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Assessment of the toxicity effects of nicotine on sperm and IVF and the potential protective role of silymarin—an experimental study in mice

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Abstract

Background: Male infertility is usually caused via the inability to produce adequate quantities of healthy and active sperms. Nicotine (NIC) is an alkaloid organic compound, predominantly found in the leaves of the tobacco plant. The major part of the nicotine is not ionized, so it can easily pass through cell membranes. Meanwhile, most of the disorders are caused by oxidative stress due to oxygen free radicals and other reactive species. Antioxidant supplements and antioxidant-rich foods can reduce induced oxidative stress without becoming destabilized themselves. This study aimed to examine spermato-protective potential of silymarin (SIL), on sperm and in vitro fertility (IVF) rate in nicotine-treated mice.

Results: Our results show a significant increase in the number of abnormal sperm morphology after nicotine exposure, when compared to control groups ($p < 0.05$). On the other hand, SIL had a significant effect on the sperm count at each of the treated doses. Further, in the mice that received nicotine plus silymarin, the viable sperm percentage and the progressive sperm motility were significant ($p < 0.05$). Also, a significant reduction in the number of two-cell embryos and blastocyst-derived embryo was seen with increment in the number of dead embryos in mice receiving nicotine alone ($p < 0.05$).

Conclusions: In conclusion, SIL could support prevention of the adverse reproductive effects of nicotine. Moreover, SIL200 mg/kg may be therefore considered as a spermato-protective agent in dietary and herbal supplements.

Keywords: Fertility, Nicotine, Silymarin , In vitro fertility, Spermatogenesis, Antioxidant

Background

Spermatogenesis is a complex and special process involving a large number of consecutive and regular stages in which germ cells (spermatogonia) undergo proliferation and differentiation, eventually resulting in the production of active sperm [1]. The internal surfaces of seminiferous tubules are covered by spermatogonies with 2 to 3 layers. Sertoli cells, which cover seminiferous tubules, play a pivotal role in the control of germ cell survival, maintenance of spermatogenesis regulate, and release of spermatozoa [2]. Mammalian spermatozoa contain n-3 and n-6 polyunsaturated fatty acids (PUFA), and it has been shown that the head and tail membrane regions of spermatozoa are rich in PUFA. It appears, PUFA are used as a critical nutrients for male reproductive improvement due to alteration in fatty acid composition and maintenance of structure of sperm [3]. The genome of

