Administration of Nicotine Exacerbates the Quinine-induced Structural and Functional Alterations of Testicular Tissue in Adult Rats: An Experimental Study

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Purpose: In this study the role of nicotine (NCT) administration on the intensity of rat testicular tissue alterations induced by quinine (QU) was evaluated.

Materials and Methods: Forty adult Wistar rats were divided into four groups. Control (CON), NCT administrated (4 mg/kg) (NCT), QU treated (25 mg/kg for 7 days) (QU), and nicotine with quinine received (NCT+QU). After 28 days, serum testosterone and malondialdehyde (MDA) levels were measured. Testes and epididymides samples were prepared for determining tissue MDA levels, histomorphometry, microscopic indices of spermatogenesis, immunohistochemistry of p53 and sperm analysis.

Results: Testosterone levels were decreased significantly ($P = .0004$) in treated groups compared to CON group. Serum MDA levels were increased significantly ($P = .0004$) in NCT and QU groups compared to CON group. Tissue MDA levels were increased significantly ($P = .0012$) in NCT+QU group in comparison to CON group. These parameters were changed significantly in NCT+QU group compared to QU group. Seminiferous tubules diameter decreased significantly ($P < .0001$) in treated groups compared to CON group and in NCT+QU group compared to QU group. The height of germinal epithelium decreased significantly ($P = .0001$) in NCT and NCT+QU groups compared to CON and QU groups. The number of Sertoli cells, spermatocytes, and spermatids decreased significantly in treated groups compared to CON group. The number of spermatogonia decreased significantly ($P = .0017$) in NCT and NCT+QU groups compared to CON group. The number of Sertoli cells, spermatogonia, and spermatocytes decreased significantly in NCT+QU group compared to QU group. All indices of spermatogenesis decreased in treated groups compared to CON group. The lowest mean of these indices was observed in NCT+QU group. The sperm viability decreased significantly ($P < .0001$) in treated groups compared to CON group. Sperm count and motility decreased significantly in NCT and NCT+QU groups compared to CON group. All experimental groups showed the over-expression of p53 compared to CON group.

Conclusion: The administration of nicotine could be involved in the exacerbation of testicular tissue alterations related to quinine therapy.

Keywords: nicotine; quinine; male infertility; testicular tissue; toxicity

INTRODUCTION

Complex cellular and endocrine/paracrine procedures regulate the process of spermatogenesis[1]. This process and likewise the androgen synthesis which is completed in testes are susceptible to a wide range of environmental stresses. In this regard, the endogenous and exogenous factors could induce various types of alterations in this process[2]. Testicular toxicity has become a serious concern due to the industrialization of societies and the ever-increasing use of chemicals and drugs[3,4]. Moreover, exposure to different xenobiotics can affect the male reproductive system by making structural and functional changes in testicular tissue.

Malaria is one of the most common parasitic diseases seen worldwide. A wide range of medications is used to treat malaria disease. Quinine (QU) is one of the most common medications used for the treatment of malaria disease. The quinine treatment is associated with side effects such as irreversible deafness, amblyopia, and cinchonism syndrome[5]. Some studies have reported the quinine-induced toxicity including neurotoxicity, cardiotoxicity, and testicular toxicity[6,7]. Histological alterations of the seminiferous tubules, reduction of testosterone levels, a decrease of sperm count and motility, and the changes in lipid peroxidation indices were reported following the administration of QU in experimental animal studies[6,7].

The abuse of narcotics such as opioids and nicotine is common in some regions of the world. It has been well established that these narcotics have side effects on the

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Table 1. The effect of NCT and QU on testes weight, organ relative weight, serum testosterone and the lipid peroxidation levels.

