N-acetyl-L-Cysteine ameliorates oxidative stress and reproductive toxicity induced by nicotine in male rats

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Abstract

Background: The current study assessed the protective role of N-acetyl-L-Cysteine in alleviating the detrimental effect of nicotine on reproductive functions in male rats.

Methods: twenty four adult male albino rats were divided into four groups of six rats. Control group was treated orally with 0.5ml/kg normal saline, nicotine treated group received 1.0 mg/kg of nicotine i.p, N-acetyl-L-Cysteine treated group received 100 mg/kg and 200 mg/kg i.p for four weeks.

Results: Nicotine caused significant reduction in serum level of total antioxidant, testosterone and luteinizing hormone. It also caused a significant reduction in sperm count. There was impairment in testicular histology of rats treated with nicotine. N-acetyl-L-Cysteine improved the reduction in sperm count, hormone levels and testicular alterations observed in nicotine treated rats.

Conclusion: The study shows that nicotine exerts significant deleterious effects on male reproductive system and the concurrent administration of N-acetyl-L-Cysteine ameliorated these detrimental effects.

Key words: Nicotine, N-acetyl-L-Cysteine, Sperm count, FSH, LH, Testosterone and Total antioxidant.

Introduction

Although there are conflicting reports on the adverse effect of cigarette smoking on male fertility, several studies suggested that there is a detrimental effect of smoking on reproductive system. Chronic smoke exposure has been positively correlated to lower spermatozoal concentration, decreased motility and increased abnormal morphology (Grief, 2011). Moreover, cigarette smoking inhibits spermatogenesis and decrease steroidogenesis in men (Mlynarcikova et al., 2005). Tobacco acts as an endocrine disruptor on the male hormone profile, specifically on LH, testosterone and prolactin levels (Blanco et al., 2012).

N –acetyl-L-cysteine (NAC) is a metabolite of the sulphur-containing amino acid cysteine. Currently it is used as an antioxidant and a mucolytic agent and administered orally or by intravenous infusion and can also be inhaled using a nebulizer (Therapeutic Goods Administration, 2008). N–acetyl-L-cysteine has been used as a chelator of heavy metal to protect against oxidative stress and prevent damage to cells. It plays an important role in the production of glutathione, which provides an intracellular defense against oxidative stress, and it participates in the detoxification of many molecules (Ibru Isik et al., 2012). N–acetyl-L-cysteine improves sperm motility and prevents sperm DNA oxidative damage (Walczak-Jedrzejowska et al., 2013)..

Material and methods

Induction of nicotine toxicity: Testicular toxicity was induced in all groups of rats (four groups) except the negative control group by daily i.p administration of nicotine 1mg/kg/rat which dissolved in saline (0.5 ml/rat), nicotine administration was done every day at the same time half an hour (Sarabia et al., 2011) after administration of the protective drugs;

N-acetyl-L-Lcysteine in two different doses (100 mg and 200 mg/kg/rat). Duration of nicotine induction with previously prescribed protective drug was for four weeks for all rats and termination of the experiment was done.

Experimental groups: twenty four male albino rats were divided randomly into four groups; six rats each. They received N-acetyl-L-Lcysteine at different two doses (table1) via i.p and the duration of treatment was four weeks for all groups.

Table (1): Treatment and dosage of the experimental groups

Group	Treatment	Dose
1 (negative control)	- NaCl (0.9%)	- 1ml/kg/day
2 (positive control)	- Nicotine	- 1mg/kg/day
3	- Nicotine	- 1mg/kg/day
	- N-acetyl-L-Lcysteine	- 100 mg/kg/day
4	- Nicotine	- 1mg/kg/day
	- N-acetyl-L-Lcysteine	- 200 mg/kg/day

At the end of the four weeks, all rats were sacrificed by decapitation and blood samples from neck veins were collected in pre-labeled centrifuge tubes. Blood samples were centrifuged at 3500 rpm for 15 minutes. Serum was separated and aliquots were prepared and stored quickly at -80˚c for biochemical analysis and hormonal assay. Diagnostic biochemical kits and Enzyme-linked immunosorbant assay (ELISA) Kits were purchased and have been used according to manufacturer instructions.