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spermatozoa can undergo changes and mutation due to endogenous and exogenous agents which, in the case of inappropriate repair, leads to production of morphologically abnormal sperms, reduced spermatozoid (oligospermia), and absence of spermatozoid (azoospermia), culminating in male infertility [4–6].Typically, during spermatogenesis in mammalian testicles, the process of apoptosis of germ cells that occurs spontaneously can act as a normal physiological mechanism followed by abnormal germ cells' elimination, after which finally high-quality sperms are produced [7]. In addition to physiological apoptosis, there are many different factors that can affect the process of apoptosis in germ cells of sperms (spermatogonia).These factors include X-ray radiation, toxic matters, and some medications [8]. Increasing apoptosis in germ cells impairs the balance between proliferation and death of germ cells and leads to disorders in the process of spermatogenesis. An increase in apoptosis seems to be one of the important factors of potential infertility. In most cases, this disorder is associated with factors such as aging, inadequate dietary intake, air pollution, environmental exposure to pesticides, tobacco use, exposure to radiation, spinal damage, genital infectious diseases, orchitis, and drug abuse [9–12]. Different chemical compounds increase production of reactive oxygen species (ROS) as well as free radicals, inducing oxidative stress followed by loss of mitochondrial membrane potential (MMP) as well as increased lipid peroxidation in testicular tissue [5]. Spermatic germ cells are very sensitive to oxidative damage because of having high levels of unsaturated fatty acids and small amounts of cytoplasmic antioxidants [6]. Tobacco use directly causes about 6 million deaths and 600,000 deaths indirectly per year [13]. Cigarettes contain various gases, vaporized liquid, and particles. About 4000 compounds are released from cigarettes during chemical processes: hydration, pyrolysis, oxidation, and decarboxylation [14]. Among 4000 compounds of cigarettes, three matters have the most harmful effects on male fertility including nicotine (NIC), heavy metals, and benzopirin, where nicotine is known as the most dangerous matter and deadly toxin in tobacco that is detectable in both the serum and semen of smokers whose level is directly related to the cigarette use dose [15]. Cotinine is a nicotine metabolite and an alkaloid in tobacco. In a study in vitro, it was found that cotinine and 3HC levels reduce sperm motility [16]. Although nicotine cotinine had no harmful effect on sperm motility at a concentration of less than 1 mM/l, at a high concentration of 1 mM/l, it significantly reduced sperm motility and its survival [17]. Leydig cells located around seminiferous tubules play a crucial role in the generation of testosterone and development of secondary sexual characteristics. It has been reported that nicotine induces Leydig cell apoptosis and inhibits biosynthesis of androgens in mice and can cause male infertility [18]. Nicotine reduces the activity of testicular androgenic enzymes, plasma testosterone concentration, and testicular tissue. It also changes plasma concentration of gonadotropins and level of testicular antioxidants and finally leads to disruption of spermatogenesis process and lack of fertility [19]. Meanwhile, researchers found that various chemical and plant agents have potential antioxidant and anti-inflammatory properties that are used to prevent and reduce the effects of oxidative stress and tissue deterioration [20–23]. Silymarin is a plant flavonoid extracted from Silybum marianum (milk thistle). Silymarin is a mix of flavonolignans which includes silydianin, silychristin, isosilybine A, isosilybine B, isosilychristin, silibinin A, and silibinin B. Silibinin is the major part of silymarin, which has also antioxidative and protective properties [24, 25]. Silymarin, in addition to its anti-inflammatory and anti-fibrotic properties, is a potent antioxidant which is capable of scavenging free radicals ROS, thereby reducing cell toxicity. Also, through inhibiting lipid peroxidation, it prevents progression of cellular degradation and apoptosis [26]. Smoking is a global health concern, with nicotine being the most important compound of cigarette. Although many studies have frequently been reported of the effects of cigarette smoking and nicotine on male fertility in both experimental animals and humans, there is yet no reported case of the protective role of silymarin. Therefore, this study provides the first assessment on the protective effects of potential of Silymarin on sperm parameters including number, motility, morphology, and viability of sperm as well as on in vitro fertility (IVF) rate in nicotine-treated mice.

Methods

Animals handling, ethics and experimental design

To evaluate the adverse impacts of nicotine on reproductive system and the possible protective capability of Silymarin, experiments were performed on sixty male and ten female adult NMRI mice (33.4 ± 3.0 g, 8–12 weeks old and pathogen free). Animals (purchased from the Pasteur Institute, Tehran, Iran) were also housed under standard conditions (23–25 °C temperature, 50–55% humidity, and 12:12 h light–dark cycle) with easy access to water and food. Experiments were conducted in accordance with the guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals.

In this study, to estimate the sample size we used from previous studies and pilot study, at the 95% confidence

level (α = 0.05) for an 80% power (β = 0.2), the required sample size is 8 mice for each group (n = 8). Considering 20% of mice may die during the experiments, so 10 mice were taken in each group (n = 10). Sixty adult male NMRI mice were randomly divided into six groups and each group contained 10 mice and grouped as follows:

Group I (Control group), each mice of this group neither received SIL nor nicotine but received intraperitoneal (IP) 0.2 ml/kg of 0.9% saline water as placebo for 28 consecutive days once daily to examine their morphological, biochemical, and histopathological parameters as the reference comparable values. Group II (Nicotine-treated group), each mouse was treated with 2.5 mg/kg bwt/day of nicotine dissolved in 0.9% saline water via the i.p. route for 28 consecutive days once daily. This group served as nicotine-treated positive control. Group III (SIL100-treated group), each mice was treated with 100 mg/kg bwt/day of SIL dissolved in 0.9% saline water via the i.p. route for 28 consecutive days once daily, without exposure to nicotine. Group IV (SIL200-treated group), each mice was treated with 200 mg/kg bwt/day of SIL dissolved in 0.9% saline water via the i.p. route for 28 consecutive days once daily, without exposure to nicotine. Group V (SIL100 plus Nicotine-treated group), the mice of this group at first were treated with SIL (as in Group-III), and then received nicotine (as in Group-II). Group VI (SIL200 plus Nicotine-treated group), the mice of this group were treated with SIL (as in Group-IV), and then received nicotine (as in Group-II). The effective dose of silymarin and nicotine as well as duration of injection in the groups was determined according to previous studies [27–30].