Variables[a]	Control	Nicotine	Quinine	Nicotine+Quinine
Testes weight (g)	2.28 ± 0.23	1.65 ± 0.36b	1.92 ± 0.21	1.43 ± 0.24[bc]
Organ relative weight (%)	0.98 ± 0.12	0.83 ± 0.21	0.90 ± 0.13	0.73 ± 0.11[b]
Testosterone (ng/ml)	0.66 ± 0.039	0.50 ± 0.054[b]	0.56 ± 0.053[b]	0.48 ± 0.044[bc]
Serum MDA (nmol/mg protein)	0.67 ± 0.103	1.01 ± 0.151[b]	0.79 ± 0.144	1.19 ± 0.355[bc]
Tissue MDA (nmol/mg protein)	0.73 ± 0.067	1.04 ± 0.130	0.98 ± 0.275	1.33 ± 0.388[bc]

Abbreviations: MDA, Malondialdehyde.
[a]. Data are expressed as means ± SD. [b]. $P < .05$ compared to the control group. [c]. $P < .05$ compared to the quinine group.

structure and the function of the male reproductive system[2,4,8].

Cigarette smoking a common public health problem, leads to exposure of nicotine (as the important toxic chemical compounds) to various body organs. As well, human and experimental studies revealed the negative effects of nicotine on the male reproductive system[2, 4,9-12].

Nicotine (NCT) is an alkaloid compound that is exposed through different routes including tobacco products and insecticides[9]. The alteration of sexual function and spermatogenesis, reduction of semen quality, and malfunction of the pituitary-testis axis have been reported following the administration of NCT or smoking in experimental and clinical studies[10-12].

It is believed that following the administration of NCT or QU, an increase of lipid peroxidation could induce ROS over production which can interact with cellular lipids and inducing cellular damage[2,6,9,10].

Both nicotine and quinine have gonadotoxic effects on testicular tissue[4,6,7]. Consequently, in this study, the potential role of nicotine administration, as a model of high-risk lifestyle (exposure to gonadotoxic compounds), was evaluated on the proportion of quinine-induced alterations of testicular tissue through an experimental study.

MATERIALS AND METHODS

Chemicals

Nicotine, quinine hydrochloride, EmbryoMax Human Tubal Fluid (HTF medium) and Tris hydrochloride (Tris-HCL) were purchased from Sigma-Aldrich (St Louis, MO 63178, USA). Enzyme immunoassay kit for measurement of serum testosterone was obtained from (Monobind Inc. USA). Primary antibody (rabbit polyclonal anti p53 antibody) was purchased from St John's Laboratory Ltd, UK. Secondary antibody (goat anti-rabbit IgG) was purchased from Agrisera Antibodies, SE-911 21 Vännäs, Sweden.

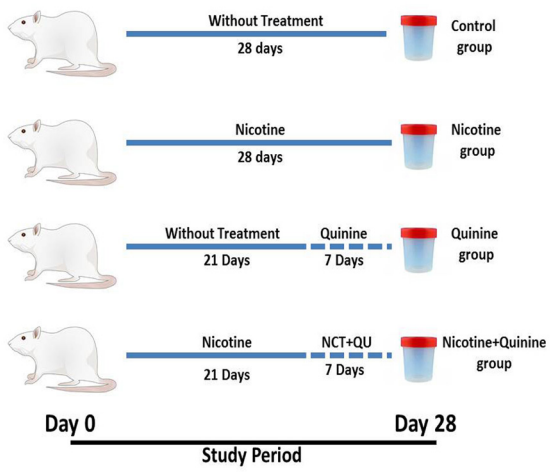

Figure 1. Diagram of animal grouping and time schedule of the administration of nicotine and quinine.

Animals

Forty adult male Wistar rats weighing 180-200 grams were used in this study. The animals were placed in standard cages under controlled temperature (22 ± 2 ºC) and 12 h light/dark cycle. During the period of the experiment, the standard laboratory chow and water were available ad-libitum to animals.

All animals' procedures used in this study were approved by the University of Tabriz standards for care and use of laboratory animals (ethical code: IR.TABRIZU.REC.1398.034), in accordance with the animal ethical committee (AEC) of the ministry of health and medical education of Iran (adopted on April 17, 2006) based on the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) based on the Helsinki Protocol (Helsinki, Finland, 1975).

