

Research article

Therapeutic effect of curcumin against nicotine-induced reproductive dysfunction in male rats

Neveen A. Salem^{1,2*}, Hanan S. Alnahdi¹, Ghada S. Ibrahim^{1,3}

¹Biochemistry Department, Faculty of Science, Al Faisalia, King Abdulaziz University, Jeddah, Saudi Arabia.

²Narcotics, Ergogenic Aids and Poisons Department, Medical Division, National Research Centre, Cairo, Egypt.

³Microbial Biotechnology Department, National Research Center, Cairo, Egypt.

Key words: Nicotine, Curcumin, Oxidative stress, Cytokines, antioxidants, Testicular function.

***Corresponding Author:** Neveen A. Salem, National Research Centre, El-Bohouth Street, Cairo, Egypt, Post. Code: 12622.

Abstract

Objectives: Nicotine is the major component of cigarette smoke, which causes injurious effects on reproductive system and adversely affects spermatogenesis and fertilizing potential of sperm. The current study aimed to clarify the possible protective effect of curcumin against nicotine-induced testicular toxicity, inflammation and oxidative stress in rats. **Material and Methods:** Forty male wistar rats were divided into four groups. (1) Control, (2) Nicotine, (3) Curcumin and (4) Nicotine + curcumin. Cytokines, inflammatory mediators, sexual hormones, oxidant and antioxidant parameters were assessed in all groups. **Results and Conclusion:** Intraperitoneal injection of adult rats with nicotine for eight weeks showed significant reductions in the weight of testes, epididymis, seminal vesicles, ventral prostate, sexual hormones, antioxidants, sperm concentration and sperm motility. While significantly increased cytokines and inflammatory mediators, superoxide anion and lipid peroxides as well as the percentage of dead sperms and the rate of abnormal sperms. However, co-administration of curcumin with nicotine prevented the degenerative changes induced by nicotine, significantly reduced oxidative stress parameters, cytokines and inflammatory mediators and restored the biochemical changes occurred in the testis tissues as well as sperm characteristics.

Introduction

Smoking cigarettes provide a great potential for exposure of the organism to the toxic compounds. Nicotine is the principal alkaloid of the cigarette smoke which causes injurious effects on reproductive system and adversely affects spermatogenesis, epididymal sperm count, motility and fertilizing potential of sperm [1], reduces the weights of testis, epididymis, seminal vesicles and prostate, sperm count, sperm motility, increases the percentage of abnormal sperms, inhibits testosterone biosynthesis and reduces testicular androgenic enzymes [2]. Nicotine had been found to disturb the antioxidant defense mechanism [3], increased testicular lipid peroxidation, hydrogen peroxide and hydroxyl radical generation, while reduced the level of glutathione, the activities of antioxidant enzymes and the mitochondrial membrane potential of testis [4].

Medical plants play an important role in the management of different diseases. Curcumin is an important constituent of plant *curcuma longa* rhizomes which is a member of family zingiberaceae. It has been claimed to be a potential anti-inflammatory agent with phytonutrient and bioprotective properties [5], a potent

scavenger of reactive oxygen species (ROS), including hydroxyl radicals, nitrogen dioxide radicals and superoxide radicals [6]. It has also shown to abrogate various forms of reproductive disorders in male animals [7].

Based on these evidences, the current study was performed to evaluate the effect of nicotine on the biochemical indices related to oxidative stress inflammation and reproductive dysfunction of adult male rats and to clarify the protective effect of curcumin on these biomarkers.

Materials and Methods

Experimental animals

The present study was performed on forty adult male albino rats of similar age with an average weight of 190 ± 20 g. The animals were obtained from the National Institute of Vaccination, Hellwan, Egypt. They were maintained under standard conditions of temperature and humidity with an alternating 12h light/dark. Animals were provided with basal diet and clean water *ad libitum*. All animals procedures were in accordance with the guidelines of Ethical Guide for Care and use of

Laboratory animals (Publication No 85-23 revised 1996). All rats were acclimatized for two weeks prior to the beginning of the study.