Sperm parameters evaluation

One day after the last injection, the mice were anesthetized with ether and killed by cervical dislocation. The testicles and epididymal tissues of all the mice were excised from the body of the mice, and the tail section was placed in Ham's F10 culture medium. They were then cut with scissors, incubated at 37 °C for 5 min, and after freeing sperms, they were stained with aniline blue with the percentage of live sperm calculated under a microscope. In order to investigate the sperm morphology, a 20-microliter droplet of sperm suspension was spread on a slide; after drying in the air and once stained with Eosin-Nigrosin (E and N staining), the sperm structure was examined (Fig. 1). Ten slides in each animal were examined, and cases such as abnormal shape of the head, double head, separated head from tail, tail swirling, bending tail, and two tails were considered as abnormal forms. The purpose of evaluation of sperm motility was to determine the percentage of motile sperm and sperm rate. For this purpose, a small drop of the sample was placed on the slide, then examined under the microscope with a magnification of × 400. In order to determine the percentage of motility of sperm, first, the percentage of sperm motility was estimated in a few microscope fields, after which, their mean was recorded as the percentage of mobility. Several microscopic fields were examined to determine the sperm rate. In order to evaluate the number of sperms, sperms were diluted at 1:9 normal saline 3% to make the sperm completely immobilized for counting. Then, a drop of the above solution was transferred slowly onto Neobar slide using a micropipette and covered with a slide. After 5 min for sperm deposition, 40 sperms with head, mid-piece, and tail were counted through magnification [31, 32].

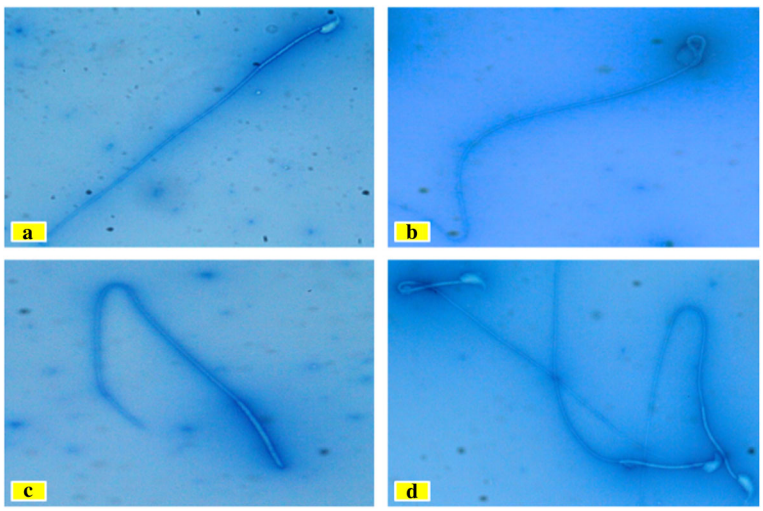

Fig. 1 Evaluation of sperm morphology profiles by using aniline blue staining. **a** Healthy sperm. **b** Sperm with curvature medial anomalies. **c** Sperm with amorphous malformations (without head). **d** Sperm with curvature tail abnormality

Sperm collection for IVF

The testis, epididymis, and left ventricular ducts of male mice killed by cervical swirling were isolated and transferred to Petri dishes containing HTF culture medium.

Stimulation of ovulation (superovulation) and collection of oocytes

The hormonal stimulus was used for inducing reproduction and evolution of oocytes as previously described [11].Briefly, peritoneal 5 international units (IU) of the pregnant mare's serum gonadotropin (PMSG) was injected. Forty-eight hours after the administration of PMSG, 5 units of the human chorionic gonadotropin (hCG) was injected. The female mice, after 12 h of HCG injection, were anesthetized and killed by cervical dislocation; then, oocytes are aspirated and collected from uterine tubules of both sides and transferred to the human tubal fluid (HTF) culture medium. Finally, hundred oocytes were obtained from the female mice.