Study design

The animals were randomly divided into four experimental groups (10 animals per group): control (CON), nicotine-received (NCT), quinine-received (QU), nicotine and quinine received (NCT+QU). In NCT+QU group, the 28-days period of study were divided into two time sections consisted of day 0 to day 21 which only NCT was administrated, and day 22 to day 28 which both the NCT and QU were administrated (**Figure 1**). Quinine hydrochloride was solubilized in distilled water (20 mg/mL as stock treatment solution) and was administered orally (gavage) at a dose of 25 mg/Kg once a day for a period of seven days[6]. Nicotine was administrated at a dose of 4 mg/Kg intraperitoneally once daily for 28 days[13].

Biochemical analysis

Twenty four hours after the final treatment, the animals were anesthetized with xylazine hydrochloride (10 mg/Kg i.p.) and ketamine hydrochloride (100 mg/Kg i.p.). The blood samples were collected through cardiac puncture.

Serum testosterone

The assessment of the serum testosterone levels was carried out through the standard ELISA method with a commercial assay kit (Monobind Inc. USA)[7].

Sampling and preparation

The animals were euthanized through sodium thiopental (100 mg/Kg i.p.). The left and right testicles were separated and dissected from their epididymis and were

Table 2. The effect of NCT and QU on testicular tissue morphometry and the germinal epithelium population.

Variables[a]	Control	Nicotine	Quinine	Nicotine+Quinine
ST Diameter (µm)	276.4 ± 18.11	200.3 ± 27.77[b]	226.0 ± 25.37[b]	177.5 ± 27.18[bc]
GE Height (µm)	124.4 ± 9.81	86.63 ± 16.04[bc]	108.4 ± 13.79	76.88 ± 14.94[bc]
Sertoli cells(# / 20 tubules)	26.33 ± 4.38	17.00 ± 3.00[b]	21.22 ± 3.83[b]	15.67 ± 2.95b[c]
Spermatogonia (# / 20 tubules)	58.63 ± 7.23	43.50 ± 8.40[b]	51.50 ± 4.81	42.13 ± 6.46b[c]
Spermatocyte (# / 20 tubules)	65.88 ± 6.33	47.25 ± 7.18[b]	55.63 ± 5.29[b]	45.00 ± 7.13b[c]
Spermatids (# / 20 tubules)	230.5 ± 26.37	145.6 ± 31.11[b]	164.4 ± 23.78[b]	132.6 ± 20.38[b]

Abbreviations: ST, seminiferous tubule; GE, germinal epithelium.
[a]. Data are expressed as means ± SD. [b]. $P < .05$ compared to the control group. [c]. $P < .05$ compared to the quinine group.

weighed as total testes weight. Moreover, the organ relative weight (organ relative weight = organ weight/ body weight × 100) was recorded. The left testicles were used for histologic studies, and the right testicles were prepared for tissue lipid peroxidation measurement and immunohistochemistry. The sperm analysis was prepared on the left epididymides.

Serum and tissue lipid peroxidation levels

The quantification of the serum and tissue lipid peroxidation was completed by the determination of thiobarbituric acid levels[14]. Testicles samples were homogenized in 50 mM Tris/HCl, pH 7.5 (1/10, w/v) and centrifuged at 3000g for 10 minutes. An aliquot of serum or tissue samples were incubated (95 ºC) for two hours with thiobarbituric acid. The sample coated microplates were analyzed by a microplate reader and the absorbance was measured at 532 nm.

Histology

The testicular tissues were immediately fixed in 10% formaldehyde in buffered solution containing 54 mM NaH2PO4 and 28 mM Na_2HPO_4 (pH 7.4) and kept at 4°C. After 48 hours, the transverse section was made on the middle part of each testis and kept immersed in the fixative solution for the completion of tissue fixation. Then, formaldehyde-fixed samples were embedded in paraffin and sliced with thickness of 6-7 micrometer and were mounted onto albumin-pre-coated glass slides. The mounted tissue samples were deparaffinized with xylene and stained by the Hematoxylin and Eosin method for histological observations by light microscopy (Olympus CX22, Tokyo, Japan).