Semen extraction and Testicular histopathology: The caudal epididymis of both testes were carefully separated from the testes and sliced manually in 2ml of normal saline in a petri dish. The obtained sperms suspension was diluted at a 1:20 ratio before counting the sperms using Neubauer hemocytometer. The two testes of each animal were dissected and fixed in Bouin`s solution for a minimum of 24 hours. The tissue samples were dehydrated and embedded in Paraffin. Sections of 4µm thickness were prepared, deparafinized in two changes of xylene before rehydration. Then, the sections were washed stained with H&E. Finally, the sections were dehydrated. The testes were examined for presence of tissue damage, morphology of seminiferous tubules and maturity of spermatogenesis. The proportion of tubules harboring spermatogonia, primary spermatocytes and mature spermatids were calculated for each animal.

Results

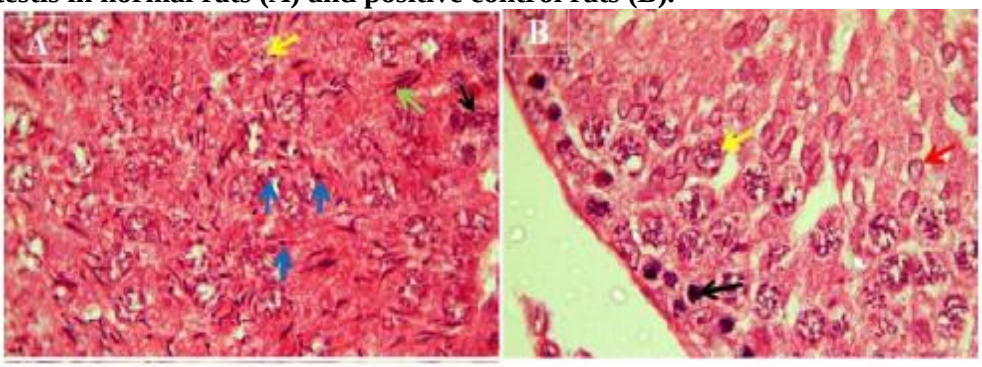
The toxic effect of nicotine was induced by administration of nicotine at a dose of 1mg/kg/day for four weeks via i.p rout. There was a significant reduction in serum total antioxidant level, testosterone, LH and sperm count in positive control rats in comparison to normal rats. On the other hand, nicotine produced insignificant change in serum FSH.

Table (2): Effects of nicotine on serum levels total antioxidant, testosterone, FSH, LH and sperm count

Parameter	Normal rats (Negative control)	Nicotine treated rats (Positive control)
Serum total antioxidant level:		
- Mean ± SD	1.89 ± 0.11	0.40 ± 0.09*
- Median	1.94	0.37
Serum testosterone level:		
- Mean ± SD	6.75 ± 0.88	2.87 ± 1.04*
- Median	6.45	3.04
Serum FSH level:		
- Mean ± SD	6.15 ±0.49	5.20 ± 0.82
- Median	6.11	5.32
Serum LH level:		
- Mean ± SD	5.43 ± 0.37	3.46 ± 0.92*
- Median	5.44	3.16
Sperm count (millions)	$26x10^6$ $\pm7.9x10^6$	$84.6x10^6$ $\pm9.2x10^6$*

Data represent mean ± SD and median of 6 observations. * Significant result at $p<0.05$ from normal control.

Figure (1): Comparison between effect of nicotine on histological sections of testis in normal rats (A) and positive control rats (B).



Tubules of normal rats (**A**) showed stages of spermatogonia (black arrow), primary spermatocytes (yellow arrow), elongated spermatid (green arrows) and mature spermatozoa (blue arrows). Tubules of positive control rats (**B**) showed spermatogenic arrest at round spermatid (red arrow) with deficiency of elongated spermatid and mature spermatozoa (H&E, x 1000 for A and B).

