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MELATONIN AND N-ACETYL-L-CYSTEINE AMELIORATE OXIDATIVE STRESS ON NICOTINE INDUCED REPRODUCTIVE DYSFUNCTION IN MALE RATS

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Background and purpose: Smoking has a hugely detrimental impact on one's health everywhere. Infertility is a major health issues, and smoking has been related to lower fertility. This study evaluates potential role of melatonin and N-acetyl-L-cysteine (NAC) to protect male rats from testicular toxicity of nicotine.

Materials and methods: An LD_{50} value was assessed. Nicotine (1 mg/kg) to cause testicular toxicity. Measurements are serum testosterone, FSH, LH and total antioxidant capacity (TAC). All groups underwent sperm count and histopathology.

Results: Nicotine's LD_{50} was estimated. TAC, testosterone, LH, sperm count, and proportion of seminiferous tubules with mature spermatogenesis all significantly decreased after nicotine administration and no significant alteration in FSH. Significant increases in TAC, sperm count, and proportion of tubules containing mature sperms after giving melatonin (5 and 10 mg/kg), while insignificant changes in FSH and LH. N-acetyl-L-cysteine produce significant rise in TAC and sperm count (100 and 200 mg/kg), testosterone (high dose) and insignificant change in FSH, LH and percentage of seminiferous tubules. Their combinations produced significant increase in TAC, testosterone level, sperm count and percentage of tubules containing mature sperms and insignificant change in FSH and LH (low and high doses). The withdrawal group exhibited a significant rise in TAC, testosterone, sperm count, and seminiferous tubule percentage. Minimal shift in LH and FSH levels.

In conclusion: These findings implied that melatonin, NAC and their combination might combat the intoxication caused by nicotine and can effectively prevent testis toxicity complications. In addition, smoking cessation could beneficially recover testicular dysfunction.

Keywords: Nicotine, smoking, melatonin, N-acetyl-L- cysteine, testicular dysfunction, NAC.

INTRODUCTION

Nicotine has shown deleterious effects on male reproductive system and testicular damage, which is a non-apoptotic regulated cell death[1]. Smokers and their offspring have a significant developing risk of genetic and other reproductive abnormalities. These abnormalities may not be manifest immediately at birth but they may become noticeable later in life[2]. Chronic smoking resulted in low sperm quality and quantity mainly through oxidative stress

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and direct attack by smoking metabolites[3]. Moreover, smoking inhibits spermatogenesis and decrease steroidogenesis in men[4]. Tobacco can adversely adjust seminal parameters and sex hormones (FSH, LH and testosterone) and result in fertility problems in males[5].

The pineal gland secretes the neuroendocrine hormone melatonin. Melatonin binds with two distinct membrane-bound receptors known as MT1 and MT2. Additionally, melatonin acts as a powerful antioxidant scavenges free radicals[6]. Enhanced release of melatonin stimulates the production of GnRH, testosterone, LH, and promotes sexual activity. This induces testicular functions and spermatogenesis[7] by suppressing local inflammatory processes and generation of ROS[8].

N-acetyl-L-cysteine (NAC), the acetylated precursor of L-cysteine, is a mucolytic agent used in medicine to treat drug overdose or toxicity. It regulates various pathophysiologic processes related to disease in addition to oxidative stress. These consist of inflammation, apoptosis, and mitochondrial dysfunction[9]. Glutathione is a well-known direct antioxidant and a substrate of multiple antioxidant enzymes. The antioxidant effect of NAC is due to its capacity to act as a reduced glutathione precursor. Moreover, in some circumstances where a significant reduction of endogenous GSH occurs, NAC can act as a direct antioxidant for some oxidant species like NO_2 and HOX[10].

The present study designed to assess lethal dose of nicotine and investigate its effect on male reproductive function and evaluation of the possible protective effect of melatonin and NAC in different doses and their combination. Also, study the effect of nicotine withdrawal after four weeks on serum levels of TAC, testosterone, FSH, LH and sperm counts at the end of the experiment.

MATERIALS AND METHODS

Animals

Male adult albino rats weighing 220±20 grams used. Animals were gained from the animal house, Faculty of Medicine, Sohag University and were housed in animal place with room temperature being preserved at 24±2°C. Animals were kept under normal light/dark cycles and fed commercial pellet diets. Animals were allowed unlimited access to food and water for their own use. The experimental protocol approved by the Institutional Animal Care and Use Committee of Faculty of Medicine, Sohag University.

Chemicals

Nicotine ($C_{10}H_{14}N_2$, 99%, 1 gm/ml) purchased from Sigma Aldrich Company, England. Melatonin ($C_{13}N_2O_2$, 99%, 1 gm. powder) purchased from Pharco Company, for pharmaceuticals, Egypt. N-Acetyl-L-Cysteine (NAC) (25 gm. powder) and Physiological saline (0.9% Na Cl) purchased from Nile Company for pharmaceuticals, Egypt. Biochemical kits purchased from Sigma Aldrich Company, England, (total antioxidant capacity, testosterone, FSH and LH).

