Review Article

Clinical Role of Silymarin in Oxidative Stress and Infertility: A Short Review for Pharmacy Practitioners

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Abstract

Silymarin (SMN) as an ancient plant has various therapeutic usage in many diseases. Almost all of its properties attributed to antioxidant and anti-inflammatory properties. Currently, infertility problems impose a heavy burden on many developing countries. As a result, effective infertility treatment is indicated. The role of oxidative stress in both male and female infertility has been revealed. Many studies have shown protective and antioxidative properties of SMN against adverse effects of chemotherapy medications and environmental toxins in sperms and oocytes. The antioxidative and clinical role of SMN in infertility has been reviewed. The use of antioxidants such as SMN can help to improve fertility rate by scavenging free radicals and inhibiting nuclear factor kappa B transcription factor. Animal studies in both male and female have indicated a beneficial effect of SMN on fertility recovery. Further clinical studies are needed considering the phytoestrogenic property of SMN, to determine the right dose and duration of treatment.

Keywords: *Antioxidants, infertility, Silymarin, therapy*

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Introduction

A great number of factors may affect sperms motility, numbers, DNA structure, and ultimately fertility. Last but not least factor is oxidative stress. Destructive environmental factors, inflammation, and infections trigger reactive oxygen species (ROS) generation by white blood cells and immature sperm cells in the semen. ROS dysregulate cell signaling, and it can be harmful to cellular functions, cells' proliferation, and finally increase apoptosis. Enzymatic and nonenzymatic antioxidant system protect cells against oxidative stress. Glutathione (GSH), pantothenic acid, coenzyme Q-10, carnitine, zinc, selenium, copper, and vitamins (A, E, C, and B complex) are nonenzymatic defense. Many surveys indicate and recommend that antioxidants consumption can improve fertility.[1]

Milk thistle,[2] from Asteraceae family and Carduoideae subfamily, is an old phytotherapic plant which has several medicinal applications.[3,4] Regarding geographical distribution, it is native to southern Europe, Asia Minor, North Africa, and south Russian Federation; introduced to North and South America, Australia, China, and Central Europe.[5] The main active polyphenolic components are flavonolignans, silychristin, silydianin, silybin, and isosilybin (entirely known as silymarin [SMN]).[4]

Due to its protective effects, good pharmacokinetics profile, and safety, SMN has been used in widespread area such as Alzheimer's disease, Parkinson's disease, sepsis, burns, osteoporosis, diabetes, ulcerative colitis, cholestasis, hypercholesterolemia, cancers, neurotoxicity, nephrotoxicity, cardiotoxicity, Amanita phalloides poisoning, hepatic and lung disease, depression, prostate disease, and *in vitro* fertilization (IVF). Until now, the hepatoprotective effect of SMN has been shown clinically.[3,6-16]

SMN does not have significant adverse effects and drug interactions. Gastrointestinal upset is the most common side effect, and infrequent allergic reactions

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such as pruritus, rash, eczema, and anaphylaxis have been reported.[17,18] Infertility is a global concern. Based on the World Health Organization definition, infertility is defined as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse." It is defined as a disability, and in global ranking for severe disability, female infertility is in the fifth rank.[19] In every four couples, one couple suffered from disability to have a child in developing countries.[20] Approximately 48.5 million couples (15% of couples) are affected by infertility worldwide. The highest infertility rate was observed in Africa and Central/Eastern Europe. 20%–30% of infertility is attributed to male factors, and 50% of them are due to female factors. Although, the percentage range of male infertility can be varied from 20-70% in different countries, but overally 20-30 % of infertility is attributed to male factors, and 50% of them are due to female factors.[21] The etiology and pathogenesis of male infertility have not been identified. Beside anatomical abnormalities and neurological disorders, some constant and environmental factors may influence male fertility. Alcohol abuse, smoking, obesity, chronic stress, urogenital trauma, reproductive system inflammation, chemicals, heavy metals, pesticides, heat, and electromagnetic radiation – these factors by triggering oxidative stress process may impact on spermatogenesis and induce infertility.[1] There is not enough evidence for confirming oxidative stress and its role in female infertility. Endometriosis, polycystic ovary syndrome (PCOS), unexplained infertility, spontaneous abortion, recurrent pregnancy loss, and preeclampsia can be attributed to pro-oxidants and antioxidants imbalance. Environmental contaminants, obesity, and some inappropriate habits such as cigarette smoking, alcohol use, and drug abuse lead to increase in ROS[22] production and probable infertility.[23,24] Based on a review, which is written by Sekhon *et al*. in 2010, over half of the recurrent pregnancy loss is owing to oxidative stress.[25] Oxidative stress generates pro-oxidants or ROS by complicated interactions among cytokines, hormones, and other factors such as irradiation. These molecules can damage cell cycle proliferation and differentiation. Antioxidants counteract ROS and prevent cell injuries.[26] Nowadays, infertility problems impose a heavy burden on many developing countries. As a result, effective infertility treatment is indicated. The role of oxidative stress in both male and female infertility has been revealed. Many studies have shown protective and antioxidative properties of SMN against adverse effects of chemotherapy medications and environmental toxins in sperms and oocytes. Considering the antioxidant effects of SMN and the potential role of oxidants in the induction of infertility, this review was conducted to investigate the antioxidant function of SMN in helping to improve fertility.