The in vitro fertilization process

The IVF process was performed as previously described [11]. Briefly, a total of 5 μl of sperm was added to oocyte-containing drops. Approximately 5 h after adding sperms, the embryos were transferred to Petri dishes. For the quantitative evaluation of IVF process, 24 h after insemination, the number of two-cell embryos were counted and recorded using stereomicroscope. Embryo assessment was followed, until the embryos had reached the blastocyst stage of development.

Data analysis

The statistical analyses were done using the SPSS software (Version 18; SPSS Inc., Chicago, USA). All data were expressed as mean values ± standard deviation (SD). Significant differences among various groups were performed by one-way analysis of variance (ANOVA) using Tukey's test. The *P* value 5% or lower was considered statistically significant.

Results

Sperm concentration

The results revealed that injection of nicotine (Group II) significantly reduced the number of sperms compared with Group I (control) (43 ± 1.52 and 58.5 ± 1.75, *P* = 0.000). The number of sperms in Group V (receiving nicotine plus 100 mg/kg of silymarin) increased compared to Group II but was not statistically significant (*P* = 0.12) (Table 1). The results suggested that increasing silymarin dose to 200 mg/kg (Group VI) had a significant effect on the rise in the number of sperms compared to Group II (51 ± 1.28 and 43.1 ± 1.52, *P* = 0.009).

Sperm mobility

Grade 1 (Immotile): The study results indicated that nicotine injection (Group II) significantly increased the percentage of immotile sperm (16.5 ± 1.17 and 7.6 ± 1.05, *P* = 0.000). In Group VI, following administration of 200 ml of silymarin, the number of immotile sperms dropped compared to Group II (10.8 ± 0.75 and 16.5 ± 1.17; *P* = 0.000). Grade II (In situ): The results showed that injection of nicotine (Group II) significantly augmented the percentage of in situ sperm motility (17.6 ± 1.1 and 9.2 ± 0.85, *P* = 0.000, respectively). Although the results revealed that 100 mg of silymarin (Group V) do not significantly change the percentage of sperm in this grade compared to Group II (P = 0.99), 200 mg (Group VI) lowered sperm motility in relation to Group II (*P* = 0.004) and Group V (*P* = 0.018). Grade 3 (Progressive): The study results indicated that injection of nicotine (group II) compared to Group I significantly reduced the percentage of sperms in this grade (*P* = 0.000). The results exhibited that injection of 200 mg of silymarin increased progression of sperm both in healthy mice (Group IV) (*P* = 0.043) and in nicotine-receiving mice (Group VI) as compared to Group II (*P* = 0.035) (Fig. 2). The study results indicated that nicotine injection (Group II) significantly reduced the percentage of living sperms compared to Group I (control) (*P* = 0.000) (Table 2). The results also suggested that two doses of silymarin had no significant effect on improving the quality of living sperms in either healthy mice (Groups III and IV) or those exposed to nicotine (Groups V and VI). The most important results in the sperm morphology were as follows (Fig. 1):

Sub-group I: Head abnormalities: The results showed that injection of nicotine into Group II mice significantly increased the percentage of sperms with abnormal morphology of the head compared to Group I (control) (*P* = 0.000). Administration of 200 mg of silymarin in

Table 1 Quantitative comparison of sperm count in study groups

Sperm parameter						
Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Sperm count	58.5 ± 1.75	43 ± 1.52[a]	59.6 ± 1.55	63.7 ± 1.88	48.7 ± 1.41	51 ± 1.28[b]

Group 1, Control group; Group 2, Nicotine group; Group 3, Silymarin 100 mg group; Group 4, Silymarin 200 mg group; Group 5, Silymarin (100 mg/kg) plus Nicotine (2.5 mg/kg); and Group 6, Silymarin (200 mg/kg) plus Nicotine (2.5 mg/kg). Sperm count × 10^6 per mL

Values are means ± SD (*n* = 10)

[a] *P* < 0.001 compared to control group (G1)