Chemical

Nicotine (C₁₀H₁₄N₂) and curcumin (C₂₁H₂₀O₆) were obtained from sigma chemical corporation (Sigma Aldrich St. Louis, Mo, USA). All other chemicals and reagents were of analytical grade and obtained from standard commercial suppliers. The nicotine was diluted by normal saline. The dose of nicotine (4 mg/kg) was injected intraperitoneally (IP) daily for eight weeks. Curcumin powder was grinded and suspended in distilled water and administered by gavage at a dose of 80 mg/kg b.w./day for eight weeks.

Experimental

The forty animals were randomly classified into four groups, ten rats for each:

1. Control group: rats received a daily oral administration of distilled water and injected with saline for eight weeks and served as negative control.
2. Nicotine (Nic.) group: rats were injected IP with nicotine (4 mg/kg b.w. daily) for eight weeks and served as positive control.
3. Curcumin (Curc.) group: rats were administered by gavage with curcumin (80 mg/kg b.w. daily) for eight weeks.
4. Curcumin (Curc.) + nicotine (Nic.) group: rats were administered by gavage with curcumin (80 mg/kg b.w. daily) and injected IP with nicotine (4 mg/kg b.w. daily) for eight weeks.

Blood collection

At the end of experimentation, All animals were fasted overnight, euthanized and blood samples were drawn from the retro-orbital venous in centrifuge tubes for serum separation, centrifuged at 3000 rpm for 15 minutes (4°C) and stored at -20°C as aliquots for further determination of cytokines, inflammatory mediators, testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and androgen binding protein (ABP).

Tissue samples

After blood collection, all rats were rapidly sacrificed and testes were immediately excised, washed several times from blood by ice-cold isotonic saline, weighed and blotted dry. Testes, epididymis, seminal vesicles and ventral prostate were dissected from any adhering connective tissue, weighed and shock-frozen in liquid nitrogen (-170°C) and stored at (-20°C). The specimens of testes were homogenized individually with tissue homogenizer to make 10% of homogenate. The homogenate was prepared for analysis by centrifugation

at 18000 rpm (4°C) for 30 minutes and the supernatant was kept at -2°C for analysis of oxidative stress parameters and antioxidant enzymes.

Biochemical analysis

Serum analysis

Cytokines interleukin-1β (IL - 1β) and tumor necrosis factor alpha (TNF-α) as well as inflammatory mediator monocyte chemoattractant protein-1 (MCP-1) were determined by Enzyme-Linked-Immuno-Sorbent-Assay(ELISA) using rat IL-1β, TNF- α and MCP-1 kits (Biosource International USA) and microtiter plate reader, Fisher Biotech. (Germany) according to the methods of Zajkowska *et al.*, [8] and Ono *et al.*, [9]. Serum sexual hormones including luteinizing hormone (LH), follicle-stimulating hormone (FSH) (Cusabio ELIA kit, (PRC), total testosterone (DRG ELISA kit, Germany) and androgen binding protein (ABP) were estimated using enzyme linked immunosorbent assay kits following the manufacturer recommendations.

Tissue homogenate analysis

Tissue superoxide anion was determined according to the modified method of Hassoun and Stohs [10]. The amount of malandialdehyde (MDA) in tissue homogenate of testes as index of lipid peroxidation (LP) was determined [11] based on the reaction with thiobarbituric acid. Superoxide dismutase (SOD) activity was estimated [12], based on inhibiting pyrogallol autooxidation by SOD. Catalase (CAT) activity was determined [13], based on decomposition of hydrogen peroxide by catalase enzyme. Reduced glutathione (GSH) level was estimated spectrophotometrically [14].

Sperm characteristics

The number of sperms was counted according to the modified method [15], using a hemo-cytometer and light microscope at x 200 magnification. Sperm motility was determined [16], on the basis of visual estimation under light microscope at x 400 magnification. Sperms were assigned as either motile or nonmotile. The percentage of abnormal spermatozoa was estimated according to Turk *et al.*, [17]. Slides were stained with India ink and visually estimated under light microscope at x 400 magnification and any head, tail and total sperms abnormalities were expressed as percentage.

Statistical analysis

The obtained data were presented as Mean ± SD. Homogeneity of variance for each variable was analyzed using the Levine test. One way analysis of variance (ANOVA), followed by Duncan's multiple rank test were performed using the mSTAT-c computer program to determine the statistical significance between the

different groups. The difference was considered significant at $P < 0.05$.

