchil Livingstone, New York, London, San Francisco, Tokyo, p.109.
4. Borek, C (2001). Antioxidant Health Effects of Aged Garlic Extract. J. Nutr 131:1010S-1015S
5. Centre for Disease Control and Prevention (CDC) (2006). Smoking and Tobacco use. Fact sheet: Health effects of cigarette smoking. Available online at www.cdc.gov
6. Cooper, A.R and Moley, K.H (2008). Maternal tobacco use and its pre-implantation effects on fertility: More reasons to stop smoking. From Seminars in Reproductive Medicine. http://journal.medscape.com/viewarticle/572258_1
7. El-Zayadi A, Selim O, Hamdy H, El-Tawil A, Badran HM, Attia M, Saeed A (2004). Impact of cigarette smoking on response to interferon therapy in chronic hepatitis C Egyptian patients. World J Gastroenterol 10(20):2963-2966.
8. Gaur DS, Talekar M, Pathak VP (2007): Effect of cigarette smoking on semen quality of infertile men. Singapore Med J 48(29):119e23.
9. Guven MC, Can B, Ergun A, Saran Y, Aydos K(1999): Ultrastructural effects of cigarette smoke on rats' testes. Eur Urol 36(6):645-9
10. Hull MG, North K, Taylor H, Farrow A, Ford WC (2000). Delayed conception and active and passive smoking: The Avon Longitudinal Study of Pregnancy and childhood study team. Fertil Steril., 74:725-33.
11. Hung PH, Froenicke L, Lin CY, Lyons L, Miller MG, Pinkerton KE, VandeVoort CA (2009). Effects of environmental tobacco smoke in vivo on rhesus monkey semen quality, sperm function and sperm metabolism. Reprod. Toxicol., 27:140-148
12. Jensen MS, Mabeck LM, Toft G, Thulstrup AM, Bonde JP (2005): Lower sperm counts following prenatal tobacco exposure. Hum Reprod 20(9):2559e66.
13. Kapawa A, Giannakis D, Tsoukanelis K, Kanakas N, Baltogiannis D, Agapitos E (2004). Effects of paternal cigarette smoking on testicular function, sperm fertilizing

capacity, embryonic development, and blastocyst capacity for implantation in rat. Andrologia, 36:57-68.

14. Mehrannia T (2007): The effect of cigarette smoking on semen quality of infertile men. Pakistan Journal of Medical Sciences 23(5)
15. Mirza A, Eder V, Rochefort GY, Hyvelin J, Machet MC, Fauchier L, Bonnet P (2005): CO Inhalation at Dose Corresponding to Tobacco Smoke Worsens Cardiac Remodeling after Experimental Myocardial Infarction in Rats. Toxicological Sciences 85(2):976-982
16. National Cancer Institute (2006). Health consequences of involuntary exposure to tobacco smoke: a report of the surgeon general. US National Institute of Health. www.cancer.gov/tobacco
17. Olatunji-Bello II, Olayemi SO, Daramola AO, Ogungbemi AO (2008): Ascorbic acid and the effect of cigarette smoke on tissues--a preliminary report. West Afr J Med. 27(2):78-81.
18. Ozyurt H, Pekmez H, Parlaktas BS, Kus I, Ozyurt B, Sarsilmaz M (2006): Oxidative stress in testicular tissues of rats exposed to cigarette smoke and protective effects of caffeic acid phenethyl ester. *Asian Journal of Andrology* **8:**189–193
19. Richthoff J, Elzanaty S, Rylander L, Hagmar L, Giwercman A (2008): Association between tobacco exposure and reproductive parameters in adolescent males. Int J Androl 31(1):31e9.
20. Saalu LC, Osinubi AA, Jewo PI, Oyewopo AO and Ajayi GO (2010): An evaluation of influence of *Citrus paradise* seed extract on doxorubicin-induced testicular oxidative stress and impaired spermatogenesis. Asian Journal of Scientific Research **3**(1):51-61.
21. Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas Jr AJ (2002): Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. Fertil Steril 78(3):491e9.
22. Siegel MS (1993): The male infertility investigation and the role of the andrology laboratory. J. Reprod Med 38(5):317-334
23. Smith HA, Ogunfeibo O (2004): Effect of Cigarette Smoke on Rabbit Testicular Lipid Peroxidation, Glutathione Peroxidase and Alkaline Phosphatase Activities. Nigerian Journal of Health and Biomedical Sciences 3:1

24. Sonmez M, Turk G, Yuce A (2005). The effects of ascorbic supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology **63**:2063-2072.
25. Swan GE, Lessov-Schlaggar CN (2007). The Effects of Tobacco Smoke and Nicotine on Cognition and the Brain. Neuropsychology Review 17(3):259-273.
26. Yamamoto Y, Isoyama E, Sofikitis N, Miyagawa I (1998): Effects of smoking on testicular function and fertilizing potential in rats. Urol Res 26:45-8.
27. Yokoi K, Mayi ZK (2004). Organ apoptosis with cytotoxic drugs. Toxicology 290:7885.