[b] *P* < 0.05 compared to nicotine group (G2), using ANOVA followed by Tukey-Kramer as post ANOVA test

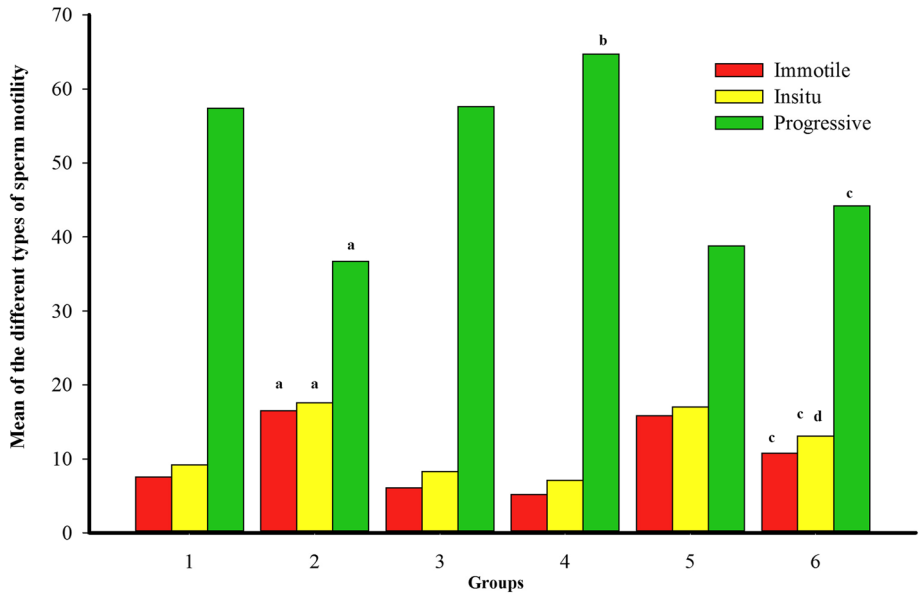

Fig. 2 Comparison of various types of sperm motility grades in study groups. Data show the mean ± SD (n = 10). [a]$P < 0.001$ and [b]$P < 0.05$ as compared to normal control group, respectively, [c]$P < 0.05$ as compared to nicotine-treated group (Group 2) and [d]$P < 0.05$ as compared to 5 group

Group IV significantly reduced sperms with abnormal head compared to Group I though it was not statistically significant (P = 0.068). In addition, 200 mg of silymarin resulted in a significant reduction in sperms with an abnormal head in Group VI compared to Group II (P = 0.001). Sub-group II: Mid-piece defects: It was observed that injection of nicotine (Group II) led to a significant elevation in the percentage of this type of morphology compared to Group I (P = 0.000). Further, 200 mg of silymarin resulted in a significant reduction in sperms in the abnormal mid-piece in Group VI compared to Group II (P = 0.003) (Table 2).

Sub-group III: Faulty sperm: The results showed that the percentage of sperms with defective tail morphology in Group II was significantly higher than in Group I (control) (P = 0.000). Injecting 200 mg of silymarin reduced the defective tail sperms in healthy mice (Group IV) compared to Group I (P = 0.028). Further, the number of mice receiving nicotine (Group VI) also declined (P = 0.035) compared to Group II (P = 0.000) and Group V (P = 0.001) (Table 2).

IVF finding

The results presented that injection of nicotine significantly decrease the number of two-cell embryos and increased the number of dead embryos compared to Group I (control) 1 day after IVF (P = 0.000 and P = 0.000, respectively). It was also found that the number of blastocyst embryos in Group II was lower than in Group I (P = 0.000) (Fig. 3). Administrating 200 mg of silymarin

Table 2 Comparison of quantitative sperm viability and morphology of sperm in study groups

