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Review Article

Health Management and Policy Section

The Impact of Oxidative Stress on Testicular Function and the Role of Antioxidants in Improving it: A Review

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ABSTRACT

Oxidative stress is an important factor for development of male infertility because of very high rate of cell division and mitochondrial oxygen consumption in testicular tissue as well as comparably higher levels of unsaturated fatty acids in this tissue than in other tissues. Moreover, the level of oxygen pressure is low due to the weakness of testicular artery; therefore, there is a severe cell competition for oxygen. Therefore, the testicular tissue and male reproductive system are particularly susceptible to oxidative stress. On the other hand, exposure to X-ray, toxins and chemicals found in the environment as well as specific physical conditions such as varicocele can exacerbate the oxidative stress and induce apoptosis of germ cells and subsequently spermatogenesis. However, under normal conditions, the body's capacity to produce antioxidants for inhibiting adverse effects of oxidative stress is affected by metabolic process and genetic structure. Besides that, environmental factors such as diet, pollutants, and chemicals can affect this capacity. Thus, the body's antioxidant system alone is not able to neutralize all free radicals and prevent harmful complications of oxidative stress. Therefore, use of antioxidants and development of antioxidant therapy can break down the oxidative chain reaction and play a very significant role in increasing the body's capacity to fight free radical-induced oxidative stress, and therefore improve the process of spermatogenesis.

Keywords: Medicinal plants, Traditinal medicine, Testice

INTRODUCTION

Most problems that threaten the health of reproductive system, especially testicular function, are associated with free radical-induced oxidative stress. Life-threatening attacks of free radicals may cause arterial occlusion and serious damage to the reproductive system cells, and consequently defects in spermatogenesis. In other words, increased production of free radicals and per oxidants as well as weakened antioxidant defense system lead to oxidative stress [1]. Therefore, it is necessary to create a balance between produced free radicals and its metabolism for appropriate function of testicular cells, because if the testicular biological system fails to detoxify or repair the adverse effects of free radicals, the cells and tissue are damaged seriously [2]. In this regard, antioxidants can avoid this damage by counteracting free radicals or preventing their formation in the testicular cells. It is noteworthy that a part of the body's antioxidant defense system, called preventive antioxidant system, is related to antioxidant enzymes such as Superoxide Dismutase (SOD), catalase, and Glutathione Peroxidase (GPX) [3-6]. These enzymes avoid oxidation by decreasing the rate of chain formation. These antioxidants can stop an oxidation chain forever by finding primer free radicals. In addition, through stabilizing metal radicals such as copper and iron, they can inhibit their oxidation, preventing various diseases [3-5]. Another part of the body's antioxidant defense system is called scavenging antioxidant system. These antioxidants delete Reactive Oxygen Species (ROS) produced in the body's different tissues to prevent peroxidation of plasma membrane lipids [6]. Vitamins such as E and C are some examples of these antioxidants. These antioxidants neutralize free radicals and prevent them from damaging the cell and tissues. Given that the antioxidants produced by the body are not able to neutralize all free radicals, then use of antioxidant supplements can play an important role in increasing the body's capacity to fight free radicals [7,8]. In this article other than presenting the role of oxidative stress on testicular function, the plants with antioxidant activities, which have positive effects on testicular function, are reviewed.

The Effect of Oxidative Stress on Sperm Morphological Characteristics

Damage to sperm morphological characteristics: Various parameters of sperm such as count, motility, and morphology are significantly susceptible to free radicals and hence free radicals can reduce sperm fertility [9]. The free radical-induced oxidative stress contributes significantly in producing and increasing abnormal sperm and decreasing sperm count and transformation and fragmenting sperm DNA. These changes in sperm DNA result in infertility. In this regard, incubation of spermatozoa under high oxygen pressure reduces the rate and motility of sperm; however, adding catalase to the culture medium prevents this effect [10]. Increased production of Hydrogen Peroxide (H_2O_2) by spermatozoa under high oxygen pressure may be a factor for reducing sperm motility [11]. Moreover, the Reactive Oxygen Species (ROS) produced by leukocytes or spermatozoid have fatal effects on sperm function in infertile men. Thus, the ROS produced in the sperm must be inactivated continuously such that ROS concentration constantly remains low enough to allow for the normal performance of cells, because a lack of balance between the production and elimination of ROS causes oxidative stress in sperm and subsequently reduces fertility potential. Researchers believe that the sperm is more susceptible to oxidative stress than other cells due to the limited amount of cytoplasm in a mature sperm and the concentration of ROS-suppressing antioxidants in the sperm as well as high levels of unsaturated fatty acids in the sperm structure [9]. In addition, according to the particular morphology of sperm, antioxidant enzymes in the sperm fail to protect plasma membrane surrounding acrosome and tail. Therefore, the health and fertility of sperm are greatly dependent on the availability of the antioxidants, which mostly is related to the antioxidant systems in the seminal plasma. If the antioxidants are separated from the semen for any reason such as washing, sperm becomes susceptible to oxidative damage. Researchers believe that sperms can fight oxidative stress conditions mainly due to the antioxidant properties of semen [12] such that ROS generated in the semen is constantly deactivated by seminal antioxidants under normal conditions. Therefore, one of the reasons for establishment of

oxidative stress conditions is the imbalance between the production of ROS and its inactivation by antioxidants of semen.