Results and Discussion

Results

Reproductive organ weights

As shown in table (1) injecting adult male rats with nicotine caused statistically significant reduction in the weights of both right and left testis, right and left epididymis, seminal vesicles and ventral prostate as compared to the control group. These reductions were -18%, -18%, -23%, -22%, -27% and 38% in the same respect. However, co-administration of curcumin with nicotine showed significant elevation in the weights of testes, epididymis, seminal vesicles and ventral prostate comparable with the rats receiving nicotine alone. Thus curcumin restored the weight values of reproductive organs to reach approximately the values of control group.

Cytokines and inflammatory mediator

Data presented in Figure (1) showed that nicotine stimulated the release of IL-1 β , TNF- α and MCP-1 when compared to the control group. Injection male rats with nicotine resulted in higher levels of cytokines (IL-1 β and TNF- α) and inflammatory mediator (MCP-1) with values of 58%, 72%, and 78%, respectively. However, administration of curcumin to nicotine treated group significantly attenuated the levels of IL-1 β , TNF- α and MCP-1 as compared to nicotine group. Thus, curcumin restored the values of these parameters to be approximately near the control group.

Sexual hormones

It is evident from the data given in Figure (2) that injecting adult rats with nicotine showed significant reductions in serum levels of testosterone (-35%), LH (-42%), FSH (-41%) and ABP (-14%) as compared to the control group. Otherwise, co-administration of curcumin to nicotine group revealed significant elevations in the levels of studied sexual hormones as compared to the nicotine group. Thus, curcumin brought back the levels of testosterone, LH, FSH and ABP to approximately close to the control group.

Oxidant and antioxidant parameters

Data given in Figure (3A, 3B) elucidated that nicotine treated rats resulted in significant increase in the levels of superoxide anion (45%) and MDA (35%) and significant decrease in the levels of antioxidants, i.e. SOD (49%), CAT (-50%) activities and GSH (-57%) as compared the control group. On the other hand, co-administration of curcumin to the rats of nicotine group showed significant attenuation in the levels of oxidative stress parameters i.e superoxide anion and MDA, while significant elevations in SOD and CAT activities as well as GSH level were

observed as compared to nicotine group. Thus, curcumin mitigated the adverse effect of nicotine on oxidant and antioxidants parameters.

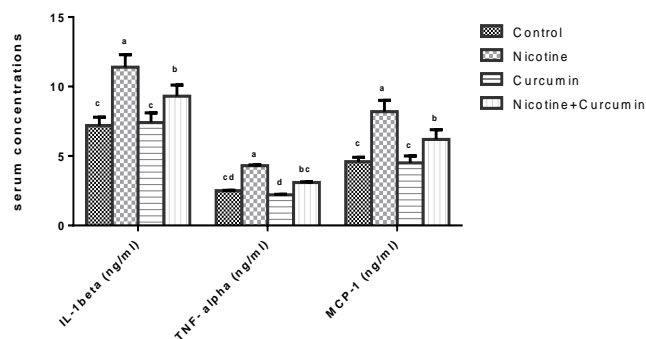


Figure 1. Effect of nicotine, curcumin and curcumin-nicotine on cytokines and inflammatory mediators in serum of male rat. Values are expressed as means \pm SE. Columns followed by the same alphabetical letter are not significantly different at $p < 0.05$

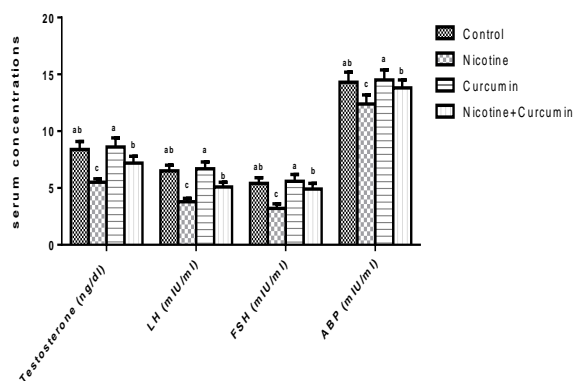


Figure 2. Effect of nicotine, curcumin and curcumin-nicotine on sexual hormones in serum of male rat. Values are expressed as means \pm SE. Columns followed by the same alphabetical letter are not significantly different at $p < 0.05$.