Discussion

Antioxidant and infertility

Many investigations have suggested that oocyte and sperm modality might improve using antioxidants, which result in successful pregnancy increment.[25,26] A distinct amount of ROS is required for natural cell operation; the imbalance exists between ROS production and antioxidants can lead to cell cycle disruption and apoptosis progression. Endogenous factors such as mitochondrial respiratory chain and exogenous factors such as alcohol, cigarette, and environmental pollutants exposure play a vital role in producing of ROS.[23,24,27,28] ROS such as oxygen ions, free radicals, and peroxides which is generated due to oxidative stress in the male and female reproductive system damage cells and can cause subfertility and infertility. Most studies have been done on males, and the mechanisms of the destructive effects of oxidative stress are almost more obvious on sperm than oocyte. Two primary mechanisms were identified for male infertility induced by oxidative stress: (1) sperm membrane impairment, sperm motility reduction, and then disturbance in fusion with the oocyte and (2) DNA damage and embryo defects.[29,30] In many female complications, for instance, abortion, pregnancy loss, preeclampsia, endometriosis, PCOS, and unexplained infertility might be attributed to an imbalance between antioxidants and pro-oxidants.[23] Besides, some studies have shown that in preovulatory follicles, oxidative stress induces cell apoptosis [Figure 1].[31]

Enzymatic (metal-activated enzymes such as superoxide dismutase (SOD), GSH peroxidase (GPX), catalase (CAT), and GSH oxidase) and nonenzymatic antioxidants consumption (supplements and synthetic antioxidants such as Vitamin C, Vitamin E, Vitamins A, and B complex, pantothenic acid, coenzyme Q10, carnitine, zinc, selenium, copper, GSH, taurine, hypotaurine, beta-carotene, and carotene) can recline ROS and help to provide balance.[1,23] Antioxidants' consumption and lifestyle modification are convenient and effective suggested solutions which can help reduce DNA and RNA damages and improve fertility.[32]

Oxidative stress and the inflammatory process can impact on sperm and oocyte, which is associated with DNA damage or any disturbance in motility, fusion, and viability of these cells. All the mentioned changes might result in infertility, abortion, and fetus anomaly.[1,23,24,29,32,33]