There was focal detachment of the basal spermatogenic cells from their basement membrane. Statistically, nicotine administration induced a highly significant reduction of tubules that have mature spermatozoa compared to normal rats. Minimal focal testicular necrosis was observed in positive control rats.

N-acetyl-L-cysteine: Administration of low dose NAC (100 mg/kg) induced a significant increase of serum antioxidant level (1.03 ± 0.37) when compared to positive control rats (0.40 ± 0.09). While low dose NAC (100 mg/kg) produced insignificant change in serum levels of testosterone, FSH and LH.

Treatment with high dose NAC (200 mg/kg) induced a significant rise of serum antioxidant, testosterone levels (1.14 ± 0.27 and 4.36 ± 0.88, respectively)when compared to positive control rats (0.40 ± 0.09 and 2.86 ± 1.04, respectively), while there was no significant changes of serum levels of FSH or LH after administration of high dose NAC (200 mg/kg). Administration of two different doses of NAC at 100 mg/kg and 200 mg/kg doses induced significant increase of the sperm count $26.2x10^6$ $\pm7.9x10^6$ sperms, $47.5x10^6$ $\pm4.3x10^6$ sperms, respectively when compared to positive control rats $50.0x10^6$ $\pm5.5x10^6$). Treatment with both low and high doses of NAC

(100 mg/kg and 200 mg/kg) showed insignificant change of percentages of tubules with mature spermatogenesis compared to positive control.

Discussion

Smoking has enormous negative health consequences worldwide and it is one of the most significant preventable causes of human morbidity and mortality. The relationship between nicotine and male infertility is still a matter of controversy. The present study was undertaken to re-assay the toxic effect of nicotine on testicular function and the possible protective role of N-acetyl-L-Cysteine. Nicotine administration induced marked oxidative impact as shown by the significant decrease of serum level of antioxidant enzymes. This finding was in agreement with results of Oyeyipo and his colleaguees (2014) who demonstrated reduction of both antioxidant and nitric oxide levels after nicotine administration. They explained their findings by reduction of serum level of SOD that catalyzes the dismutation of superoxide (O_2^-) radical to hydrogen peroxide (H_2O_2). The observed decrease of serum SOD activity may induce reduction of *de novo* synthesis of antioxidant enzymes (Oyeyipo et al., 2014).

Decrease level of testosterone is one of indicators of chemical toxicity in male reproduction (Yoshida et al., 2002) as it is essential to maintain spermatogenesis, structure and function of male accessory sex glands. In this study, the reduced sperm counts were accompanied by a significant decrease of testosterone level. Other studies showed decreased testosterone level after administration of nicotine in rats (Hruskovicova et al., 2013; Oyeyipo et al., 2013). The observed decrease level of testosterone could be due to inhibition of testicular expression of 3 and 17β-hydrosteroid dehydrogenase enzymes as these molecules are the key enzymes for testicular androgenesis (Jana et al., 2010). In the same context, the reduced testosterone level was concomitant with decreased serum level of LH that are necessary for maintaining testosterone level through the hypothalamo-pituitary-testicular axis. The observed reduction of LH level could be due to oxidative stress of rats` brain induced by nicotine. There are several examples of experimental data that supported the adverse effect of nicotine on hypothamic-pituitary-testicular axis (Funabashi et al., 2005; Tweed et al., 2012 and Oyeyipo et al., 2013). In contrast to our findings, other studies observed reduction of serum testosterone level in nicotine treated rats with concomitant elevation of serum level of FSH and LH. They assumed that nicotine has local testicular damaging effects and explained the rising of FSH and LH by compensatory feedback mechanisms after testosterone reduction (Ramlau-Hansen et al., 2007 and Heidary et al., 2012).