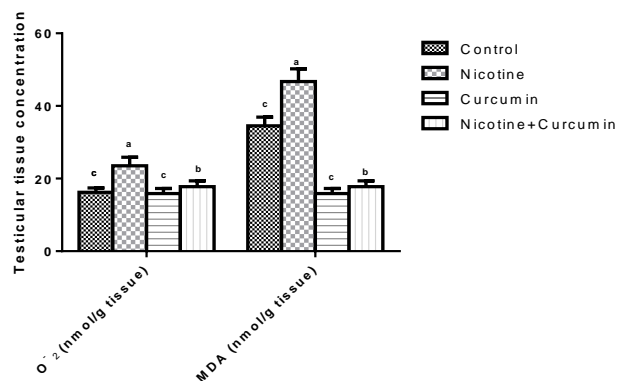


Figure 3(A). Effect of nicotine, curcumin and curcumin-nicotine on oxidants level in testicular tissues of male rats. Values are expressed as means \pm SE. Columns followed by the same alphabetical letter are not significantly different at $p < 0.05$.

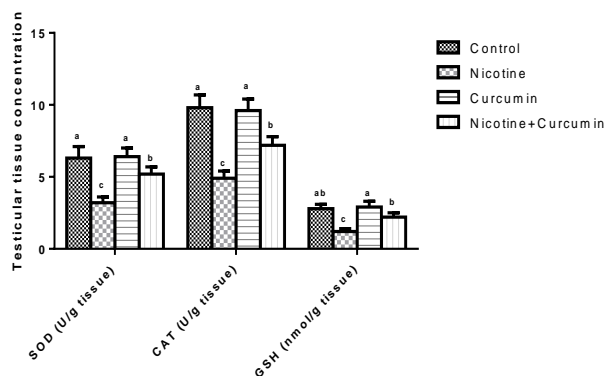


Figure (3B). Effect of nicotine, curcumin and curcumin-nicotine on antioxidants level in testicular tissues of male rats. Values are expressed as means ±SE. Columns followed by the same alphabetical letter are not significantly different at $p < 0.05$.

Sperm characteristics

As shown in Table (2), the treatment of male rats with nicotine caused significant decrease in sperm concentration (-35%) and motility percentage (-23%), while significantly increased the percentage of dead sperms (65%), head (194%), tail (65%) and entire sperm abnormalities (113%) as compared to the control rats. Supplementing curcumin to nicotine treated group significantly elevated sperm concentration and sperm motility, while significantly attenuated the percentage of dead sperm, head, tail and entire sperm abnormality in comparison with that in the nicotine alone treated group.

Table 1. Effect of nicotine, curcumin and curcumin-nicotine on the weight of reproductive organs of male rat.

	Testes (g)		Epididymis (g)		Seminal vesicles (g)	Prostate (g)
	Right	Left	Right	Left		
Control	1.65 ± 0.14 ^{ab}	1.60 ± 0.13 ^{ab}	0.48 ± 0.05 ^{ab}	0.46 ± 0.04 ^{ab}	0.62 ± 0.05 ^{ab}	0.32 ± 0.02 ^{ab}
Nicotine	1.35 ± 0.09 ^c	1.32 ± 0.10 ^c	0.37 ± 0.04 ^c	0.36 ± 0.06 ^c	0.45 ± 0.03 ^c	0.19 ± 0.01 ^c
Curcumin	1.72 ± 0.17 ^a	1.70 ± 0.15 ^a	0.50 ± 0.06 ^a	0.49 ± 0.05 ^a	0.64 ± 0.06 ^a	0.33 ± 0.03 ^a
Curcumin + Nicotine	1.58 ± 0.12 ^b	1.56 ± 0.12 ^b	0.45 ± 0.03 ^b	0.44 ± 0.03 ^b	0.59 ± 0.04 ^b	0.29 ± 0.02 ^b

Values are expressed as means ±SE. Means followed by the same alphabetical letter are not significantly different at $p < 0.05$.

Table 2. Effect of nicotine, curcumin and curcumin-nicotine on sperm characteristics of male rats.