Methods

Detection of nicotine LD_{50}

Assessment of lethal dose (LD_{50}) of nicotine according to Lorke[11].

$$LD_{50} = \sqrt{D_0 \text{ x } D_{100}}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

Induction of testicular toxicity by nicotine and evaluation of nicotine withdrawal effect

Testicular toxicity induced by daily IP administration of nicotine (1 mg/kg) for 4 weeks. Melatonin given in two doses IP (5 mg/kg and 10 mg/kg) half an hour before nicotine[12], NAC in two different doses IP (100 mg/kg and 200 mg/kg)[13] or their combination in both low and high doses. The experiment was terminated after four weeks except for the withdrawal group which continued for another four weeks without treatment.

Experimental groups

Fifty four male albino rats were divided randomly into nine groups; six rats each. They received melatonin and NAC at different doses

via IP administration half an hour before nicotine 1 mg/kg daily for 4 weeks for treated groups.

- First group (negative control): 0.5 ml of saline.
- Second group (positive control): Nicotine (1 mg/kg) .
- Third group and fourth group: Melatonin 5 mg/kg and 10 mg/kg respectively.
- Fifth group and sixth group: N-acetyl-L-cysteine 100 mg/kg and 200 mg/kg respectively.
- Seventh group: Melatonin 5 mg/kg and NAC 100 mg/kg.
- Eighth group: Melatonin 10 mg/kg and NAC 200 mg/kg.
- Ninth group (withdrawal effect): Induction of testicular dysfunction by IP nicotine administration 1 mg/kg, daily for four weeks and no treatment was given for another four weeks before scarification.

Blood samples collection

After four weeks, the rats of groups 1-8 were decapitated and their blood samples were taken. For withdrawal group, the animals were left for another four weeks without treatment before scarification. For fifteen minutes, blood samples were centrifuged at 3500 rpm. For hormonal assay and biochemical analysis, serum was separated and promptly stored at -80°C.

Biochemical analysis of TAC

Diagnostic biochemical kits have been used according to manufacturer instructions to measure serum level of TAC.

Assessment of testosterone, FSH and LH levels by ELISA

Enzyme-linked immunosorbant assay (ELISA) kits have been used according to manufacturer instructions.

Sperm count and histopathological evaluation

Semen extraction and sperm count

The caudal epididymis of both testes carefully cut off from the testes and and placed in a petri dish with two milliliters of normal saline. The obtained suspension of sperm was diluted 1:20 and then the sperm were counted using a Neubauer hemocytometer[14].

Testicular histopathology

Preparations of testicular histological sections

The two testes of each rat were removed and examined grossly for any pathological abnormalities. After cutting each testis into thin slices that were 3 mm thick, the tissue was fixed by allowing it to sit in Bouin's solution for at least 6 hrs[15]. The tissue samples dehydrated in upgraded concentrations of alcohol. After deparaffinizing the sections, downgraded alcohol concentrations were used to rehydrate them. The sections stained with haematoxylin and eosin (H&E).

Evaluation of testicular morphology and spermatogenesis

The testes inspected using Olympus light microscope (CX21). One section of each testis was examined by low power magnification (x100) for existence of tissue damage and for the general morphology of seminiferous tubules. Different stages of spermatogenesis up to mature spermatids were assessed by high power magnification (x400). For every animal, the percentage of tubules containing mature spermatids, primary spermatocytes, and spermatogonia was determined.

Statistical analysis

The commercially available statistical analysis software (IBM-SPSS version 22.0) used for data analysis. Results oulined as mean ± standard deviation (SD). Differences between two continuous variables evaluated by Mann-Whitney U test. The differences were considered significant if $p< 0.05$ and highly significant if $p< 0.01$.

RESULTS

LD_{50} of nicotine

Based on observations of the two phases of Lork`s method; highest dose that give no

mortality was 10 mg/kg and the lowest dose that induced mortality of the rats was 20 mg/kg. Accordingly, LD_{50} of nicotine calculated according to the following formula:

$$LD_{50} \text{ of nicotine} = \sqrt{D_0 \text{ x } D_{100}} =$$

14.142 mg/kg

1- Effect of nicotine on TAC, testosterone, FSH, LH, sperm count and percentage of tubules

Administration of nicotine (1 mg/kg) for four weeks IP resulted in significant decrease of TAC, testosterone and LH hormones with insignificant change in serum level of FSH. Moreover, sperm count significantly decreased after nicotine treatment. Nicotine caused some histopathological alterations in the testis such as focal detachment of the basal spermatogenic cells from their basement membrane, these alterations characterized by frequent lack of elongated spermatids and mature spermatozoa in seminiferous tubules (Table 1 & Fig. 1).

