Ameliorative Impact of Silymarin on the Male Reproductive System: 
An Updated Systematic Review

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Abstract

Background and objectives: Numerous studies have evaluated the effects of silymarin on sperm quality and its neutralization impact of various toxins on the male reproductive system. However, these studies as a whole have not been summarized and categorized yet. Silymarin is a flavonoid and known as a powerful antioxidant compound in the treatment of many diseases including liver disorders, rhinitis, diabetes, and testis disorders. The study aimed to discuss the impact of silymarin on the male reproductive system.

Material and Methods: From Apr 1998 to Feb 2020, related articles were extracted from databases of Web of Science (WOS), PubMed, Google Scholar, Science Direct, Scopus, EBSCO, and grey literature by seeking MeSH words including Silymarin, Milk thistle, Silybum marianum, testis, Spermatogenesis, and Sex hormones.

Results: Silymarin withholds damage to the testicular germinal epithelium and comforts the spermatogenesis process by amplification the antioxidant system, decreasing lipid peroxidation and oxidative stress, and preventing the expression of pro-apoptotic genes, increasing testosterone and gonadotropins.

Conclusion: In outcome, based on the results, silymarin can boost fertility in sterility males by its talented antioxidant features.

Keywords: Silymarin [MeSH], Spermatogenesis [MeSH], Gonadal Steroid Hormones [MeSH], Testis [MeSH]

DOI: 10.29252/jorjanibiomedj.10.1.10
Introduction

Silybum marianum is widely used in the treatment of many disorders (Fig 1) (1). Silymarin (C25H22O10), a flavonoid and polyphenolic molecule, is extracted from the seeds of Silybum marianum (Fig 2) (1, 2, 3). Silymarin can be effective in many including infertility in men and women (4). In addition to having other biological properties, these compounds are recognized as antioxidants (5, 6). In 1959, silybin was discovered as the first member of a new family of natural compounds called flavonolignans and was known as the active and dominant compound in silymarin (1, 7, 8). Many studies have shown the protective and antioxidant properties of silymarin against the side effects of chemotherapy drugs and environmental toxins on sperm (9-12).

Oxidative stress is one of the important factors in male reproductive system disorders. Also, the most common way to deal with oxidative stress is to use antioxidants today. Hence, the present review intended to contribute a summary of prior research regarding silymarin antioxidant role in male reproduction disorder carried out.

Materials and Methods

Inclusion criteria in the present study included the evaluation of spermatogenesis, testicular tissue, blood hormones, and enzymes from Apr 1998 to Feb 2020. The electronic search was performed on the databases of WOS, PubMed, Science Direct, Scopus, EBSCO, and grey literature. Finally, the Google Scholar Database was used to ensure complete content and this study lasted
from Jan 2021 to Jul 2021. The inclusion criteria of this study were contained andrological studies (spermatogenesis, spermiogenesis, and sperm parameters), histological and morphometrical studies (testicular tissue), and endocrinological studies (sex hormone). The present study was performed using the words MeSH including Silymarin, Milk thistle, Silybum marianum, testis, spermatogenesis, and sex hormones. The search results of the databases involved a total of 264 articles. The number of these articles was reduced to 78 after passing the identification, screening, and eligibility stages. Exclusion criteria included invalid journals, duplication, and lack of access to the full text (Fig 3). The steps related to searching for articles, results, and extracting data were performed independently by two researchers and in case of differences in each of the steps, they were reviewed by a third researcher. A search syntax was developed by using: Search ((("silymarin"[MeSH Terms] OR silymarin [Text Word]) OR ("milk thistle"[MeSH Terms] OR Milk thistle [Text Word])) OR ("milk thistle"[MeSH Terms] OR Silybum marianum [Text Word])) AND ("testis" [MeSH Terms] OR testis [Text Word])) OR ("spermatogenesis"[MeSH Terms] OR Spermatogenesis [Text Word])) AND ("gonadal steroid hormones"[MeSH Terms] OR sex hormones [Text Word]).

**Fig.3.** Preferred Reporting Items for Systematic Analysis (PRISMA) diagram: the flow of information through the different phases of a Systematic Review

**Results**

Increased ROS during oxidative stress is known to impair oxidant-antioxidant balance and contribute to the peroxidation of unsaturated fatty acids in the sperm membrane and disorder in spermatogenesis and sperm function (16, 17, 31). The quantity and quality of spermatogenesis are determined based on the evaluation of parameters such as count, motility, viability, DNA damage, normal morphology, and population of Sertoli and spermatogenic cells (32-34). Numerous studies
have shown that silymarin compensates for the integrity of plasma membranes and acrosomes, motility, viability, and sperm DNA fragmentation (4, 30-38).