Lipid peroxidation of sperm plasma membrane: Lipid peroxidation in the cell membrane can disrupt fluidity and permeability of cell membranes and damage all cells. In other words, when the cell membranes are damaged by free radicals, their protective cell is lost and thus the total cell is exposed to risk. In this regard, increased production of ROS induces lipid peroxidation in spermatozoa, which has two important effects: 1) Reducing sperm combination with oocyte; 2) Increasing spermatozoa ability to bind to the transparent area (zona placida) [13]. As well, lipid peroxidation caused abnormality in the middle section of sperm and loss of acrosome capacity of fertilization Malondialdehyde (MDA) molecules cause asymmetric distribution of lipid membrane components by penetrating into the cell membrane structure. Notably, the rate of lipids peroxidation is determined according to the resulting product of secondary failure of primary lipid hydro peroxides [13]. MDA is produced due to the degradation of the peroxides of unsaturated fatty acids. It is used as a marker (biomarker) to determine the rate of oxidative damage to lipids that differs depending on biotic and abiotic stress. This issue has been one of the used indicators in the studies on lipids peroxidation in humans and animals. It is noteworthy that currently, the damage caused by lipid peroxidation is the most important factor for testicular dysfunction [14].

Damage to DNA sperm: It is important to protect integrity and accuracy of DNA in the sperm nucleus to transfer genetic material completely from one generation to another, because genetic material disorder causes defective transmission of genetic information to embryo. Oxidative stress causes increase in DNA breakdown [15]. Besides that, evidence indicates that fragmentation of DNA is, commonly seen in infertile people's spermatozoa, is due to the ROS high concentration in the sperm [16]. In a study on DNA samples in people with teratozoospermia, the DNA damage rate was higher in the samples of people with spermatozoospermia than in those of healthy people; moreover, this damage was demonstrated to be mainly due to the amount of ROS produced by these sperms which can be the cause of infertility in people with spermatozoospermia. Therefore, a main reason for infertility is believed to be excessive production of ROS or decreased antioxidant capacity in semen that causes oxidative stress conditions and ultimately decrease in sperm motility, increase in sperm death, and fragmentation of DNA [13]. A study reported that the oxidative damage to DNA is 100 times higher in infertile men than in fertile men. Relevantly, ROS concentration is very high in the sperm of the men whose wives have previous abortion; therefore, increased oxidative stress in these people's testicles leads to destruction of sperm membrane and hence damage to DNA. This can be associated with abortion among such people's wives [17]. In this regard, semen has been reported to contribute significantly to maintaining sperm DNA's health such that gonads are thought to be one of the main sources of sperm-protective antioxidants [13]. In a study, the effect of Hamster gonads removal was investigated on sperm health. The findings demonstrated that removing the gonads caused increase in damage to hamster DNA [18]. Therefore, gonads can be considered the main source of antioxidants in the semen.

Factors for Inducing Testicular Oxidative Stress

Despite the role of antioxidant agents in protecting testicular function throughout spermatogenesis process, a wide range of internal and external factors can cause disturbance in antioxidant defense and subsequently induce oxidative stress. Some of these factors are as follows:

Testicular torsion: Testicular torsion is a rare disease, which is mainly seen in mature men and leads to disturbed testicular blood flow. The incidence rate of testicular torsion is estimated to be one per 158 people at the age of 25 years, which is associated with decreased quality of ejaculation in over 35% of this population [19]. If testicular torsion is not treated within 3-4 hours after incidence, it can lead to permanent testicle shrinkage [10]. Long-term testicular torsion results in testicular ischaemia (decrease in testicular blood flow), increase in levels of oxidative stress in the testicles of the same side, increase in production of NO and H_2O_2, formation of lipids peroxidation, accumulation of isoprostane, decrease in antioxidant enzymes, and increase in apoptosis rate [19]. Even, short periods of ischaemia, for three hours or shorter, can lead to high levels of testicular oxidative stress, decrease in testicular glutathione level, and spermatogenesis-induced disorder [20]. The tissue damage due to testicular torsion can be significantly reduced by pretreatment with certain antioxidants such as selenium, resveratrol, L-carnitine, caffeic acid phenethyl ester and *Allium sativum* extract [21].