Histomorphometry of seminiferous tubules

For performing morphometric assessments, 10 microscopic fields (200×) and at least 20 seminiferous tubules (STs) were studied in each section. The measurement of the height of germinal epithelium (GEH) and the diameter of seminiferous tubules (STD) was performed on the images obtained via AmScope digital camera (AmScope MD500). The images were processed by the

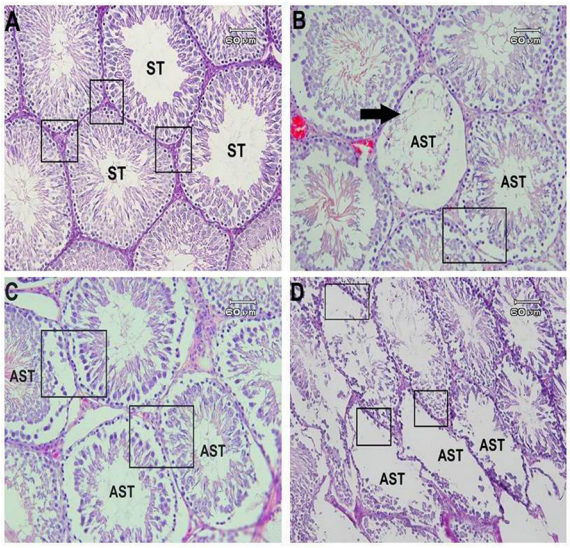

Figure 2. Cross-sections of seminiferous tubules in experimental groups. **(A)** Control group: normal architecture of seminiferous tubules (ST) with consistent arrangement of spermatogenic cells is visible. Narrow interstitial connective tissue (squares) has been situated between seminiferous tubules. **(B)** NCT-administrated group: Atrophied seminiferous tubules (AST) and depletion in height of germinal epithelium (black arrow) are visible. The normal arrangement of germ cells is demolished (square). **(C)** QU-treated group: AST with a decline in the germinal cell population (squares) are evident. **(D)** NCT+QU-treated group: Tubular atrophy and disruption with a prominent reduction of the cellular population (squares) were the noticeable changes. H&E staining. Magnification: ×200

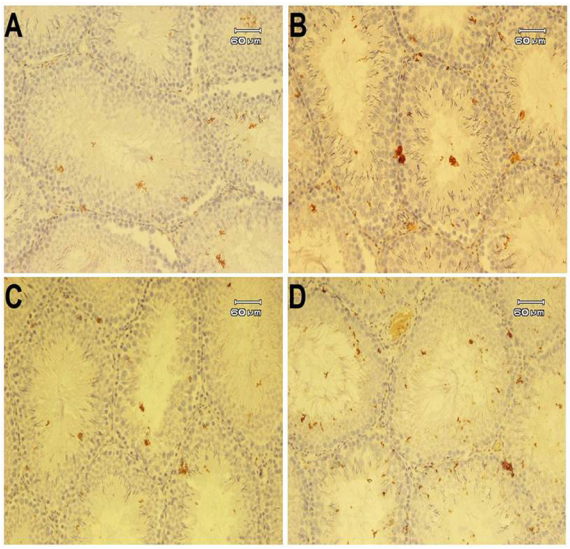

Figure 3. Immunohistochemical staining for p53 expression in testicular tissue. (A) Control group: faint positive reaction immunostaining to p53 expression is visible in seminiferous tubules. (B) NCT-administrated group: The positive reactions increased compare to the control group. (C) QU-treated group: An increase in areas with a positive reaction is visible compare to the control group. The positive reaction is less than NCT-administrated group. (D) NCT+QU-treated group: The positive reaction to p53 expression is higher in comparison to other groups. Magnification: ×200

Table 3. The effect of NCT and QU on the microscopic indices of spermatogenesis and epididymal sperm analysis.