2- Effect of melatonin on TAC, testosterone, FSH, LH, sperm count and percentage of tubules

Melatonin at both low and high doses (5 mg/kg and 10 mg/kg) significantly increased TAC however, there was no significant change in serum level of testosterone, FSH, or LH at either dose. Furthermore, both high and low doses of melatonin significantly increase the number of sperms. The percentage of tubules with mature spermatogenesis did not significantly change after treatment with a low dose of melatonin (5 mg/kg). On the other hand, compared to positive control rats, a high dose of melatonin (10 mg/kg) significantly elevated the percentage of tubules with mature sperms (Table 2 & Fig. 2).

3- Effect of N-acetyl-L-cysteine on TAC, testosterone, FSH, LH, sperm count and percentage of tubules

Results of the present study revealed that treatment with low and high dose of NAC produced a significant increase of TAC and insignificant change in serum levels of FSH and LH. Treatment with low dose produced insignificant effect while high dose NAC resulted in a significant rise of testosterone level. Treatment with both low and high doses of NAC showed negligible changes in the percentage of tubules with mature spermatogenesis, the administration of two different doses of NAC was successful in increasing the sperm count significantly when compared to positive control rats (Table 3 & Fig. 3).

4- Effect of combined melatonin and N-acetyl-L-cysteine on TAC, testosterone, FSH, LH, sperm count and percentage of tubules

Serum testosterone and TAC levels increased significantly when melatonin and NAC were administered concurrently at low and high doses. Conversely, the administration of low and high dosages of melatonin and NAC together resulted in negligible alterations in serum concentrations of FSH and LH. Combined administration of low and high doses of melatonin and NAC showed significant rise of sperm count and percentages of tubules with mature spermatogenesis (Table 4 & Fig. 4).

5- Effect of nicotine withdrawal on TAC, testosterone, FSH, LH, sperm count and percentage of tubules

When compared to positive control, withdrawal of nicotine caused a significant increase in TAC and testosterone levels. While serum levels of FSH and LH showed negligible changes upon cessation of nicotine use. Nicotine withdrawal induced a significant increase of sperm count and the percentages of seminiferous tubules with mature sperms that is comparable to normal rats' values (Table 5 & Fig. 5).

Table 1: Effect of nicotine (1 mg/kg) on TAC, testosterone, FSH, LH, sperm count and percentage of tubules.

Parameter	Normal rats (Negative control)	Nicotine (Positive control)
Serum total antioxidant capacity (mM/L) Mean ± SD	1.89 ± 0.120	0.41 ± 0.090$^{\#}$
Serum testosterone level (ng/ml) Mean ± SD	6.75 ± 0.890	2.86 ± 1.041$^{\#}$
Serum FSH level (ng/ml) Mean ± SD	6.15 ± 0.491	5.20 ± 0.821
Serum LH level (ng/ml) Mean ± SD	5.44 ± 0.370	3.46 ± 0.921$^{\#}$
Sperm count (millions/ml) Mean ± SD	84.6 x 10^6 ± 9.2 x 10^6	26 x 10^6 ± 7.9 x $10^{6\#}$
Percentage of tubules with mature sperms (%) Mean ± SD	80.7% ± 3.51%	42.5% ± 4.41%$^{\#\#}$

Data represent mean ± SD of 6 observations. $^{\#}$Significant result at $p< 0.05$ from normal control. $^{\#\#}$Highly significant result at $p< 0.01$ from normal control.

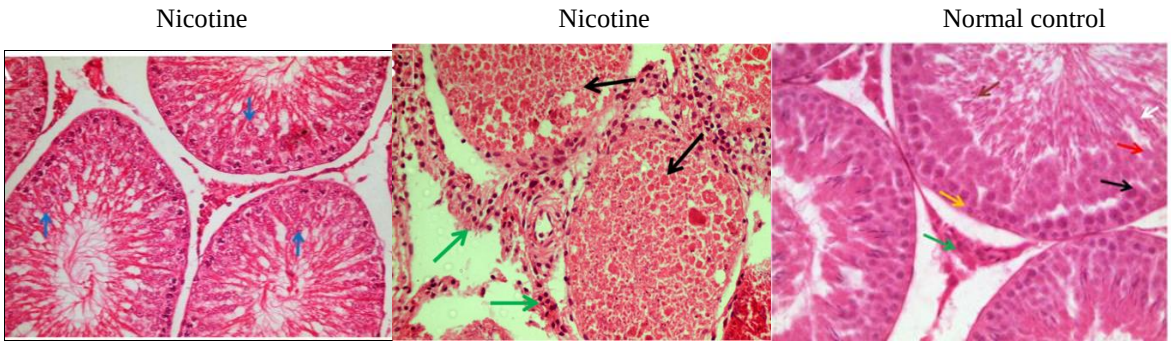

Fig. 1: Effect of nicotine (1 mg/kg) on stages of spermatogenesis.
A photomicrograph of a section in the testis of **normal control** rats showing regular seminiferous tubules with interstitial tissue (green arrow), tubules showed basal spermatogonia (yellow arrow) primary spermatocytes (black arrow), round spermatid (red arrow), elongated spermatid (white arrow) and mature spermatozoa (brown arrow). Tubules of positive control rats (**nicotine**) showed spermatogenic arrest at round spermatid (blue arrows) with deficiency of elongated spermatid and mature spermatozoa (black arrows) the necrotic tubules were separated by non-necrotic interstitial tissue stroma (green arrows). (H&E, x 400).