Malondialdehyde (MDA) levels are considered an indicator of lipid peroxidase activity and the end product of lipid peroxidation (39). Studies have shown that silymarin increases total antioxidant capacity and decrease MDA (4, 36, 40, 41). Silymarin due to its antioxidant properties can prevent lipid peroxidation of cell membranes and maintain sperm membrane integrity (26).

Silymarin can also increase testosterone levels, which in turn promotes sperm health and maintains cell division (3, 42). It has been shown that silibinin may improve germinal epithelium function and spermatogenesis by preventing oxidative stress (3).

Moreover, silymarin as a potent antioxidant can eliminate free radicals including ROS, and subsequently reduce DNA fragmentation (1, 14). Research has shown that silymarin increases Bcl2 gene expression and decreases Bax and caspase 3 expression, thereby reducing apoptosis in testicular tissue (43).

Transcription factor E2F1 is one of the factors involved in the apoptosis, count, and viability of sperm. Silymarin can reduce E2F1 expression and thus prevents overstimulation of apoptosis in sperm cells (41) (Table 1).

Sperm are vulnerable to ROS at different stages of spermatogenesis (25). Several internal and external factors can interact with lipids and proteins to cause oxidative stress and ROS production following that occur lipid peroxidation, DNA fragmentation, raise superoxide ions in mitochondria, decreased antioxidant activity, and ultimately spermatogenesis disorder (46).

Numerous studies have shown that low testosterone levels reduce testicular function (32). Moreover, increased apoptosis is one of the results of oxidative stress that reduces motility and count of sperm (25, 46). Silymarin increases testicular weight, epithelial height, spermatogenesis, and total antioxidant capacity, and decreases MDA, apoptosis, and edema (3, 47-53).

Numerous studies have shown that silymarin is a powerful antioxidant that can protect testicular tissue from the adverse effects of oxidative stress (3, 54). Table 2 evaluates and compares the effects of silymarin on testicular tissue (Table 2).

Many studies have illustrated that silymarin can increase LH, FSH, and GnRH hormones (43, 47, 55-57). Testosterone is important in the starting and continuing of spermatogenesis and its reduction contributes to defects in spermatogenesis (58). Silymarin can also increase testosterone levels, which in turn increases spermatogenesis (47, 56, 59). Moreover, silymarin can regulate serum levels of LH, FSH, inhibin B, and testosterone against toxins, and vis-a-vis can reduce lipid peroxidation and MDA (60-62).