Variables[a]	Control	Nicotine	Quinine	Nicotine+Quinine
Tubular differentiation index (%)	71.63 ± 8.81	56.88 ± 6.05[b]	60.25 ± 9.45	50.75 ± 10.50[b]
Spermiogenesis index (%)	74.75 ± 8.067	49.75 ± 7.83[bc]	62.38 ± 9.63[b]	50.38 ± 8.94[bc]
Repopulation index (%)	70.13 ± 7.60	57.25 ± 8.82[b]	60.63 ± 6.25	46.25 ± 6.69[bcd]
Sperm count (106/mL)	16.63 ± 2.32	12.88 ± 1.72[b]	14.25 ± 2.12	11.50 ± 2.44[b]
Sperm viability (%)	78.50 ± 7.85	58.38 ± 9.53[b]	65.13 ± 5.35[b]	51.75 ± 6.01[bc]
Sperm motility (%)	76.00 ± 7.502	60.25 ± 8.031[b]	66.13 ± 9.387	55.38 ± 5.069[bc]

[a]. Data are expressed as means ± SD. [b]. $P < .05$ compared to the control group. [c]. $P < .05$ compared to the quinine group. [d]. $P < .05$ compared to the nicotine group.

image analysis software (AmScope 3.7).

The population of spermatogenic cells lineage

For quantitative calculating of the populations of the cells, the number of Sertoli cells, the spermatogonia, the spermatocyte, and the round spermatids was counted in every tubule and this process repeated for at least 20 tubules. Then the whole number of counted cells for 20 tubules presented as the mean of the cell population.

Microscopic indices of spermatogenesis

The quantitative investigation of spermatogenesis in testicular tissue was completed by the measurement of three indices: tubular differentiation index (TDI, the number of seminiferous tubules with more than three layers of germinal cells derived from type-A spermatogonia), spermiogenesis index (SPI, the ratio of seminiferous tubules with spermatozoids to the empty tubules) and repopulation index (RI, the ratio of active spermatogonia to inactive cells)[15].

Immunohistochemistry of p53

Paraffin-embedded testicular tissue sections were prepared for immunostaining of p53 protein. Briefly, antigen retrieval was conducted on deparaffinized and rehydrated slides kept in 10 mM sodium citrate solution (pH 6.0) at 95 ºC in a water bath for 40 minutes. Immunohistochemical staining was performed in terms of the manufacturer's protocol (St John's Laboratory Ltd, UK). Briefly, endogenous peroxidase activity was blocked with 0.3% H_2O_2. Tissue slides were washed with phosphate buffered saline solution (PBS) (pH 7.2) and then incubated with rabbit polyclonal anti p53 antibody (as primary antibody) (1:500) at 4 ºC overnight. Sections were treated with horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG (as secondary antibody) (Agrisera Antibodies, SE-911 21 Vännäs, SWEDEN) in 37 ºC incubator humidified chamber container with a wet paper towel for 1 hour. Diaminobenzidine (DAB) chromogen was added to the tissue sections and incubated for 5 minutes. Also, the tissue slides were dehydrated and cover-slipped after hematoxylin counterstaining.

Epididymal sperm analysis

Sperm count

The cauda epididymis was cut into small pieces. The contents of epididymis were diluted by the HTF medium (1:20 v:v). Approximately 10 microliters of the specimen were transferred to a haemocytometer, and were placed for five minutes in the humidified chamber. The number of cells was counted with a light microscope at 400× magnification and was expressed as the sperm concentration (×106/ml)[16].

Sperm motility percentage

One drop of the specimen was placed on an incubated glass slide (37 ºC) and covered with a lamella. The percentage of motile cells was recorded in four different microscopic fields at 400× magnification[17].

Sperm viability percentage

The evaluation of sperm viability was carried out by adding 20 µl of Eosin Y-1% solution and Nigrosin 10% solution into an equal volume of the specimen. The examination was done on the slides were incubated for two minutes at room temperature. The head of dead sperm cells was stained with pink while the head of live cells appeared pale[18].

Statistical analysis

Non-parametric (Kruskal Wallis test) and parametric (one-way ANOVA analysis of variance followed by Tukey's multiple comparison test) methods were performed for the evaluation of differences between experimental groups. All data were expressed as the mean ± SD. Statistical analyses were performed using GraphPad Prism software (version 5.04; GraphPad Inc., CA, USA). The P values < .05 were considered significant statistically between experimental groups.