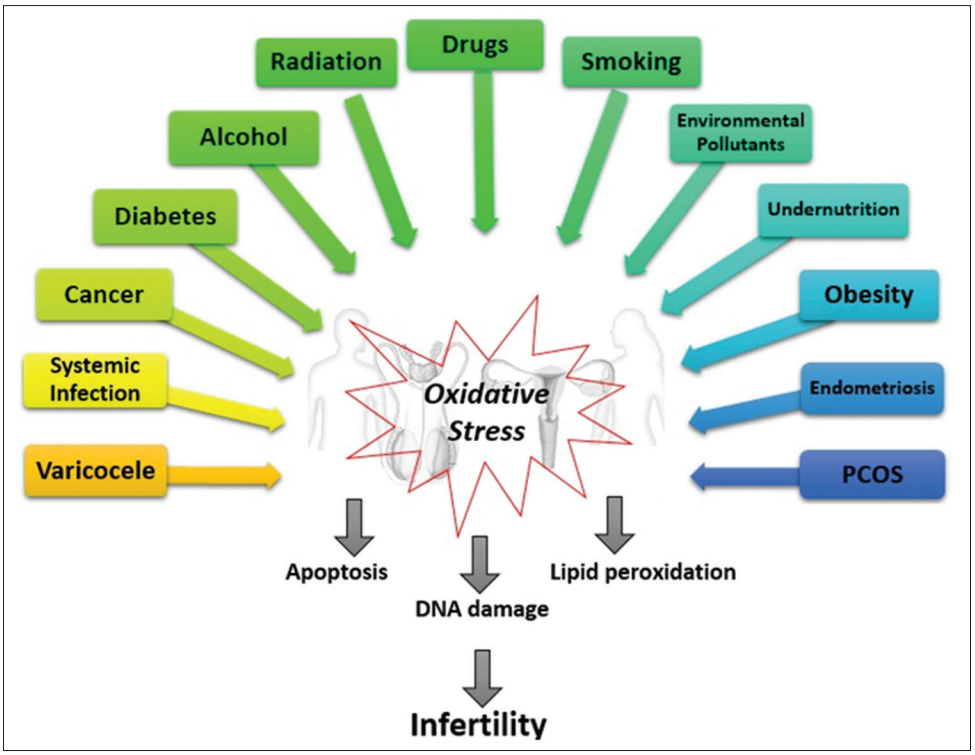

Figure 1: Role of oxidative stress, reproductive systems, and infertility

Anti-inflammatory effect of silymarin

Studies have shown antioxidant, anti-inflammatory, anti-fibrotic, detoxifying, and regenerative properties of SMN.[6,34] Beside antioxidant properties of SMN, dose- and time-dependent immunomodulatory effects of SMN have been investigated in several studies. Its anti-inflammatory effects are induced by nuclear factor kappa B (NF-κB) inhibition and tumor necrosis factor α (TNF-α) activation. Although low-dose SMN by inhibiting T lymphocytes showed immunomodulating activity, superior doses showed stimulatory effects on inflammatory processes. Furthermore, SMN has dual impacts on growth and death of cells with diverse nature.[2,9,35] Studies have demonstrated that it prevents cellular inflammation by suppression of the mammalian target of rapamycin signaling and activation of activating transcription factor 4 and adenosine monophosphate protein kinase. Cellular studies show that more exposure to SMN (24 h) can cause an increase in anti-inflammatory effects by inhibition of pro-inflammatory mRNA and signaling pathways such as NF-κB and forkhead box O.[36]

NF-κB is a regulatory transcription factor which can lead to interleukins 1 and 6, TNF-α, lymphotoxin, granulocyte-macrophage colony-stimulating factor, and interferon production.[37] Inflammatory mediators such as TNF-α, nitrous oxide, interleukin-6, and interleukin-1 receptor antagonist are modulated by SMN.[17]

Silymarin effects on male fertility

Comet assay, a sensitive test for evaluation of DNA strand breaks in eukaryotic cells, showed that DNA damage was diminished due to the antigen-toxic activity of flavonoids in human lymphocytes and sperm cells which were exposed to food mutagens (3-amino-1-methyl-5*H*-pyrido (4,3-*b*) indole (Trp-P-2), 2-amino-3-methylimidazole-(4,5-*f*) quinoline)[32] and flavonoids (SMN, myricetin, quercetin, kaempferol, rutin, and kaempferol-3-rutinoside). SMN, myricetin, and quercetin had antigenotoxic impacts on the sperm. Antioxidant effects were ascribed to phenolic hydroxyl groups, and an incremental pattern of these effects was observed by the addition of hydroxyl groups to A and B rings. Antigenotoxic effects in the equivalent dosage in lymphocytes and sperms showed that this protective outcome would obtain in either somatic or germ cell lines.[38]