### Table 1. Evaluation of the effect of silymarin on men and different species of animals (Spermatogenesis)

<table>
<thead>
<tr>
<th>Author, year (ref)</th>
<th>Species</th>
<th>Type of response</th>
<th>Dose of SM &amp; Duration of treatment</th>
<th>Mot</th>
<th>Abn</th>
<th>Cou</th>
<th>Via</th>
<th>Other Parameters</th>
<th>T &amp; D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aghashahi et al., 2020 (9)</td>
<td>Human</td>
<td>1 μM – 180 min in vitro</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Acrosome and plasma membrane integrity, ↑ total antioxidant capacity, ↓ MDA</td>
<td>Aluminum</td>
<td></td>
</tr>
<tr>
<td>Aghashahi et al., 2020 (9)</td>
<td>Human</td>
<td>1 μM – 180 min in vitro</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Acrosome and plasma membrane integrity, ↑ TAC, ↓ MDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etemadi et al., 2020 (10)</td>
<td>Human</td>
<td>2 μM-180 min</td>
<td>↓ SDF, ↑ nucleus diameter, ↑ MMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cadmium</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Species/Organism</td>
<td>Treatment</td>
<td>Duration</td>
<td>Effects</td>
<td>Chemical or Condition</td>
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<tr>
<td>Rahimi-Madisch et al., 2020 (11)</td>
<td>NMRI mice</td>
<td>100 and 200 mg/kg – 28 days</td>
<td>↑ ↓ ↑ ↑</td>
<td>↑ Immotile sperm, ↑ Progressive motility, ↑ testes weight, ↓ SDF</td>
<td>Nicotine</td>
<td></td>
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<tr>
<td>Fatehi et al., 2017 (30)</td>
<td>NMRI mice</td>
<td>50 mg/kg – 7 days</td>
<td>↑ ↓ ↑ ↑</td>
<td>↑ Acrosome and plasma membrane integrity, ↓ SDF</td>
<td>γ-ray</td>
<td></td>
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<td></td>
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<tr>
<td>Fatehi et al., 2017 (30)</td>
<td>NMRI mice</td>
<td>50 mg/kg – 7 days</td>
<td>↑ ↓ ↑ ↑</td>
<td>↑ Acrosome integrity</td>
<td>Sodium arsenite</td>
<td></td>
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<tr>
<td>Ali Mehr &amp; Parisough, 2016 (35)</td>
<td>Taleshi rat</td>
<td>0, 50, 100, 150 and 200 µg/ml – 72 hours \textit{in vitro}</td>
<td>↑</td>
<td>↑ Acrosome and plasma membrane integrity, ↓ MDA</td>
<td>Sodium arsenite</td>
<td></td>
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<tr>
<td>Ziaeirad et al., 2015 (36)</td>
<td>Rooster</td>
<td>100 µg/mL – 48, 72 hours</td>
<td>↑</td>
<td>↑ ↓ MDA</td>
<td>Sodium arsenite</td>
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<tr>
<td>Eskandari et al., 2017 (37)</td>
<td>Ram</td>
<td>20 µM – 180 min \textit{in vitro}</td>
<td>↓</td>
<td>↑ SDF</td>
<td>Sodium arsenite</td>
<td></td>
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<tr>
<td>Eskandari et al., 2016 (38)</td>
<td>Ram</td>
<td>20 µM – 180 min</td>
<td>↑</td>
<td>↑ Acrosome integrity</td>
<td>Sodium arsenite</td>
<td></td>
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<tr>
<td>Shafici-Roudbari et al., 2017 (41)</td>
<td>Wistar rats</td>
<td>50 mg/kg – 10 days</td>
<td>↑ ↓</td>
<td>↓ Sperm DNA fragmentation, ↓ nitric oxide, ↑ TAC</td>
<td>Doxorubicin</td>
<td></td>
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<tr>
<td>Heidari Khoei et al., 2018 (43)</td>
<td>Wistar rats</td>
<td>100, mg/kg - 200 mg/kg - 5 weeks</td>
<td>↑ ↑ ↑</td>
<td>↓ SDF, ↑ Normal sperm morphology (non-significant)</td>
<td>Diabetic</td>
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<tr>
<td>Abo El-atta et al., 2020 (47)</td>
<td>SD rat</td>
<td>200 mg/kg – 30 days</td>
<td>↑</td>
<td>↑ ↑</td>
<td>Malathion</td>
<td></td>
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<tr>
<td>Hamid et al., 2016 (48)</td>
<td>Albino rat</td>
<td>150 mg/kg - 35 days</td>
<td>↑</td>
<td>↑ ↑</td>
<td>CCl₄</td>
<td></td>
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<tr>
<td>Yaman et al., 2018 (50)</td>
<td>Wistar albino rats</td>
<td>200 mg/kg – 6 weeks</td>
<td>↑ ↓</td>
<td>↑ ↓ MDA, ↑ Spermatogenesis</td>
<td>Methotrexate</td>
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<tr>
<td>Khalil, 2002 (51)</td>
<td>Albino rat</td>
<td>151.2 mg/kg – 1 month</td>
<td>↑</td>
<td>↑ Spermatogenesis</td>
<td>Methotrexate</td>
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<tr>
<td>Chen et al., 2015 (53)</td>
<td>ICR mice</td>
<td>5, 10, 20 mg/mL – 120 min \textit{in vitro}</td>
<td>↓</td>
<td>↓ Glucose-activated sperm motility, ↓ VAP &amp; VCL</td>
<td>High glucose (HG)</td>
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<tr>
<td>Abedi et al., 2016 (55)</td>
<td>Wistar rats</td>
<td>150 mg/kg – 28 days</td>
<td>↑</td>
<td>↑</td>
<td>Spermatids, ↑ spermatozoa cells</td>
<td>Methotrexate</td>
<td></td>
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<tr>
<td>Attia et al., 2017 (56)</td>
<td>Rabbit bucks</td>
<td>5 and 10 g/kg – 8 weeks</td>
<td>↑</td>
<td>↑ ↑</td>
<td>CCl₄</td>
<td></td>
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</tr>
<tr>
<td>Al-Moziel MS et al., 2020 (57)</td>
<td>Albino rat</td>
<td>100 mg/kg – 30 days</td>
<td>↑ ↓ ↑ ↑</td>
<td>↑ Spermatogenesis</td>
<td>Tadalafil</td>
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<tr>
<td>Sahreen et al., 2013 (59)</td>
<td>SD rat</td>
<td>50 mg/kg – twice a week for eight weeks</td>
<td>↓</td>
<td>↓ SDF</td>
<td>CCl₄</td>
<td></td>
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<tr>
<td>Malekinejad et al., 2012 (61)</td>
<td>Wistar rat</td>
<td>50 mg/kg – 4 weeks</td>
<td>↓</td>
<td>↓ SDF, ↓ carbonyl stress</td>
<td>Doxorubicin</td>
<td></td>
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<tr>
<td>Eskandari et al., 2016 (66)</td>
<td>Ram</td>
<td>20 µM – 180 min \textit{in vitro}</td>
<td>↑</td>
<td>↑ Intact mitochondrial membrane</td>
<td>Sodium arsenate</td>
<td></td>
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<tr>
<td>Choobineh et al., 2018 (67)</td>
<td>Ram</td>
<td>0.05, 0.1 and 0.15 mM – 180 min \textit{in vitro}</td>
<td>↓</td>
<td>↓ SDF, ↓ apoptosis</td>
<td>Lithium chloride</td>
<td></td>
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</tr>
<tr>
<td>Choobineh et al., 2018 (68)</td>
<td>Ram</td>
<td>0.1 and 0.15 mM – 180 min \textit{in vitro}</td>
<td>↑</td>
<td>↑ Acrosome integrity</td>
<td>Lithium chloride</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Momeni et al., 2018 (69)</td>
<td>Ram</td>
<td>0.5 µM – 180 min \textit{in vitro}</td>
<td>↑</td>
<td>↑ Intact mitochondrial membrane</td>
<td>Aluminum</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Table 2. Evaluation of the effect of silymarin on men and different species of animals (testicular tissue)