RESULTS

Weight of testicles

The mean of testicular weight was altered in all treated groups compared to the control group (Table 1). The reduction of testes weight was significant between the control group with the NCT and NCT+QU groups ($P = .0003$). As well, the weight of testes was reduced significantly in NCT+QU group compared to the QU group. In this regard, the organ relative weight was changed in treated groups in comparison to CON group. The reduction of organ relative weight was observed significantly in NCT+QU group ($P = .0222$). Moreover, the administration of the NCT prior and during to the QU therapy led to more decrement of the organ relative weight compared to QU group.

Serum testosterone levels

The blood concentration of testosterone was reduced significantly ($P = .0004$) in all treated groups in comparison to CON group (Table 1). Also, this parameter was reduced significantly in NCT+QU group compared to QU group.

Lipid peroxidation

The levels of serum malondialdehyde (MDA) were increased in all treated groups compared to control group (Table 1). This increase of MDA levels was observed significantly in NCT and NCT+QU groups ($P = .0004$). In this regard, the serum MDA levels were increased significantly in NCT+QU group compared to QU group. Accordingly, the most increase in serum MDA levels was observed in NCT+QU group (**Table 1**). The

measurement of MDA levels in testicular tissue showed the increase of this parameter in all treated groups in comparison to CON group (**Table 1**). However, this increase of tissue MDA levels was significant between NCT+QU group and control group ($P = .0012$). In this way, the MDA levels in testicular tissue were increased significantly in NCT+QU group compared to QU group.

Testicular tissue morphometry

The mean diameter of the seminiferous tubules was reduced significantly ($P < .0001$) in treated groups compared to CON group (**Table 2**). Accordingly, tubular diameter was reduced significantly in NCT+QU group in comparison to QU group. In this regard, the height of the germinal epithelium was decreased in treated groups compared to the control group. This reduction was observed significantly ($P = .0001$) between CON group with NCT and NCT+QU groups as well. Moreover, this index was reduced significantly in NCT and NCT+QU groups compared to quinine treated group.

The population of spermatogenic cells

The mean of the germ cells population was changed in all treated groups in comparison to the control group (**Table 2**). In this regard, the population of the Sertoli cells, spermatocytes and round spermatids was decreased significantly in treated groups ($P = .0001$, $P = .0002$ and $P = .0002$ respectively). The population of spermatogonia was reduced significantly in NCT and NCT+QU groups compared to CON group ($P = .0017$). The administration of NCT before and during the QU therapy led to a significant reduction in the population of the Sertoli cells, spermatocytes and spermatogonia compared to QU group however, the mean of round spermatids was not reduced significantly.

Microscopic indices of spermatogenesis

The mean of all indices of spermatogenesis was reduced in treated groups in comparison to CON group (**Table 3**). This reduction was significant between NCT and NCT+QU groups with CON group for TDI and RI indices ($P = .0016$ and $P = .0003$, respectively) and between all experimental groups with the control group for SPI index ($P = .0002$). The microscopic indices of spermatogenesis were reduced in NCT+QU group compared to QU group. This decrement was observed significantly for SPI and RI and was observed non-significantly for TDI (**Table 3**).

Sperm analysis

The results obtained from epididymal sperm analysis showed that all parameters of sperm analysis were declined in treated groups in comparison to the control group (**Table 3**). This reduction was observed significantly in all treated groups for sperm viability ($P < .0001$) while, sperm count and sperm motility parameters were reduced significantly in NCT and NCT+QU groups compared to CON group ($P = .0024$ and $P = .0010$, respectively). Moreover, treatment of animals with QU after the administration of NCT induced more reduction in sperm analysis parameters compared to QU group. This reduction was recorded significantly for sperm motility and viability between above mentioned groups (**Table 3**).