Groups	Sperm viability	Sperm morphology		
		Head abnormalities	Mid piece defects	Faulty tail
G1; (Control)	72.9 ± 0.54	6.4 ± 0.63	6.3 ± 0.42	7.3 ± 0.55
G2; (Nicotine)	48.1 ± 1.11[a]	12.6 ± 1.24 [a]	12.7 ± 1.06 [a]	21.8 ± 1.06 [a]
G3; (SIL 100)	73.3 ± 1.4	5.5 ± 0.58	5.2 ± 0.62	5.8 ± 0.55
G4; (SIL 200)	74.2 ± 1.63	3.3 ± 0.51	3.7 ± 0.63	3.6 ± 0.7 [b]
G5;(SIL 100 plus Nicotine)	49 ± 1.22	10.4 ± 0.81	11.3 ± 0.83	18.4 ± 0.99
G6; (SIL 200 plus Nicotine)	50.7 ± 1.7	7.9 ± 0.62 [c]	8.8 ± 0.38 [c]	13.2 ± 0.91 [c,d]

Values are means ± SD (n = 10)
[a]$P < 0.001$
[b]$P < 0.05$ compared to control group (G1)
[c]$P < 0.05$ compared to nicotine group (G2)
[d]$P < 0.05$ compared to SIL 100 plus nicotine group (G5), using ANOVA followed by Tukey-Kramer as post ANOVA test

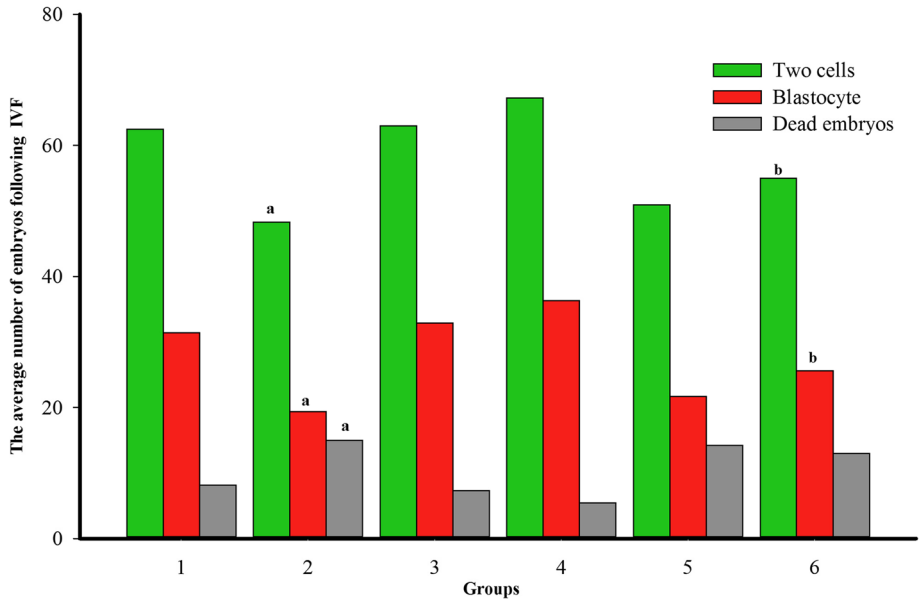

Fig. 3 Effect of silymarin and nicotine alone or in combination on the number of embryos and fertility in study groups. Data show the mean ± SD (n = 10). [a]$P < 0.001$ as compared to normal control group. [b]$P < 0.05$ as compared to nicotine-treated group

in Group IV raised the number of two-cell embryos compared to Group I; however, this increase was not significant (P = 0.063). In addition, in comparison with Group II, administrating 200 mg of silymarin in Group VI not only causes a significant increase in the number of two-cell embryos (P = 0.003), but also leads to considerable development of cellular evolution and formation of blastocyst embryos (P = 0.01) (Fig. 3).