According to study results, oxidative stress signaling pathway induction and free radical species production were imposed on ram sperm by sodium arsenite; cells which are treated with SMN had better motility. SMN as an antioxidant by scavenging free radical and promoting antioxidant enzyme capacity can improve sperm viability, motility, and mitochondrial membrane potential.[34,39] In other research which is conducted by Eskandari and Momeni, plasma membrane and acrosome integrity of ram epididymal spermatozoa which were exposed to

arsenite increased significantly compared to the control group that was not treated with SMN.[40] These two studies showed that strong antioxidant properties of SMN protect ram sperm against the disruptive effects of arsenite. During sperm storage, lipid peroxidation (LPO) and ROS production increase and detrimental products accumulation lead to sperm damage. SMN as ROS scavenging polyphenols can counteract with this destructive process. Addition of SMN as a supplement for ram semen storage showed that sperm quality was improved. Supplementation with caproic acid had a better impact.[41] In another similar study, the addition of SMN to sperm maintenance medium showed positive effects on bull sperm preservation, in both chilled and frozen condition.[42]

Polyunsaturated fatty acids, which are found abundantly in the mammalian spermatozoa cell membrane, provide a vulnerable state that results in ROS production by LPO and cell's detriment. Antioxidants neutralize ROS and defend cells against injury. By assuming antioxidant activity of SMN, an investigation of the impacts of silibinin, the most biologically active flavonoids of SMN, on the testicular tissue of mice was conducted. With a dose-dependent manner, a significant improvement in testosterone level and diameter of spermatid, and testicular associated factor were observed.[43] In an animal study, which is conducted by Abedi *et al*., the effects of SMN on spermatogenesis, changes of testicular tissue, and hormones of the hypothalamic–pituitary–gonadal axis (luteinizing hormone [LH], follicle-stimulating hormone [FSH], gonadotropin-releasing hormone, and testosterone) in male rats were evaluated. Compared to the control group, experimental groups which were treated with SMN showed a significant increase in LH, FSH, gonadotropin-releasing hormone and testosterone levels, and the number of spermatids and spermatozoa cells.[44] Male albino rats were treated with testosterone intramuscularly and SMN orally to evaluate the protective effects of SMN against testosterone damages in the reproductive system. In testosterone group, caspase-3 and P53 overexpression were observed. Oral SMN had a significant role in the prevention of testosterone biochemical and histopathological adverse reactions.[45] Based on the study results which is conducted to evaluate the reproductive alteration of rabbits bucks fed milk thistle seeds and rosemary leaves, in SMN group (10 g/kg) the sperm concentration, total sperm output, live sperm, total live sperm, total motile sperm, testosterone level, and fertility rate were significantly improved.[22]

Accordingly, SMN showed an impressive role in the improvement of sperm-related factors and fertility. Varicocele with several assumed mechanisms can lead to induce infertility in men, such as hypoxia (due to venous stasis), Leydig cell degeneration, testosterone level decrement, and testicular temperature increase and androgen receptor impairment. Furthermore, tissue inflammation, leukocyte infiltration and venous stasis-induced hypoxia, inducible nitric oxide (NO) synthase upregulation, production of malondialdehyde (MDA), and NO in the varicocele testis have been observed. Phospholipids' accumulation in mammalian spermatozoa cell membrane makes a susceptible condition which resulted in ROS production rise. Some studies showed ROS augmentation to correlate with cell damage in the varicocele. Level of E2f1 transcription factor as a potent apoptosis inducer increased in varicocelized animals, which contribute to hypoxia. Investigations indicated that SMN not only can regulate E2f1 transcription factor but also has a potential to decline hypoxia harm. Furthermore, SMN increased spermatogenesis and ameliorated carbohydrate depletion in germinal cells.[46]

In another similar study in varicocelized rats which were exposed to oxidative stress, SMN administration increased spermatozoa nuclei maturity and viability significantly compared to the control group.[47] Furthermore, SMN increased the serum total antioxidant capacity and total thiol molecules in varicocelized rats. Therefore, sperm motility increment and reduction in DNA damage were observed.[48] Mazhari *et al*. evaluated the effect of SMN and celecoxib on varicocelized rats. Results showed that these two agents with different mechanisms play a decisive role in decreasing varicocele-induced injuries. Inflammation downregulation by cyclooxygenase enzymes inhibition and antioxidant activities enhancement to protect RNA of germinal cells are proposed mechanisms of celecoxib and SMN, respectively.[49,50] Consequently, varicocele-induced oxidative stress can be improved by SMN due to its antioxidant activities.