Histology of testicular tissue

The histologic study showed various alterations in testicular tissue of treated groups in comparison to the control group (**Figure 2**). The atrophy of seminiferous tubules and the loss of tubular architecture, a decrease of the germ cells population, impaired cellular arrangement, and the increase of interstitial connective tissue were the most prominent alterations observed in treated groups. The most prominent changes were observed in NCT and NCT+QU groups as well. The structural changes of testicular tissue were observed in lower degrees in QU group in comparison to NCT+QU group.

Immunohistochemistry of p53

Immunostaining of testicular tissue for detection of the p53 expression showed an increase of the positive reaction areas to p53 expression in treated groups in comparison to the control group (**Figure 3**). In this regard, the expression of p53 was higher in NCT+QU group compared to NCT and QU groups.

DISCUSSION

The results of this study showed that, the administration of nicotine exacerbates the structural and functional alterations of testicular tissue induced by quinine therapy. In the present study, the existence of significant difference in most quantitative and qualitative evaluated parameters between the QU-treated group and the NCT+QU treated group indicates that nicotine could involve in exacerbating of testicular tissue changes following quinine therapy in adult rats.

Based on the results of previous studies and the results of this experiment, it can be established that the main mechanism for tissue alterations following nicotine or quinine administration is increased oxidative stress. So, it can be suggested that the administration of nicotine before and during quinine therapy increases the structural and functional changes in testicular tissue through increasing the amount of ROS and its related cellular and tissue alterations.

Both nicotine and quinine have toxic effects on the male reproductive system[7,13]. It has been known that the administration of nicotine or quinine leads to the over-production of reactive oxygen species (ROS) in the male reproductive system[6,19]. In this regard, oxidative stress-related cellular and tissue damages play a major role in male infertility problems[20]. Over production of ROS could induce various damages in the cellular membrane of spermatozoa which lead to peroxidation of plasma membrane lipids and subsequently cellular structural abnormalities[21]. The results of this study showed the increase of serum and tissue MDA levels (as one of the final products of lipid peroxidation) in all treated groups. Our results are in parallel to the previous reports indicating the increase of ROS in testicular tissue following the administration of nicotine or quinine (2,4,6,7). In this study, it was also found that the most increase in MDA levels occurs in the NCT+QU group. This result indicates that the administration of the NCT prior to QU therapy can induce more tissue damage related to lipid peroxidation compared to individual quinine therapy.

Various studies demonstrated the cellular and tissue alterations of the seminiferous tubules following the administration of nicotine or quinine[6,10,22]. In this study, the results obtained by the histologic and morphometric evaluations showed different changes in the seminiferous tubules in treated groups. These changes included varieties of cellular destruction, impaired tubular architecture and the spermatogenic alterations which are closely related to cell population decline. Testicular tis-

sue and spermatozoa due to theirs considerable amount of lipids are susceptible to cellular lipid peroxidation [7,19]. In the present study the alterations of testicular histomorphometry and the germ cells population were observed in parallel to the changes of MDA levels as a marker of cell and tissue lipid peroxidation and its related cellular damage and cell loss. These results demonstrated that the administration of nicotine before and during the period of quinine therapy could involve in exacerbation of the QU-related changes of the structure and the function of testicular tissue.

This study showed a decrease of the serum testosterone levels in all treated groups compared to control group. Also, this reduction was significant in the NCT+QU group in comparison to the QU-received animals. Testosterone through interaction with the Sertoli cells has an essential role in progressing of the spermatogenesis[23,24]. The reduction of testosterone levels has been noted following the administration of nicotine or quinine[6,22-24]. Decreased testosterone levels may be due to seminiferous tubular damage and subsequently decrease in diameter of tubules[7]. Moreover, one of the possible mechanisms which is involved in the reduction of testosterone levels following the administration of NCT or QU is the raise in the activity of testosterone hydroxylases which result in increased hepatic metabolism of testosterone[25]. However, it has been documented that the elevation of testosterone levels leads to improvement of the function of the reproductive system in metabolic diseases such as diabetes which indicates the positive role of testosterone in the process of spermatogenesis[26]. According to the significant decrease of the Sertoli cells population and testosterone levels in the NCT+QU received group compared to the QU received group, it could be proposed that the administration of the NCT before or during the QU therapy could involve in exacerbation of the structural changes of testicular tissue through diminished spermatogenesis process and subsequent decrease of tubular cellularity. In this way, the microscopic indices of spermatogenesis were reduced more in the NCT+QU received group in comparison to the QU treated group.