Table 2: Effect of nicotine and melatonin (5 mg/kg and 10 mg/kg) on TAC, testosterone, FSH, LH, sperm count and percentage of tubules.

Parameter	Normal (Negative control)	Nicotine (positive control)	Nicotine and melatonin (5 mg/kg)	Nicotine and melatonin (10 mg/kg)
Serum total antioxidant capacity (mM/L) Mean ± SD	1.89 ± 0.120	0.41 ± 0.09$^{\#}$	0.88 ± 0.340*	1.13 ± 0.142*
Serum testosterone level (ng/ml) Mean ± SD	6.75 ± 0.890	2.86 ± 1.041$^{\#}$	2.85 ± 0.710	3.73 ± 0.80
Serum FSH level (ng/ml) Mean ± SD	6.15 ± 0.491	5.20 ± 0.821	5.29 ± 0.620	5.24 ± 0.63
Serum LH level (ng/ml) Mean ± SD	5.44 ± 0.370	3.46 ± 0.921$^{\#}$	3.52 ± 0.730	3.42 ± 1.00
Sperm count (millions/ml) Mean ± SD	84.6 x10^{6} ± 9.2 x 10^{6}	26 x 10^{6} ± 7.9 x $10^{6\#}$	42.2 x 10^{6*} ± 3.7 x 10^{6}	47.3 x 10^{6*} ± 5.1 x 10^{6}
Percentage of tubules with mature sperms (%) Mean ± SD	80.7% ± 3.51%	42.5% ± 4.41%$^{\#\#}$	51.7% ± 8.1%	60.3%* ± 3.5%

Data represent mean ± SD of 6 observations. *Significant result at $p < 0.05$ from positive control. $^{\#}$Significant result at $p < 0.05$ from normal control. $^{\#\#}$Highly significant result at $p < 0.01$ from normal control.

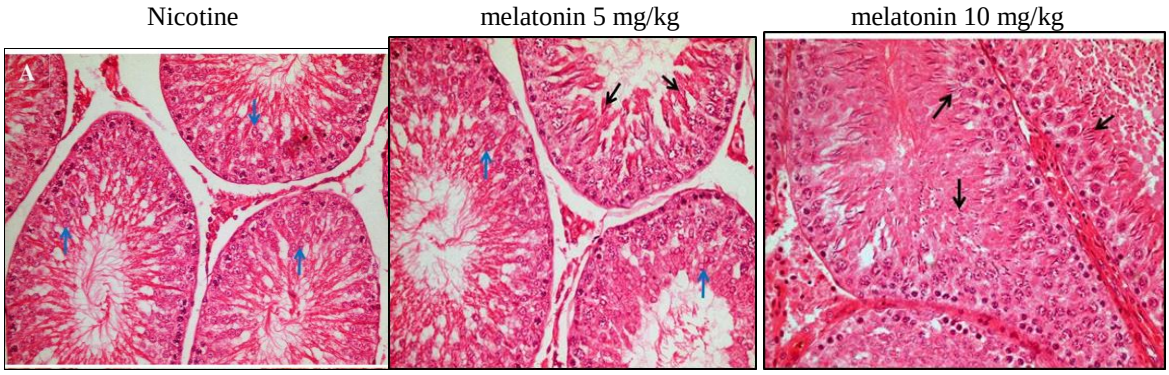

Fig. 2: Effect of nicotine (1 mg/kg) and melatonin (5 mg/kg and 10 mg/kg) on stages of spermatogenesis. Focal spermatogenesis up to level of round spermatids (blue arrows) with focal maturation up to mature spermatozoa (black arrows). (H&E x400).

Table 3: Effect of nicotine and N-acetyl-L-cysteine (100 mg/kg and 200 mg/kg) on TAC, testosterone, FSH, LH, sperm count and percentage of tubules.