<table>
<thead>
<tr>
<th>Author, year (ref)</th>
<th>Species</th>
<th>Type of Response</th>
<th>Dose of SM &amp; Duration of treatment</th>
<th>T &amp; D</th>
<th>Oxidative stress &amp; Apoptosis</th>
<th>Histology, Testicular biochemistry &amp; PCR</th>
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<tbody>
<tr>
<td>Moshtagheion et al., 2013 (40)</td>
<td>Wistar rat</td>
<td></td>
<td>50 mg/kg - 42 days</td>
<td>Varicocele</td>
<td>↓ Ap, ↓ OS</td>
<td>↓ MDA, Maintain TTM, ↓ E2H, ↓ dissociated germinal epithelium in the seminiferous tubules, ↑ negative TDI, ↓ edema of connective tissue, ↑ Number of Leydig cells&amp;Sertoli cells, ↑ percentage of seminiferous tubules and spermiogenesis indices</td>
</tr>
<tr>
<td>Shafeie-Roudbari et al., 2017 (41)</td>
<td>Wistar rats</td>
<td></td>
<td>50 mg/kg – 10 days</td>
<td>Doxorubicin</td>
<td>↓ Ap, ↓ OS</td>
<td>↓ NO, ↓ E2F1 expression, ↑ TAC</td>
</tr>
<tr>
<td>Heidari Khoei et al., 2018 (43)</td>
<td>Wistar rats</td>
<td></td>
<td>100, 200 mg/kg – 5 wk</td>
<td>Diabetic</td>
<td>↓ Ap, ↓ OS</td>
<td>↓ MDA, ↑ SOD, ↑ CAT (non-significant), ↓ LPO, ↑ Bcl-2, ↓ Bax, ↓ Caspase-3</td>
</tr>
<tr>
<td>Abo El-atta et al., 2020 (47)</td>
<td>SD rat</td>
<td></td>
<td>200 mg/kg – 30 days</td>
<td>Malathion</td>
<td>↑ Spermatogenesis, ↓ necrotic seminiferous tubules, ↑ Testicular weight</td>
<td></td>
</tr>
<tr>
<td>Hamid et al., 2018 (48)</td>
<td>Albino rat</td>
<td></td>
<td>150 mg/kg – 35 days</td>
<td>CCl4</td>
<td>↓ OS</td>
<td>↑ Testis weight, the tail of the sperm are thin and the sperm offspring are similar to the normal state</td>
</tr>
<tr>
<td>Sheweita SA et al., 2016 (49)</td>
<td>Rat</td>
<td></td>
<td>25 mg/kg –1 wk</td>
<td>B[a]P</td>
<td>↑ CAT, ↑ GPx, ↑ SOD, ↓ TBARS, ↑ edema, ↓ necrotic seminiferous tubule, ↑ 17 HSD activity</td>
<td></td>
</tr>
<tr>
<td>Yaman et al., 2018 (50)</td>
<td>Wistar albino rats</td>
<td></td>
<td>200 mg/kg – 6 wks</td>
<td>Methotrexate</td>
<td>↓ OS</td>
<td>↓ Atrophy &amp; degeneration of germinal cells, ↓ Spermatogenesis, ↓ pathomorphological changes in the spermatogonia, ↓ interstitial edema, ↑ SOD2, ↑ GPx1, ↑ CAT</td>
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<tr>
<td>Yaman et al., 2018 (50)</td>
<td>Wistar albino rats</td>
<td></td>
<td>200 mg/kg – 6 wks</td>
<td></td>
<td>↓ OS</td>
<td>↑ GPx1, ↑ CAT, ↓ MDA</td>
</tr>
<tr>
<td>Khalil, 2002 (51)</td>
<td>Albino rat</td>
<td></td>
<td>151.2 mg/kg – 1 month</td>
<td></td>
<td></td>
<td>↑ Spermatogenesis</td>
</tr>
<tr>
<td>Çeribas et al., 2020 (52)</td>
<td>Japanese quail chicks</td>
<td></td>
<td>Diet including 1% MTS – 35 days</td>
<td>High energy diets (HED)</td>
<td>↓ OS</td>
<td>↑ Round spermatid &amp; Elongated spermatid, ↑ sperm count, ↓ MDA</td>
</tr>
<tr>
<td>El-Hady A et al., 2015 (54)</td>
<td>Albino rat</td>
<td></td>
<td>18 mg/Kg - 3 times/week for 2 months</td>
<td>Mobile phone radiation</td>
<td>↓ OS</td>
<td>↑ Number of Leydig cells, ↑ seminiferous tubules diameter, ↑ total protein, ↑ DNA content of the nuclei of spermatogenic cells and Leydig cells, ↓ collagen fibers</td>
</tr>
<tr>
<td>Sahreen et al., 2013 (59)</td>
<td>SD rat</td>
<td></td>
<td>50 mg/kg – twice a week for 8 wk</td>
<td>CCl4</td>
<td>↓ OS</td>
<td>↑ TBARS &amp; H2O2, ↑ SOD, ↑ CAT, ↑ GSH, ↑ GST, ↑ GPx, ↓ abnormality of germinative epithelium, ↓ sperm with abnormal shape</td>
</tr>
</tbody>
</table>
Table 3. Evaluation of the effect of silymarin on men and different species of animals (Endocrinology and Blood biochemistry)