Reactive oxygen species (ROS) play a critical role in the normal function of spermatozoa through the stimulation of sperm capacitation, acrosome reaction, and oocyte fusion[27]. Moreover, the functions of sperm due to its high levels of fatty acids and limited DNA repair ability are highly dependent on ROS[28]. In this study, we verified a decrease in the sperm analysis indices in all treated groups. Our results are consistent with the previous studies that have reported a reduction of sperm analysis indices following the administration of quinine or nicotine[6,9]. Moreover, the results of the present study showed a significant decrease in sperm count, sperm viability and sperm motility in the NCT+QU received group. These sperm alterations are in parallel to the changes observed in the serum and tissue MDA levels. Tumor protein p53 is one of the regulatory proteins involved in the cell cycle through the activation of DNA repair proteins or initiation of apoptosis after severe DNA damage. The p53 is often up-regulated after DNA damage resulting in the initiation of apoptosis[29]. In physiological conditions, apoptosis occurs in 20% of developing germ cells. However, the increase of apoptosis could induce some alterations in germ cell lineage and sperm parameters[30]. Consequently, the expression level of this protein may indicate the amount of oxidative stress-induced cellular damage. In this study, the immunostaining of testicular tissue showed that the administration of the NCT before the QU treatment induced more up-regulation of p53 compared to individual quinine therapy. Accordingly, the consistency of the immunohistochemical results with the histomorphometric results indicates that the increase of DNA damage of the germ cells related to oxidative stress induces the cell loss of the germinal epithelium through the induction of p53 up-regulation and the initiation of the apoptotic pathways. The negative effects of common chemotherapeutic medications and xenobiotic toxicants have been reported on the structure and function of the male reproductive system[9-11,13,19, 22,31]. According to the results of these studies, oxidative stress and DNA damage are the main factors which are involved in the structural and functional alterations of the male reproductive system organs. So, the synergistic effects of these gonadotoxic agents in conditions of simultaneous administration should be taken into consideration.

In this study, the mean testicular weight was decreased in the experimental groups compared to the control group. This reduction was observed in more degrees in the NCT-treated and the NCT+QU treated groups. Moreover, this index was reduced significantly in the NCT+QU received group compared to the QU received group. The measurement of absolute and relative organ weight is the important indices in toxicological studies[32]. Moreover, it has been demonstrated that the weight of testes depends on the mass of differentiated spermatogenic cells[33]. According to the results of testicular weight, testes relative weight and histomorphometry, we can suggest that compared to the individual QU therapy, the administration of the NCT prior to the QU therapy could induce more reduction of testicular weight through the exacerbation of the cellular depletion and structural alterations.

CONCLUSIONS

The effects of nicotine on the structure and function of testicular tissue have been demonstrated in various human and animal studies[2,4,9,10]. Moreover, in recent years, experimental studies in animal models have shown the negative effects of quinine on the male reproductive system[6,7]. Our study showed that treatment of rats with quinine hydrochloride in its common therapeutic dose induces structural and functional alterations in testicular tissue. Also, nicotine administration before and during quinine therapy exacerbates these changes. This study is the first report about the effect of nicotine administration on the intensity of testicular tissue alterations induced by quinine. Based on the results of this study, it can be proposed that nicotine may affect the level of quinine associated structural and functional changes of testicular tissue and could be involved in the exacerbation of these alterations. Finally, this subject should be given more attention that the use of xenobiotic gonadotoxic compounds such as nicotine at the time of quinine therapy can exacerbate the adverse effects of this antimalarial drug on the male reproductive system.

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CONFLICTS OF INTEREST

The authors report no conflict of interest.

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