Parameters	Normal rats (Negative control)	Nicotine (positive control)	Nicotine and NAC (100 mg/kg)	Nicotine and NAC (200 mg/kg)
Serum total antioxidant capacity (mM/L) Mean ± SD	1.89 ± 0.120	0.41 ± 0.09$^{\#}$	1.03 ± 0.370*	1.14 ± 0.27*
Serum testosterone level (ng/ml) Mean ± SD	6.75 ± 0.890	2.86 ± 1.041$^{\#}$	4.12 ± 0.570	4.36 ± 0.88*
Serum FSH level (ng/ml) Mean ± SD	6.15 ± 0.491	5.20 ± 0.821	5.14 ± 0.600	5.25 ± 0.670
Serum LH level (ng/ml) Mean ± SD	5.44 ± 0.370	3.46 ± 0.921$^{\#}$	3.53 ± 1.160	3.58 ± 1.080
Sperm count (millions/ml) Mean ± SD	84.6 x 10^6 ± 9.2 x 10^6	26 x 10^6 ± 7.9 x $10^{6\#}$	47.5 x 10^{6*} ± 4.3 x 10^6	50.0 x 10^6 ± 5.5 x 10^6
Percentage of tubules with mature sperms (%) Mean ± SD	80.7% ± 3.51%	42.5% ± 4.41%$^{\#\#}$	48.0% ± 8.2%	51.3% ± 11.6%

Data represent mean ± SD of 6 observations. *Significant result at $p< 0.05$ from positive control. $^{\#}$Significant result at $p< 0.05$ from normal control. $^{\#\#}$Highly significant result at $p< 0.01$ from normal control.

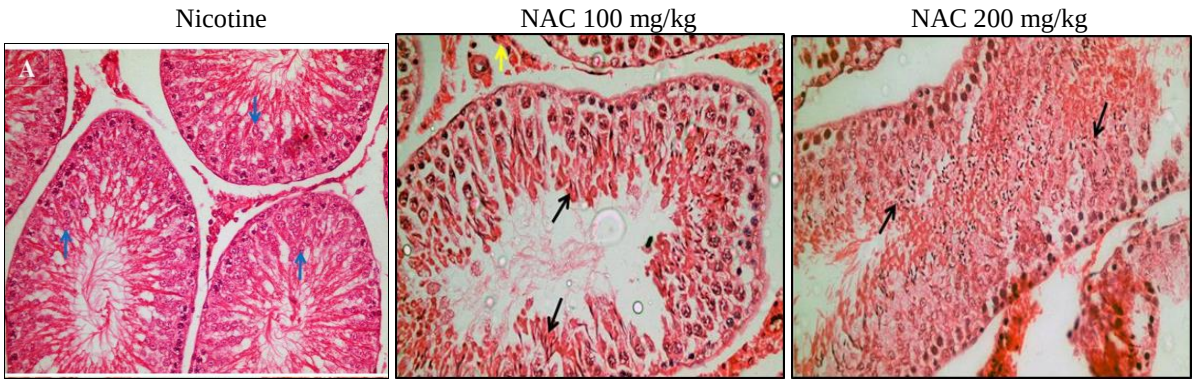

Fig. 3: Effect of nicotine (1 mg/kg) and N-acetyl-L-cysteine (100 mg/kg and 200 mg/kg) on stages of spermatogenesis.

Focal spermatogenesis up to level of round spermatids (blue arrows) with focal maturation up to mature spermatozoa, some tubules showed significant numbers of mature spermatozoa (black arrows). Interstitial leydig cells were demonstrated (yellow arrow); (H&E x400).

Table 4: Effect of combined melatonin and N-acetyl-L-cysteine on TAC, testosterone, FSH, LH, sperm count and percentage of tubules.

Parameter	Normal (Negative control)	Nicotine (positive control)	Nicotine, melatonin (5 mg/kg) and NAC (100 mg/kg)	Nicotine, melatonin (10 mg/kg) and NAC (200 mg/kg)
Total antioxidant capacity (mM/L) Mean ± SD	1.89 ± 0.120	0.41 ± 0.09$^{\#}$	1.16 ± 0.210*	1.20 ± 0.250*
Serum testosterone level (ng/ml) Mean ± SD	6.75 ± 0.890	2.86 ± 1.041$^{\#}$	5.62 ± 1.040*	4.64 ± 0.510*
Serum FSH level (ng/ml) Mean ± SD	6.15 ± 0.491	5.20 ± 0.821	5.29 ± 0.920	5.56 ± 0.580
Serum LH level (ng/ml) Mean ± SD	5.44 ± 0.370	3.46 ± 0.921$^{\#}$	5.57 ± 1.090	4.78 ± 0.510
Sperm count (millions/ml) Mean ± SD	84.6 x 10^6 ± 9.2 x 10^6	26 x 10^6 ± 7.9 x $10^{6\#}$	50.5 x 10^{6*} ± 3.8 x 10^6	50.5 x 10^{6*} ± 3.8 x 10^6
Percentage of tubules with mature sperms (%) Mean ± SD	80.7% ± 3.51%	42.5% ± 4.41% $^{\#\#}$	63.3%* ± 9.8%	64.3%* ± 11.7

Data represent mean ± SD of 6 observations. *Significant result at $p< 0.05$ from positive control. $^{\#}$Significant result at $p<0.05$ from normal control. $^{\#\#}$Highly significant result at $p< 0.01$ from normal control.