	Sperm concentration million/g	Sperm motility %	Dead sperms %	Abnormal sperms %		
				Head	Tail	Total
Control	325 ± 22.2 ^a	78.9 ± 6.2 ^a	12.5 ± 1.3 ^c	3.2 ± 0.3 ^c	5.4 ± 0.6 ^c	8.6 ± 0.9 ^c
Nicotine	212 ± 18.6 ^c	61.1 ± 4.4 ^c	20.6 ± 2.4 ^a	9.4 ± 0.8 ^a	8.9 ± 1.1 ^a	18.3 ± 1.4 ^a
Curcumin	327 ± 21.5 ^a	79.5 ± 6.8 ^a	12.2 ± 0.9 ^c	3.1 ± 0.4 ^c	5.2 ± 0.5 ^c	8.3 ± 0.8 ^c
Curcumin + Nicotine	298 ± 19.4 ^b	72.1 ± 5.3 ^b	15.3 ± 1.8 ^b	5.8 ± 0.6 ^b	6.8 ± 0.7 ^b	12.6 ± 1.1 ^b

Values are expressed as means ±SE. Means followed by the same alphabetical letter are not significantly different at $p < 0.05$

Discussion

Nicotine is the major component of cigarette smoke, which causes injurious effect on reproductive system and adversely affects spermatogenesis and fertilizing potential of sperm [17]. The present study aimed to evaluate the possible protective effect of curcumin against nicotine induced testicular toxicity, inflammation and oxidative stress in rats.

The results of the current study revealed that injecting adult rats with nicotine 4 mg/kg b.w./day for eight weeks showed significant reductions in the weights of right and left testis, epididymis, seminal vesicles and ventral prostate. The significant decrease in the weights of accessory sex organs is due to acute scarcity of androgens

[18]. Testicular weight, an excellent indicator of gonadal toxicity, was significantly decreased in male rats treated with nicotine which is consistent with the previous finding [19]. In addition, Jalili *et al.*, [18] reported that intraperitoneal injection of nicotine resulted in the decrease of testosterone hormone and testis weight and impairment of reproductive variables which supported the results obtained in the current study.

Co-administration of curcumin to the rats injected with nicotine enhanced the weight of testes and accessory sex organs. Curcumin abrogated the harmful effect of nicotine on reproductive organs weight. These results are in consistent with the findings of other studies [20].

Injecting adult rats with nicotine revealed significant elevation in the levels of cytokines (IL-1 β and TNF- α) and inflammatory mediator (MCP-1) which caused injury in the testicular tissues. In this connection, several cytokines were increased in response to nicotine exposure [21]. However, co-administration of curcumin to the rats injected with nicotine reversed the levels of IL-1 β , TNF- α and MCP-1 approximately near to the control group via its anti-inflammatory potential. Curcumin has been claimed to be a potential anti-inflammatory agent with phyto-nutrient and bioprotective properties [22].

The present results showed that nicotine significantly depressed the serum levels of testosterone, LH, FSH and ABP. In this connection several studies have been performed on the adverse effect of nicotine on the genital system of humans and rats [23]. The decrease in serum testosterone level of rat treated with nicotine may have been caused by the disruption of testicular cyto-architecture which consequently adversely affected Leydig cell number and functioning, leading to decrease serum testosterone level, since Leydig cells secrete testosterone [24].

The present study demonstrated that co-administration of curcumin with nicotine significantly augmented serum testosterone, LH, FSH and ABP concentrations with respect to nicotine alone treated rats, suggesting that curcumin has stronger influence on testicular androgenesis. Testosterone and LH hormones are essential for normal testes function and healthy spermatogenesis. These hormones were significantly improved after treatment with curcumin, suggesting that curcumin treatment improved spermatogenesis impairment and nicotine toxic effect on the testis.

The anterior pituitary gonadotrophic hormones LH and FSH play an essential role in testis physiology. FSH plays a dual role in spermatogenesis by directly affecting the Sertoli cells to stimulate and initiate germ cell number and indirectly enhances androgen production by the Leydig cells [25]. FSH stimulates the Sertoli cell to produce ABP which may serve to increase the accumulation of ABP in the seminiferous epithelium and intracellular androgen receptors [26]. The restoration of the sexual hormones activity in injected rats with nicotine by curcumin co-administration may be due to the significant protection of testicular cells by curcumin.

In the current study, injecting adult rats with nicotine revealed significant elevation in the levels of superoxide anion and MDA and significant decrease in SOD, catalase activities and GSH level in testis tissues as compared to the control group. Nicotine has been found to disturb the antioxidant defense mechanism [3], increased lipid peroxidation, hydrogen peroxide and hydroxyl radical generation, while reduced the level of glutathione, the activities of antioxidant enzymes [4] which supported the results obtained in the present study.