<table>
<thead>
<tr>
<th>Author, year (ref)</th>
<th>Species</th>
<th>Type of Response</th>
<th>Dose of SM &amp; Duration of treatment</th>
<th>Endocrinology</th>
<th>Blood biochemistry</th>
<th>T &amp; D</th>
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</thead>
<tbody>
<tr>
<td>Heidari Khoei et al., 2019 (90)</td>
<td>Wistar rats</td>
<td>↑ T</td>
<td>100 and 200 mg/kg - 5 wk</td>
<td>↑ T, ↑ LH, estradiol not changed</td>
<td>Diabetic</td>
<td></td>
</tr>
<tr>
<td>Abo El-atta et al., 2020 (47)</td>
<td>SD rat</td>
<td>↑ FSH, ↑ LH</td>
<td>200 mg/kg – 30 days</td>
<td>↑ FSH, ↑ LH, ↑ T</td>
<td>↑ BuChE, ↑ GSH, ↑ CAT, ↑ TAC, ↑ SOD, ↑ GPx, ↓ MDA</td>
<td>Malathion</td>
</tr>
<tr>
<td>Khalil, 2002 (51)</td>
<td>Albino rat</td>
<td>↑ T, ↑ LH, estradiol not changed</td>
<td>151.2 mg/kg – 1 month</td>
<td>↑ T, ↑ LH, estradiol not changed</td>
<td>Malathion</td>
<td></td>
</tr>
<tr>
<td>Abedi et al., 2016 (55)</td>
<td>Wistar rat</td>
<td>↑ FSH, ↑ LH, ↑ T, ↑ GnRH</td>
<td>150 mg/kg – 28 days</td>
<td>↑ FSH, ↑ LH, ↑ T, ↑ GnRH</td>
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</tr>
<tr>
<td>Atta et al., 2017 (56)</td>
<td>Rabbit bucks</td>
<td>↑ T</td>
<td>5 and 10 g/kg - 8 wk</td>
<td>↑ T, ↑ ALT, ↑ TAC (non-significant)</td>
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<td></td>
</tr>
<tr>
<td>Al-Moziel MS et al., 2020 (57)</td>
<td>Albino rat</td>
<td>↑ FSH, ↑ LH, ↑ T</td>
<td>100 mg/kg – 30 days</td>
<td>↑ FSH, ↑ LH, ↑ T</td>
<td>Tadalafil</td>
<td></td>
</tr>
<tr>
<td>Sahreen et al., 2013 (59)</td>
<td>SD rat</td>
<td>↑ FSH, ↑ LH, ↑ IB</td>
<td>50 mg/kg - twice a week for 8 wk</td>
<td>↑ FSH, ↑ LH, ↓ Estradiol, ↓ Prolactin</td>
<td>CCl4</td>
<td></td>
</tr>
<tr>
<td>Faraji et al., 2018 (60)</td>
<td>NMRI mice</td>
<td>↑ T</td>
<td>100 mg/kg – 24 hrs</td>
<td>↑ T</td>
<td>MDA, ↑ GSH, ↑ CAT, ↑ SOD, ↑ GPx</td>
<td>Cadmium chloride</td>
</tr>
<tr>
<td>Malekinejad et al., 2012 (61)</td>
<td>Wistar rat</td>
<td>↑ FSH, ↑ LH, ↑ IB</td>
<td>50 mg/kg – 4 wk</td>
<td>↑ipepididymal sperm, ↓ abnormal sperm rate, ↓ normal sperm rate, ↓ Interstitial edema, ↓ Depletion of the seminiferous tubules, ↓ c-myc expression</td>
<td></td>
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</tr>
</tbody>
</table>