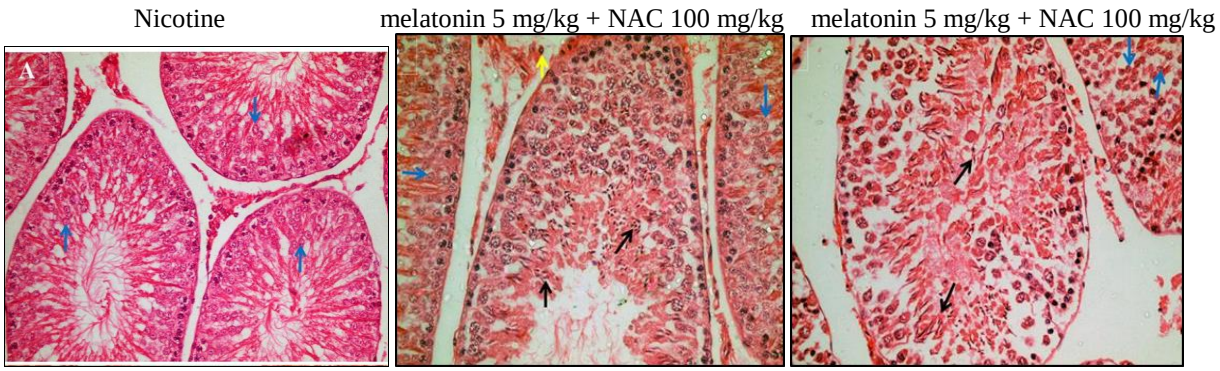

Fig. 4: Effect of combined administration melatonin and N-acetyl-L-cysteine on stages of spermatogenesis. Focal spermatogenesis up to level of round spermatids (blue arrows). Seminiferous tubules showed spermatogenesis up to level of mature spermatozoa (black arrows). Interstitial leydig cells were demonstrated (yellow arrow). (H&E x400).

Table 5: Effect of nicotine withdrawal on TAC, testosterone, FSH, LH, sperm count and percentage of tubules.

Parameter	Normal rats (Negative control)	Nicotine (Positive control)	Nicotine withdrawal
Serum total antioxidant capacity (mM/L) Mean ± SD	1.89 ± 0.120	0.41 ± 0.09$^{\#}$	1.57 ± 0.290*
Serum testosterone level (ng/ml) Mean ± SD	6.75 ± 0.890	2.86 ± 1.041$^{\#}$	5.46 ± 0.490*
Serum FSH level (ng/ml) Mean ± SD	6.15 ± 0.491	5.20 ± 0.821	5.82 ± 0.180
Serum LH level (ng/ml) Mean ± SD	5.44 ± 0.370	3.46 ± 0.921$^{\#}$	4.58 ± 0.790
Sperm count (millions/ml) Mean ± SD	84.6 x10^{6} ± 9.2 x10^{6}	26 x 10^{6} ± 7.9 x $10^{6\#}$	72.33 x 10^{6*} ± 11.2 x 10^{6}
Percentage of tubules with mature sperms (%) Mean ± SD	80.7% ± 3.51%	42.5% ± 4.41%$^{\#\#}$	74.2% ± 4.2%*

Data represent mean ± SD of 6 observations. *Significant result at $p< 0.05$ from positive control. $^{\#}$Significant result at $p< 0.05$ from normal control. $^{\#\#}$Highly significant result at $p< 0.01$ from normal control.

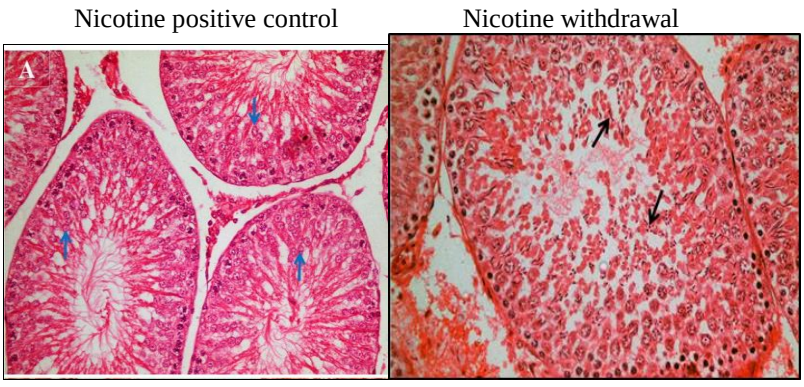

Fig. 5: Effect of nicotine (1 mg/kg) and nicotine withdrawal on stages of spermatogenesis.
Numerous mature spermatozoa were demonstrated in most seminiferous tubules (black arrows), focal spermatogenesis up to level of round spermatids (blue arrows) H&E x400.

DISCUSSION

Cigarette smoke is a major environmental health threat. The main alkaloid that found in cigarettes is nicotine that responsible for variety of biochemical and behavioral changes[16]. Nicotine induces oxidative stress and testis injury[17].