Studies have been shown radioprotective effects of SMN on rat and mice testis tissue.[51,52] Results showed that in groups of animals treated with SMN 24 h before gamma radiation exposure, testicular parameters such as frequency of spermatogonia, primary spermatocyte, round spermatid, spermatozoa, seminiferous tube and lumen diameters, the thickness of the epithelium, Leydig cell nuclear diameter and volume, epithelium height, and apoptotic cells were improved. Better outcomes were observed at a dose of 200 mg/kg.[52]

According to results of an investigation which compared antioxidant effects of SMN and thymoquinone on male rats' reproductive functions which tread with

an environmental pollutant (benzo[a] pyrene), SMN ameliorated the antioxidant enzymes activities including CAT, GPX, and SOD, as such it regulated antioxidant status and level of free radicals in testicular tissue. Thymoquinone not only had no positive effects but also diminished levels of testosterone, estrogen, and progesterone.[53]

Methotrexate as a cytotoxic agent leads to testicular damages, such as decline in diameter of seminiferous tubules and primary spermatocyte, sperms' motility and count reduction, immature and abnormal sperms' production, germ cells' apoptosis, and disruption in spermatogenesis process. In groups of mice which were exposed to methotrexate and concomitantly were treated with silibinin (the most bioactive components of SMN), percent of the dead to live sperm decreased and interstitial space and diameter of spermatid improved significantly. These protective effects can be attributed to anti-inflammatory and antioxidant and radical scavenging of SMN.[54,55]

The adverse effects of Doxorubicin, an antineoplastic anthracycline, on the heart, liver, and testis have been proven previously. The preventive and protective role of SMN in male rats, which exposed to doxorubicin-induced oxidative stress and testis injury, were indicated. A decrease in MDA production, a LPO biomarker, and improvement in sperm quality were observed due to SMN administration.[56] Doxorubicin and radiation by producing free radicals change the equivalence of antioxidants and pro-oxidants and damage cells. Administration of them altered level of some factors such as MDA, GSH, GPX, and SOD (as antioxidants in tissue) in rats.

Addition of SMN to their regimen modified factors alteration by its antioxidant properties.[57]

In male rabbits, nickle chloride can lead to negative impacts on sperm count, motility, viability, and fertility ability of sperm. Furthermore, the reduction in serum concentration of progesterone, estradiol, and testosterone was observed. Investigation showed SMN improved these factors and increased fertility rate.[58]

Silymarin effects on female fertility

In a randomized, double-blind study, the effects of SMN on granulosa cell apoptosis and folliculogenesis in forty healthy women who underwent IVF were evaluated. Results showed that using SMN and gonadotropin concomitantly since the 1st day of ovulation induction can cause a granulosa cell apoptosis decline ($P = 0.032$), Although they could not find any relationship between SMN consumption and endometrial thickness, oocyte retrieval, and follicular maturation.[59,60] PCOS is one the most common causes of infertility in the female. Considering SMN antioxidant activity and its impacts on insulin sensitivity, patients who suffered from PCOS were treated with SMN throughout 3 months. The first group ($n = 20$) received SMN (750 mg/day), the second group received metformin (1500 mg/day), and the third group received metformin (1500 mg/day) and SMN (750 mg/day) concurrently. The levels of glucose, insulin, testosterone, LH, and progesterone were significantly improved. The best consequences were observed in the group which was treated with SMN and metformin.[61] There are several factors that affect IVF of bovine embryo; one of the inhibitory factors is oxidative stress. ROS-mediated oxidative stress was induced in the bovine oviduct epithelial cell by sodium nitroprusside. The outcomes showed that SMN via antioxidative and antiapoptotic gene expression regulation could ameliorate bovine oviduct epithelial cell livability and morphology and adverse effects of LPO.