SM: Silymarin; NIMRI: Naval Medical Research Institute; SD: Sprague-Dawley; Ap: Apoptosis; OS: Oxidative Stress; CAT: Catalase; SOD: Superoxide dismutase; BPA: Bisphenol A; B[a]P: benzo[a]pyrene; LPO: Lipid peroxidation; TAC: Total antioxidant capacity; GSH-Px: Glutathione peroxidase; MDA: Malondialdehyde; GST: Glutathione S Transferase; ROS: Reactive oxygen species; T & D: Against Toxin & Diseases; AGE: Advanced glycation end products; TBARS: Thiobarbituric Acid Reactive Substances; H2O2: Hydrogen peroxide; HSD: 17β hydroxysteroid dehydrogenase; NO: Nitric oxide; TDI: tubular differentiation index; Gpx: Glutathione peroxidase; ↑: Increase or Improve; ↓: Decrease, (Comparison in the toxin/disease group with Silymarin + toxin/disease group).
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<table>
<thead>
<tr>
<th>Rafiee et al., 2016 (62)</th>
<th>Wistar rat</th>
<th>50 mg/kg – 28 days</th>
<th>↑ T</th>
<th>CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ali Shah et al., 2017 (71)</td>
<td>SD rat</td>
<td>200 mg/kg – 60 days</td>
<td>↑ T</td>
<td>CCl₄</td>
</tr>
<tr>
<td>Zahra Z et al., 2020 (74)</td>
<td>SD rat</td>
<td>200 mg/kg – 30 days</td>
<td>↑ FSH, ↑ LH, ↑ T</td>
<td>BPA</td>
</tr>
</tbody>
</table>

SM: Silymarin; NIMRI: Naval Medical Research Institute; SD: Sprague-Dawley; T: Testosterone; MDA: Malondialdehyde; CAT: Catalase; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase; TAC: Total antioxidant capacity; LH: Luteinizing hormone; GnRH: Gonadotropin-releasing hormone; FSH: Follicle-stimulating hormone; ALT: Alanine aminotransferase activity; BPA: Bisphenol A; IB: Inhibin B; BuChE: butyryl Cholinesterase; ↑: Increase or Improve; ↓: Decrease; T&D: Against Toxin & Diseases, (Comparison in the toxin/disease group with Silimarin + toxin/disease group).