Discussion

Tobacco use is an important global health issue. Exposure to tobacco smoke not only have both direct and indirect effects on the health of human but also affects all stages of human reproduction and development [33].Tobacco contains more than 4000 compounds, out of which at least 70 compounds have been identified as carcinogenic [15].According to statistics, approximately 46% of men aged 20–39 years smoke, and the actual use of cigarettes increased from 721 million cigarettes in 1980 to 967 million cigarettes in 2012 [34]. Tobacco smoking increases the risk of infertility in both male and female [33].There is significant evidence that tobacco use during paternal preconception can transfer reproductive system disorders called intergenerational effects through germ cells [35, 36]. Studies have shown that administration of nicotine in rats causes an increase in Basal layer collagen fibers, destruction of Sertoli cell intercellular connections, chromosomal abnormalities, degenerative germ cells, excessive accumulation of fat droplets in the sperm cytoplasm, and morphological abnormalities in the sperm [37]. Meanwhile, nicotine toxicity may be mediated through changes in collagen fibers that will prevent proper sperm release from germinal epithelium into middle cavity of spermicidal tubes [37, 38]. Also, studies have suggested that administration of nicotine in addition to reducing sperm number, motility, and survival can damage the cell membrane and sperm DNA and increase sperm production with abnormal morphology and finally induce apoptosis in testicular cells [39–41]. Our research indicated that exposing mice to nicotine, led to decrease percentage of viable sperm as well as an increase in the number of sperms with abnormal morphology. Similarly, in a study by Condorelli et al., nicotine led to fragmentation of testicular cell DNA, which is correlated with a reduction in the number of living spermatozoa and mature spermatozoa apoptosis [42]. Furthermore, according to a report, it was found that nicotine adversely affected spermatogenesis of mice in a dose- and time-dependent fashion. In this regard, nicotine at 1 ng/ml concentration suppresses progressive sperm motility, resulting in degenerative changes in seminiferous tubules at high concentration than 100 ng/ml, followed by tissue complications such as reduced thickness of the mass of spermatogenic cells. However, with nicotine removal, complications are reversible. As with nicotine discontinuation, regeneration of damaged seminiferous tubules was reported [43]. According to our results, nicotine also reduced the number of two-cell plus blastocyst embryos, and raised the number of dead embryos after IVF process. In this regard, a study by Sofikitis et al. showed that spermatozoa exposure to nicotine reduced sperm motility, changed

hypoosmotic swelling test, and impaired sperm penetration in the ovum. In response to these detrimental effects, the function of sperm membrane is disrupted leading to the absence of sperm-oocyte binding and finally no embryo formation [16]. Nicotine can also affect production of enzymes. In a report, it was found that daily administration of 0.6 mg/kg of nicotine for 28 days reduced sperm number, motility, cell survival, and increased abnormal changes in sperm morphology. In that study, malondialdehyde production level also increased [44]. In addition, nicotine can also affect the structure of testicles. Aydos et al. examined nicotine toxicity on the ultrastructure of the testis of mice and showed that administration of nicotine for 3 months resulted in thickening of Tunica Propria, increased collagen fibers, loss of intercellular connections in Sertoli cells, and chromatin deficiency [37]. Elshal et al. showed that the percentage of DNA fragmentation (DFI) in infertile male smokers is higher than in infertile male non-smokers. Smoking also lowers the level of supra oxide dismutase and antioxidant enzymes [45]. The results of this study showed that peritoneal injection of silymarin increased the number of motile sperm and reduced abnormal sperm, which was dose dependent. According to the results of previous studies on the compounds and active ingredients in silymarin, it can be confirmed that one of the main reasons for this phenomenon is the diverse and rich antioxidant compounds of silymarin; by inhibiting and removing free radicals, it protects spermatozoa process against oxidative damage. All macro-molecules in an organism body, proteins, nucleic acids, carbohydrates, and lipids are subject to oxidative damage. Normally, there is a balance between the concentration of reactive oxygen components and antioxidant cleansing interaction in the male genital system. Polyunsaturated fatty acids and phospholipids are the main components of the sperm membrane which are highly vulnerable to oxidation. The production of large amounts of ROS by immature sperm can overcome the antioxidant defense mechanisms of sperm and seminal fluid resulting in damage to mature sperms (i.e., lipid peroxidation of the plasmid membrane) finally culminating in their degradation [5, 6]. A large number of phytochemical compounds have been detected and characterized from various parts of the plant. Among which is more important include the following: flavonoids, alkaloids, phenols, saponins, tannins, steroids, and terpenoids. These compounds have biological and pharmacological properties. In particular, it has been demonstrated that phenolic and flavonoid compounds acts as scavenging free radicals [46, 47]. According to various human and animal studies, silymarin, as a natural antioxidant, possesses antioxidant and chemo-protective effects as well as tissue regenerative properties. Silymarin can act in several ways: (1) the direct scavenging of free radicals; (2) preventing the formation of free radicals by inhibiting the specific enzymes producing free radicals and/or preserve the integrity of the mitochondria electron transport chain during various stress conditions; (3) activating a range of antioxidant enzymes such as GST, GSH-Px, GR, SOD, and CAT as well as non-enzymatic antioxidants, generally through different transcription factors (TFs) including NF-kB-Nrf2 pathway; (4) the activation of vitagenes involved in the synthesis of protective proteins such as HSP, thioredoxin (Trx), and sirtuins [24].. Therefore, silymarin can be used as a supplement thanks to its chemoprotectant and radioprotectant properties to protect sperm cells against harmful chemical and physical factors. Further, reports suggest that silymarin, as a radioprotective, can reduce and even improve the adverse effects of radiation [48, 49].It has also been shown that silymarin is a sperm protective agent against sodium arsenite toxicity, and it is able to improve various parameters of sperm such as motility, viability, and mitochondrial membrane potential of sperm [50]. In our study, the mice that only received nicotine showed a significant increase in immotile sperms and in situ sperms coupled with a significant decrease in the number of progressive sperms. In contrast, in the group receiving silymarin, a reduction was observed in the number of immotile sperms. Further, high doses of silymarin lowered the number of immotile sperms in the mice receiving nicotine. Therefore, administrating high doses of silymarin can increase the progressive sperm even in healthy mice, and improve the number of sperms in the mice receiving nicotine. Meanwhile, the results showed that in the mice receiving nicotine, the number of two-cell and blastocyst embryos diminished while the number of dead embryos increased. In contrast, the group receiving a high dose of silymarin experienced a significant increase in the number of two-cell and blastocyst embryos after IVF. Almost similar to our study, Moosavifar et al. (2006) showed that granulosa cell apoptosis in the group receiving 210 mg of silymarin significantly improved follicular development and enhanced fertility in IVF process [51]. Similarly, in our study, the number of two-cell and blastocyst embryos increased in Group VI after administration of 200 mg of silymarin. Consequently, silymarin, as a potent antioxidant, has been able to modulate the effects of nicotine and can be used as a protective compound in smokers and addictive people to improve sperm parameters and enhance reproductive potential.