On the other hand, co-administration of curcumin to nicotine treated rats showed significant attenuation in the levels of superoxide anion and MDA, while significant increase in the activities of SOD and CAT as well as the level of GSH as compared to the rats receiving nicotine alone.

Thus, curcumin restored the oxidant and antioxidant parameters to be approximately near the control group. The protective effect of curcumin against nicotine toxicity may be due to the modulation of expressions of antioxidative system, direct scavenging of reactive oxygen species (ROS), including hydroxyl radicals, nitrogen dioxide radicals and superoxide radicals [6], reduction of the levels of several markers of oxidative stress and decreased lipid peroxidation induced by nicotine [7].

It is evident that intraperitoneal injection adult rats with nicotine showed significant decrease in sperm concentration, sperm motility and significant increase in the percentages of dead sperms and the rate of abnormal sperms as compared to the control group. However, co-administration of curcumin with nicotine revealed significant elevation in sperm concentration, sperm motility and significant attenuation in the percentage of dead sperm and the rate of abnormal sperms as compared to the rats receiving nicotine alone. In this connection, numerous studies provided further support to the present study that nicotine caused significant reduction in sperm concentration, sperm motility and normal sperm morphology [2]. In addition, nicotine has been found to disturb the antioxidant defense mechanism [3], increased lipid peroxidation of unsaturated fatty acids in the sperm plasma membrane, causing a loss of its fluidity and function [4].

Co-administration of curcumin to the rats treated with nicotine abrogated the adverse effects of nicotine on sperm characteristics and the values were approximately near to those in the control group regarding sperm concentration, motility and abnormal sperm rate. The obtained results are in consistent with the previous findings [6]. These results could be discussed on the basis of the antioxidant [4] and anti-inflammatory properties of curcumin [5]. Curcumin has proved its credentials as wonderful chemopreventive agent effectively counteract nicotine-induced oxidative stress and inflammation resulted in testicular dysfunction as represented through attenuating oxidative stress and improving antioxidant and anti-inflammatory defense system and prevent all toxic effect of nicotine.

Conclusion

The obtained results reinforced the prominent role of ROS and inflammatory mediators as contributing factors in nicotine-induced testicular toxicity and also elicited that curcumin could be effective in protecting against

nicotine-induced testicular damage for its potent anti-inflammatory and cytoprotective effect against the deleterious toxic effect caused by nicotine. Thus curcumin could be used as a supplement for healthy reproductive life.