**Discussion**

The present study was performed to investigate the antioxidant effect of silymarin in the male reproductive system that has been exposed to toxins and environmental pollutants.

Spermatogenesis is a coordinated, orderly, long, and complex process that takes place in the germinal epithelium (28, 29). On the other hand, seminiferous tubules are very sensitive to endogenous and exogenous stresses and exposure of the testicle to such conditions affects the somatic cells or germ cells at various stages of differentiation and leads to temporary or permanent irreversible infertility (30).

Many studies based on human and animal exposure to environmental toxins showed the negative impact of toxins on sperm quality and quantity (63). These toxins may also damage the DNA of sperm (63-65). Although silymarin therapy has an effective role in the improvement of sperm-related parameters and fertility against various toxins (68, 69, 71, 72). Research has shown that silymarin increases the concentration of norepinephrine. Norepinephrine is one of the factors that could influence the hypothalamus-pituitary-testis axis, and it increases GnRH and gonadotropins (LH and FSH hormones) through the synthesis of nitric oxide (48, 55). There is a relationship between the concentration of LH and the number of spermatogenic cells (48). LH by binding to Leydig cell increases the secretion of testosterone. Testosterone is an important factor in the spermatogenesis process (55). Also, FSH by binding to the Sertoli cell able to increase the concentration of ABP (androgen binding protein) and ABP can increase the concentration of testosterone in the seminiferous tubule to promote spermatogenesis (8, 55). On the other hand, silymarin can illustrate its antioxidant properties in 5 ways, such as 1. Direct scavenging of free radicals, 2. Preventing the formation of free radicals by inhibiting the specific enzymes responsible for their production of them, or by maintaining the integrity of the mitochondrial electron transfer chain, 3. by participating in maintaining the optimal redox conditions (oxidation and reduction) of the cell by activating a range of antioxidant enzymes and non-enzymatic antioxidants, 4. Activation of protective genes, responsible for the synthesis of protective molecules, including HSP (Heat shock proteins) and thioredoxin, 5. Reducing inflammatory responses by inhibiting NF-κB (Nuclear Factor-κB) pathways (22, 23).

Silymarin with its antioxidant effect can counteract the effects of various toxins such as sodium arsenite (66), lithium chloride (67, 68), aluminum (69, 70), tetrachloride Carbon (48, 71), Trp-P-2 and IQ (72), malathion (47), doxorubicin (61), acetate (73), methotrexate (50), nicotine (6), bisphenol A (74), busulfan (75) and cadmium (5, 60, 76). Silymarin also exerts similar beneficial effects on the side effects of diseases such as diabetes and varicocele (40, 43).

Spermatogenesis is a dynamic and controlled process that involves spermatogonia proliferation, meiotic divisions of spermatocytes, and differentiation of spermatids into sperm (44).

Sertoli cells and the height of the germinal epithelium regulate spermatogenesis by providing structural and nutritional support to the germ cells. Sertoli cells also control germ cell populations through apoptotic pathways (39, 45).
In addition to the physiological apoptosis of germ cells that occurs continuously throughout life, external disorders such as radiation or exposure to toxic substances increase the apoptosis (39).

Besides, silymarin can be considered a promising plant protection agent in complementary medicine that may act an important role in protecting spermatocytes against the potential effects of freezing damage and γ-rays (30, 77, 78).

Conclusion

The results of studies show that antioxidants in the male reproductive system reduce oxidative stress in the testis and improve spermatogenesis. Many studies have shown the protective and antioxidant properties of silymarin against damage from chemotherapeutic drugs and environmental toxins in sperm. Also, the main published studies show the positive effects of silymarin on increasing the quality and quantity of sperm. Therefore, it is recommended that silymarin be prescribed to treat diseases caused by the effects of oxidative stress on the male reproductive system and improve fertility.

Acknowledgments

The authors of this article consider it necessary to appreciate the efforts of Hamideh Khodabandeh Lou and Razieh Bayat.

Conflicts of interest

There is no conflict of interest.

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**How to cite:**