Conclusion

In conclusion, our results could justify the factors affecting the transformation and degradation of sperm cells, as well as the evolution of embryos for IVF by nicotine-

exposed sperm. Because the testicles are susceptible to absorption with smoking cigarette, the prolonged exposure to it causes alteration in the morphology of spermatogenic cells, sperm production, and spermatogenesis disorder. On the other hand, silymarin, as a potent antioxidant, has the ability of modulating the effects of nicotine and can be used as a dietary supplement ingredient for those who use tobacco or its derivatives, or even those who do not smoke but are exposed to second-hand cigarette smoke. Our findings suggested that silymarin may prevent cellular damage of nicotine-induced toxicity and can be useful as a novel medication in complementary medicine in improving male fertility. Overall, this research tried to offer a better understanding on the cellular mechanism of spermatoprotective and chemopreventative properties for silymarin.

Abbreviations

SIL: Silymarin; IVF: In vitro fertility; ROS: Reactive oxygen species; PMSG: Pregnant mare's serum gonadotropin; hCG: Human chorionic gonadotropin; HTF: Human tubal fluid; GST: Glutathione s-transferases; GSH-Px: Glutathione peroxidase; GR: Glutathione reductase; SOD: Superoxide dismutase; CAT: Catalase; HSP: Heat shock proteins; Trx: thioredoxin; NF-kB: nuclear factor kappa b; Nrf2: nuclear factor (erythroid-derived 2)-like 2

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Authors' contributions

AR and MM: designed experiments, and produced the manuscript. MRM performed the experiments, AH and RA analyzed and interpreted results. MSM is a contributor in writing the manuscript. All authors have read, corrected, and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

This research was approved by the Shahrekord University of Medical Sciences with the code of ethics IR.SKUMS.REC.1396.32.

Consent for publication

All authors are aware of and agree for publication of the manuscript.

Competing interests

The authors declare no competing financial interest.

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