LD_{50} used to measure acute toxicity of drugs. To screen for toxicity in various chemicals and pharmacological agents, LD_{50} evaluation is essential. When evaluating a drug's toxicity, it is important to consider its onset, duration of action, biological effects, side effects, and mortality. Acute toxicity research only provides data on a pharmacological agent's LD_{50}, therapeutic index and level of safety[18]. LD_{50} of nicotine was 14.14 mg/kg, which was consistent with findings of Irena who evaluated LD_{50} after IP injection of nicotine in rats[19].

Nicotine exhibited testicular dysfunction as evidenced by most tested parameters. Nicotine administration induced marked oxidative impact as shown by the significant decrease of serum level of TAC. This finding was in agreement with results of Saad *et al.*[17] and Oyeyipo and his colleagues[20]. Nicotine caused oxidative stress by increasing production of ROS. The production of ROS and the antioxidant system's enzymes must be balanced for parynchematous organs like testes, which have high metabolic activity and cell replication[21]. Testosterone is necessary to keep spermatogenesis function and structure of male accessory sex glands. In this study, there was a significant drop in testosterone levels along with the decreased sperm counts. Other research demonstrated that rats given nicotine had lower serum testosterone levels[22&23] because nicotine induce autophagy in Leydig cells[24]. Our finding observed reduction in serum level of LH. The reduced LH level was concomitant with decreased serum level of testosterone. Luteinizing hormone is the primary regulator of testosterone biosynthesis in Leydig cells, which is required for the hypothalamic-pituitary-testicular axis to maintain testosterone levels. This is in agreement with Bisong and his co-authors[25]. There was a significant reduction of sperm count in positive control rats compared to normal rats. Nicotine administration reduced sperm count, motility, viability and percentage of sperm normal morphology[25&26].

Nicotine induced testicular histopathological changes such as focal detachment of the basal spermatogenic cells from their basement membrane and frequent lack of elongated mature spermatozoa in seminiferous tubules. These histopathological finding are consistent with previous reports, which demonstrated that nicotine exhibited abundant seminiferous tubules with the arrest of spermatogenesis at the spermatocyte stage[25&27]. Previous research revealed that generation of ROS, lipid peroxidation and reduction in testosterone hormone in nicotine treated rats appears to be the main mechanism causing disruption in spermatogenesis[28].

Melatonin in both doses produce significant rise of TAC in treated rats. These data were in accordance with Chabra *et al.*[29] and Xu *et al.*[30]. Their findings supported our findings regarding the protective properties of melatonin, its ability to fight oxidative damage and the elevation of TAC. The current investigation found insignificant impact on the levels of testosterone, FSH, and LH in either dose of melatonin. This negligible effect of melatonin in both doses on serum level of testosterone was due to dependence of melatonin secretion on pituitary secretion of FSH and LH[31]. These negligible effects are consistent with other research reported different effect of melatonin on FSH and LH either no effect, reduction or elevation of pituitary hormones[32-35]. Melatonin in high dose enriched percentages of tubules with mature sperms. While low dose of melatonin produced insignificant alteration. These histopathological improvements of high dose of melatonin were in consistent with Ying and his coworkers[36]. Melatonin restoring histological function in rat testes via reducing oxidative stress in testicular cells[36].

The current study found that NAC in either dose produced significant elevation in TAC. This in agreement with Ahmed and his coauthors[37] who reported that NAC ameliorated oxidative stress and act as powerful antioxidant. Therefore, NAC may restore the disturbance between proxidant and antioxidant mechanisms during oxidative

stress[37]. Low dose of NAC produced negligible alteration in testosterone level. While high dose of NAC elevate testosterone level significantly, this result was in harmony with Abu-Aita and his collages[38]. NAC showed negligible alteration on serum levels of FSH and LH, this insignificant effect due to elevation of testosterone level suppress GnRH level as feedback mechanism[39]. Two different doses of NAC induced significant elevation of the sperm count. Data was in accordance with finding of El-Maddawy and El-Sayed[40]. While treatment with both low and high doses of NAC showed insignificant change of percentages of tubules with mature sperms. Administration of NAC may need long period to produce significant effect and prominent improvement in testicular tissues.

Interestingly, combined administration of melatonin and NAC in low and high doses produced significant increase of serum levels of TAC and testosterone. This elevation was in accordance with other studies[38&41]. Combination of melatonin and NAC produce testicular protective effect through scavenging and neutralizing oxidative radicals and up regulating the activities of some endogenous antioxidants[42&43]. On the other hand, they have no significant effect on LH and FSH. This result agrees with Sharonjeet and Lekha[44]. Combined administration of melatonin and NAC at two different doses induced significant increase of sperm count. These finding in accordance with other researcher that observed antioxidant protective effect of melatonin and NAC to defense this oxidative damage and succeed to elevate sperm counts[42&43]. In addition, there is significant rise of percentages of tubules with mature spermatogenesis.