References

1. Aydos, K.; Guven M.C.; Can B. and Ergun A: Nicotine toxicity to the ultrastructure of the testis in rats. *B.J.U. International*. 2001; 88: 622-626.
2. Nair, S.V.G. and Rajamohan T.: The role of coconut water on nicotine-induced reproductive dysfunction in experimental male rat model. *Food and Nutrition, Sci.* 2014; 5: 112 – 1130.
3. Dani, J.A. and Hinemann S.: Molecular and cellular aspects of nicotine abuse. *Neuron* 1996; 16: 905- 908.
4. Jana, K.; Samanta P.K. and De D.K.: Nicotine diminishes testicular gametogenesis, steroidogenesis and steroidogenic acute regulatory protein expression in adult albino rats. Possible influence on pituitary gonadotropins and alteration of testicular antioxidant status. *Toxicological Sciences* 2010; 116: 647-659.
5. Aggarwal, B.B. and Sung B.: Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. *Trends Pharmacology. Sci.* 2009; 30(2):85-94.
6. Goel, A.; Jhurani S. and Aggarwal B.B.: Multi-targeted therapy by curcumin: how spicy is it? *J. Nat. Prod.* 2008; 70(2):143-146.
7. Khorsandi, L.; Mirhoseini M.; Mohamadlour M.; Orazizadeh M.: and Khaghani S.: Effect of curcumin on dexamethasone-induced testicular toxicity in mice. *Pharm. Boil.* 2013; 51:206-212.
8. Zajkowska, T. M.; Hermanowska-Szpakowicz T.; Pancewicz S.A.; Kondruse K.M.; Swiorzbinska R. and Grygorezuk S.: Serum concentration of TNF-alpha and IL-1 beta in patient with chronic hepatitis C treated with interferon alpha a preliminary report. *Rocz.Akad. Med. Bialymst.* 2002; 47:276-286.
9. Ono, K.; Matsumori A.; Furukawa Y.; Igata H.; Shioi T. and Matsushima S.S.: Prevention of myocardial reperfusion injury in rats by antibody against monocyte chemoattractant protein-1. *Lab. Invest.* 1999; 79 (2): 195-203.
10. Hassoun, E.A. and Stohs S.J.: Cadmium induced production of superoxide anion and netric oxide, DNA single strand breaks and lactate dehydrogenase leakage in. *J. 774 A-1 Cultures. Toxicol.* 1996; 112(3): 219-226.
11. Ohkawa, H.; Ohishi N. and Yagi K.: Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 1979; 95:351-358.
12. Marklund, S.L.: Pyrogallol auto oxidation. In *CRC Handbook of Methods for Oxygen Radical Research* (Ed Greenwald R.A.). Boca Raton Florida CRC press: 1985; 243-247.
13. Clairborne A.: Catalase activity. In *CRC Handbook of Methods for Oxygen Radical Research* (Ed Greenwald R A). Boca Raton Florida CRC Press 1985; 283- 284.
14. Avi-Dor, Y. and Lipkin R.: A spectrophotometric method for the determination of reduced glutathione. *J. Boil. Chem.* 1958; 223: 69-72.
15. Yokoi, K.; Urhus E.O. and Nielsen F.H.: Nickel deficiency diminishes sperm quantity and movement in rats. *Boil. Trace Elements Res.* 2003; 91:141-153.
16. Sonmez, M.; Turk G. and Yuce A.: The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wister rats. *Theriogenology* 2005; 63: 2061-2072.
17. Turk, G.; Atessahin A.; Sonmez M.; Ceribasi A. O. and Yuce A.: Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system and the histologic structure of the rat testis by ellagic acid. *Fertile. Steril.* 2008; 89(5): 1474-1481.
18. Reddy, S.; Londonkar R.; Ravendra S. R. and Patil S. B.: Testicular changes due to graded doses of nicotine in albino mice. *Indian j. physiol. Pharmacol.* 1998; 42(2): 276-280.
19. Jalili, C.; Khani F.; Salahshoor M.R. and Roshankhah S.: Protective effect of curcumin against nicotine-induced damage on reproductive parameters in male mice. *Int. J. Morphol.* 2014; 32(3):844-849.
20. Sakr, S.A. and Badawy G.M.: Protective effect of curcumin on monosodium glutamate-induced reproductive toxicity in male albino rats. *Global. J. Pharmacol.* 2013; 7(4): 416-422.
21. Izadpanah, M.; Alizadeh R.; Minaee M.B.; Heydari L.; Babatunde A. and Abbasi M.: The effect of curcumin on sperm parameters and metric oxide production in varicocele rats. *Int. J. Morphol.* 2015; 33(4):1530-1535.
22. Hossain, M.; Sathe T.; Fazio V.; Mazzone P.; Weksler B.; Janigro D.; Rapp E. and Cucullo L.: Tobacco smoke: A critical etiological factor for vascular impairment at blood-brain barrier. *Brain Res.* 2009; 1287: 192-205.
23. Stillman, J.R.; Rosenberg M.S. and Sachs P.B.: Smoking and reproduction. *Fertil. Steril.* 1986; 46: 545-566.
24. Oyeipo, I. P.; Raji Y.; Emikpe B.O. and Bolarinwa A.F.: Effect of oral administration of nicotine on organ weight, serum testosterone level and testicular histology in adult male rat. *Nig. J. Physiol. Sci.* 2010; 25:81-86.
25. O'shaughnessy, P.J.; Monteiro A.; Verhoven G.; DeGendt K. and Abel M.H.: Effect of FSH on testicular morphology and spermatogenesis in gonadotrophin-deficient hypogonadal mice lacking androgen receptors. *Reproduction* 2010; 139: 177-184.
26. Hansson, V.; Weddington S.C.; Mc Lean W.S.; Smith A.A.; Nayfeh S.N.; French F.S. and Ritzen E.M.: Regulation of seminiferous tubular function by FSH and androgen. *J. Reprod. Fert.* 1975; 44: 363-375.