Furthermore, bovine IVF embryo growth rate enhancement was observed.[62] The reproductive effect of extracts of four phytoestrogenic plants: Cucurbita pepo, Silybummarianum, Linumusitatissmum, and Vitex agnus-castus was compared with 17 β estradiol on the ovarian tissue of immature female three spot gourami fish. Among these extracts, Silybummarianum has the best impact on fertility improvement.[63] Phytoestrogenic effects of some plants are due to the existence of compounds such as lignans, isoflavones, coumestans, and resorcylic acid lactones. Isoflavones bind to β estrogen receptors with low affinity while endogenous estradiol binds to α estrogen receptors with high affinity. When the estradiol level is too low in the body, isoflavones show agonistic effects, and antagonistic properties may be observed in high levels of estradiol.[63]

Regarding histological and functional changes in ovariectomized rats, which were treated with SMN, the estrogenic effects of SMN were indicated.[64] *In vitro* receptor gene assays showed that SMN did not activate aryl hydrocarbon receptor, but some components of it such as silybin B and taxifolin had partial to full estrogen receptor agonist activities. Silybin A and other flavonolignans did not have estrogenic features. Estrogenic effects should be considered as a clinical side effect, and extract purification based on flavonolignans without estrogenic characteristics is recommended.[65] Some malformations have been observed in mice fetus by this plant-based compound.[66] Besides, ingestion of high dose phytoestrogen for a long time may cause adverse reproductive effects in animals. Therefore, histological and hormonal changes in female and male rats, which exposed to SMN, were assessed.

Phytoestrogenic effects of SMN may modify hormonal levels; SMN with a dose (151.2 mg/kg BW) equivalent to the human therapeutic dose (420 mg/day) in female rats increased the FSH, number, and size of follicles, and endometrial epithelium hypertrophy and prevented pregnancy on days 1–5 after intercourse. In male rats, which treated with similar doses of SMN for 1 month, the increase in testosterone, LH levels, and testis sperm counts was observed. Based on these results, SMN may be useful for male fertility.[67] Estrogen has a decisive role in spermatogenesis and diet with a low level of estrogen can improve normal sperm production.[68] The agonistic or antagonistic effect of SMN on estrogen receptors may be dependent on body estradiol level, dose, and duration of treatment. Cyclophosphamide, as an oxidative stress stimulator, induced antral follicle atresia in female rats. Significantly in SMN-treated group, antral follicles' development and counts were improved.[69] Therefore, SMN may be used as a protective agent for ovarian follicles.

SMN intake in one study improved sperm parameters, reproductive performances, decreased LPO, and increased antioxidant enzyme activities. The relative mRNA expression of Bcl-2 was considerably reduced, and that of Bax, Caspase-3 was increased in the diabetic rats compared to the control group. SMN significantly increased the expression of Bcl-2 and decreased the expression of Bax, Caspase-3. Serum levels of testosterone showed a significant decreased in diabetic rats, compared to the control group and serum testosterone levels revealed an increase in groups that received SMN.[70]

Conclusion

Due to the psychological and financial burden of infertility in the community, application of new effective, feasible, affordable approaches is necessary. Investigations showed the distinct role of oxidative stress in both male and female infertility. Moreover, some plants extracts are accessible and useful to neutralize oxidants and protect cells. Therefore, administration of antioxidants such as SMN can help to improve fertility rate. Furthermore, many studies showed protective and anti-oxidative properties of SMN against damages of chemotherapy medications and environmental toxins in sperms and oocytes. Its anti-inflammatory effects are induced by NF-κB inhibition and TNF-α activation. Antioxidant effects are attributed to the presence of hydroxyl groups in the chemical structure of these compounds. Therefore, SMN by scavenging free radicals and regulating the inflammatory cytokines can neutralize adverse effects of inflammation process and oxidative stress.

Animal studies in both male and female have indicated a beneficial effect of SMN on fertility recovery. Considering controversies, further studies, particularly in a human, should be performed while considering some phytoestrogenic property of SMN, to determine the dose and duration of treatment.

Authors' Contribution

Morvarid Zarif-Yeganeh and Mansoor Rastegarpanah performed literature search and acquisition of data, prepared, edited, reviewed, discussed and contributed to the final manuscript. Mansoor Rastegarpanah; devised the project and the main conceptual ideas, designed, defined the intellectual content, and was the guarantor.

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Conflicts of interest

There are no conflicts of interest.

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