In the present study, there was a significant reduction of sperm counts in positive control rats compared to normal rats. Previous study showed that i.p nicotine (0.6 mg/kg) administration for 28 days decreased sperm count, sperm motility and sperm viability while increasing the percentage of sperm with abnormal morphology (Siti et al., 2017). Additionally, these finding was in agreement with Fairuz and his coworkers (2011) who reported that i.p administration of nicotine (5 mg/kg) for two months to Sprague Dawley juvenile rats (5 to 6 weeks old) lowered sperm motility and decreased live sperm and sperm with normal morphology. The observed reduction of sperm counts could be the ability of nicotine to induce oxidative stress

through free radical-mediated lipid peroxidation and protein oxidation in the testes and the prostate (Fairuz et al., 2011). The observed toxic effect of nicotine on sperm count was supported by testicular histopathological findings. Nicotine induced definitive testicular histopathological changes such as focal detachment of the basal spermatogenic cells from their basement membrane and frequent deficiency of elongated mature spermatozoa in somniferous tubules. These histopathological findings were compatible with (Mosbah et al., 2015) who reported that the histological examination of testes revealed atrophy, degenerative change in seminiferous tubules.

N-acetyl-L-cysteine is a potent antioxidant and free-radical scavenging agent that increases intracellular GSH; a major component which protect cells from oxidative stress (Kumamoto et al., 2001). The present study indicate that low and high dose of NAC (100 mg/kg and 200 mg/kg) produced significant elevation in total antioxidant level in positive control rats, these finding was in agreement with Kumamoto et al., 2001, who reported antioxidant property of NAC and its ability to increase antioxidant enzymes. According to (Dodd et al., 2008), NAC ameliorated oxidative stress caused by many proxidant and act as powerful antioxidant that stimulate GSH synthesis. This is confirmed by (Ahmed et al., 2014) finding. N-acetyl-L-cysteine may restore the disturbance between pro-oxidant and antioxidant mechanisms during oxidative stress and that are coincided with our findings.

In the current study, low dose of NAC produced insignificant change in serum level of testosterone. While high dose of NAC (200 mg/kg) elevate testosterone level significantly in comparison to positive control rats, this result was in agreement with (Nashwa et al., 2011). Nashwa and her collages observed significant elevation of testosterone, LH and FSH levels due to concomitant administration of NAC and ginger for 65 days after testicular toxicity induction by ciprofloxacin. Ciprofloxacin generate oxidative damage and NAC have an important role in ameliorating reproductive toxicity through restoring the oxidant-antioxidant balance. In the current study, NAC produced insignificant effect on serum levels of FSH and LH, this insignificant effect can explained by Bu-Min and his coauthors (2001) results which relate between testosterone levels and GnRH hormone; elevation of testosterone levels suppress GNRH levels as feedback mechanism. Possible protective effects of NAC in spermatogenic defects induced by nicotine was evaluated in this study, the two different doses of NAC (100 mg/kg and 200 mg/kg) induced significant elevation of the sperm count compared to positive control rats. These data was in accordance with finding of El-Maddawy and El-Sayed (2018) who observed NAC ability to improve semen quality and its role in elevation of sperm count after testicular dysfunction induced by paracetamol in male albino rats. Additionally, Reddy and his collages (2011) proved protective effect of NAC against arsenic-induced oxidative stress and reproductive toxicity in male mice, Reddy and his coauthors observed elevation of sperm count in arsenic treated rats.

In the present study; treatment with both low and high doses of NAC showed insignificant change statisically of percentages of tubules with mature spermatogenesis when compared to positive control rats. Administration of NAC may need long

period to produce significant effect and prominent improvement in testicular tissues. This explanation proved by other researcher works as Kumar and his coworkers (2013) who scheduled their experiment in three months. Additionally Ahmed et al (2013) demonstrated ameliorative effect of NAC in testicular toxicity caused by nicotine; NAC was administered for three months and at the end of the experiment improve the hisopathological defect of nicotine on testes.

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