Recovery of testicular function was observed after nicotine withdrawal for four weeks in the form of significant elevation of TAC and testosterone level. Nicotine withdrawal produced insignificant change in serum FSH and LH level. Hruskovicova and his coauthor[45] findings could support negative effect of nicotine withdrawal on serum level of FSH and LH. They observed insignificant change in serum level of FSH and LH after 6 weeks of smoking cessation, while one year of smoking cessation is associated with decrease in FSH and LH serum levels. On the other hand, other study observed that nicotine cessation succeed to regain FSH and LH level to normal values[23].

In the current study, there was an increase in the epididymal sperm count of rats in the withdrawal group showing that the effect of nicotine on sperm count may be ameliorated by nicotine cessation; these findings go in line with Oyeyipo *et al.*[28]. Nicotine withdrawal induced significant increase of proportions of tubules that exhibited complete spermatogenesis. The percentages of seminiferous tubules with mature sperms increased to a level that is comparable to normal rats. This finding was in agreement with Nesseim and his coauthors[46] finding who observed regeneration of seminiferous tubule damage after nicotine withdrawal in male albino rats.

Conclusion

The obtained results suggest that melatonin and NAC use either alone or in combination counteract nicotine-intoxication and might be efficient in the prevention of testis toxicity complications and histopathological changes induced by nicotine. They could be a potential to improve male fertility. Smoking cessation, it is a safe method and it remains the best choice to avoid testicular dysfunction caused by smoking.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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الميلاتونين وإن–اسيتيل–إل سيستين يحسنان الإجهاد التأكسدي وضعف الإنجاب الناتج عن النيكوتين في ذكور الجرذان

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الخلفية: أصبحت الآثار الضارة الكبيرة للتدخين على الخصوبة والتكاثر واضحة ولكن لم يتم تقديرها بشكل عام.

الهدف من هذه الدراسة: الدراسة الحالية تقيم الدور الوقائي المحتمل للميلاتونين وإن أستيل إل سيستين في مكافحة سمية الخصية للنيكوتين في ذكور الجرذان.

المواد والطرق: قبل إحداث سمية الخصية عن طريق النيكوتين تم تحديد LD_{50} تم استخدام أربعة وخمسون من الجرذان البيضاء. تم استخدام ستة ذكور من الجرذان كمجموعة ضابطة سالبة وإعطاؤها محلول ملح. تم استحداث سمية الخصية في ثمان وأربعين ذكرًا عن طريق الحقن في الغشاء البروتوني (١ ملجم/كجم) من النيكوتين. تم تقسيم الحيوانات الى ثمان مجموعات. تم إعطاء المجموعة الضابطة الإيجابية نيكوتين فقط. المجموعتان الثالثة والرابعة تلقت الميلاتونين في (٥ و ١٠ ملجم / كجم) . المجموعتان الخامسة والسادسة تم إعطاؤها إن استيل إل سيستين في (١٠٠ و ٢٠٠ ملجم / كجم). المجموعتان السابعة والثامنة تم إعطاؤها مزيج من جرعات منخفضة ومرتفعة من الميلاتونين وإن استيل إل سيستين. المجموعة التاسعة تم إعطاؤها نيكوتين وتم سحبه. وقد تم فحص الأنسجة وعدد الحيوانات المنوية في جميع المجموعات.

النتائج: تم حساب LD_{50} من النيكوتين. أظهر النيكوتين انخفاضاً ملحوظاً في إجمالي قدرة مضادات الأكسدة (TAC) ، والتستوستيرون ، ومستوى LH ، وعدد الحيوانات المنوية ولم يكن هناك تغير في مستوى FSH . أظهر الميلاتونين ارتفاعا ملحوظا في القدرة الكلية المضادة للأكسدة، وعدد الحيوانات المنوية ولم تحدث تغيير في مستوي FSH و LH. كما أظهر إن استيل إل سيستين ارتفاعا ملحوظا في القدرة الكلية المضادة للأكسدة، وعدد الحيوانات المنوية في الجرعتين والتستوستيرون في الجرعة العالية. ولم يكن هناك تغير في مستوى FSH و LH. وعند إعطاؤهما معا أظهرت النتائج زيادة في القدرة الكلية المضادة للأكسدة والتستوستيرون وعدد الحيوانات المنوية وتغير طفيف في مستوي FSH

و LH. في مجموعة الانسحاب هناك زيادة في القدرة الكلية المضادة للأكسدة والتستوستيرون وعدد الحيوانات المنوية ولم يحث تغير في مستوي FSH وLH.

في الختام؛ هذه النتائج تشير إلى أن التدخين يسبب ضعف الانجاب. يمكن تحسين هذا الضعف عن طريق إعطاء الميلاتونين أو الأسيتيل- سيستيين منفردين أو جمعهما معا. لكن الإقلاع عن التدخين هو الخيار الأفضل لتجنب الخلل الوظيفي الذي يحدث في الخصية بسبب التدخين واسترجاع ضعف الخصية لأنه وسيلة آمنة.