High testicular temperature: Any factors that cause abnormal increase in testicular temperature are associated with testicular oxidative stress. Besides that, increased testicular temperature is associated with decreased SOD and function of catalase [22]. The exposure of sperm germ cells to increased temperature consequently increases the H_2O_2 level and is associated with increased rate of apoptosis. Therefore, increase in the catalase level can largely prevent cell death, as they decrease the H_2O_2 level [23].

Varicocele: Varicocele, dilation of spermatic vein in the left testicle, is associated with increased rate of male infertility [24]. Varicocele is associated with induction of oxidative stress through disturbing spermatogenesis process via related mechanisms. In some clinical trials, the incidence of varicocele has been demonstrated to be associated with excessive production of ROS by sperm, high levels of damage to DNA in these cells, and drainage of seminal plasma antioxidants [25]. These studies have demonstrated that varicocele causes induction of oxidative stress, which was confirmed in animal model. Varicocele was associated with testicular and seminal oxidative stress and decrease in testicular antioxidant capacity in mice [2,26]. It is noteworthy that varicocele causes increase in testicular blood flow and subsequently testicular temperature [21,27].

Diabetes: A study showed that experimentally inducing diabetes in animal models cause induction of oxidative stress by increasing the free radicals and subsequently disturbing testicular function thereby effecting fertility. Diabetes may contribute to male infertility by increasing the production of ROS and lipid peroxidation in testicles. In this regard, diabetes-induced oxidative stress causes DNA breakdown and increase in fetal mortality. These adverse effects can be repaired by use of certain antioxidants such as vitamin C, melatonin and taurine [28]. The level of damage to DNA is more in the sperms of males with diabetes compared to those without diabetes [29].

Infection: Oxidative stress due to inflammation and inflammatory disease is a common predisposing factor for male infertility. Testicular infection causes a significant decrease in production of testosterone and disturbance in spermatogenesis [30]. Analysis of microarray data on the genes expression in infertile men's testicles indicates increased expression of inflammatory genes [31]. A study showed that the oxidative stress induced by intraperitoneal administration of Lipopolysaccharide (LPS) caused stimulation of lipid peroxidation in Leydig cell membrane and considerable increase in steroid making and beta-hydroxyl dehydrogenase activity. Besides, this administration caused mitochondrial and Leydig cell dysfunction and specifically, inhibition of cholesterol transport activity [32].

Hyperthyroidism: Hyperthyroidism is associated with oxidative stress in mice testicles, an increase in lipid peroxidation and GSH level and induction of antioxidant enzymes. This oxidative stress appears to be due to increased mitochondrial activity and concurrent release of electrons from mitochondrial electron transport chain due to increased production of thyroxine [33]. Now-a-days, the complications due to hyperthyroidism-induced oxidative

stress can be repaired by melatonin, an important antioxidant; therefore, exacerbation of oxidation can be prevented [34]. Clinical trials have shown that hyperthyroidism causes decline in seminal quality especially disturbance in sperm motility. Furthermore, hypothyroidism causes induction of oxidative stress [35]. Therefore, it can be argued that normal testicular function is heavily dependent on thyroid function.

Imbalance among reproductive hormones: The conditions of the endogenous glands of the testicles can have an immediate effect on antioxidant activity of this organ. For example, treatment with cyclophosphamide and dimethane sulfanate can cause inhibition of expression of antioxidant enzymes such as glutathione peroxidase, SOD and catalase and decrease in testosterone concentration, disturbance in spermatogenesis and increase in cell apoptosis in testicles [36]. In this regard, gonadotropinexogen can affect testosterone production inversely and cause inhibition of antioxidant enzymes expression; moreover, this hormone disturbs spermatogenesis process and causes cell death. When Leydig cells in rats are treated with human chorionic gonadotropin for the long-term, ROS are excessively produced in testicular tissue cells and subsequently lipid peroxidation is stimulated, the activity of antioxidant enzymes is reduced, the apoptosis of germinal cells is induced and spermatogenesis is disturbed [37].

Xenobytes effect: Xenobytes, including the residues of antibiotics, refers to the residues of the drugs and chemicals that are stored in the body in different ways in the long term. Although low amounts of xenobytes are less likely to cause damage, long-term accumulation of these substances can be associated with certain problems in the body. Recently, a wide spectrum of xenobytes has been demonstrated to induce testicular oxidative stress alongside suppressing antioxidant mechanism [38].

Being exposed to toxins: Environmental toxins can cause testicular oxidative stress and consequently disturbance in spermatogenesis. For example, treating mice with certain pesticides such as hexachlorocyclohexane caused a significant increase in testicular oxidative stress and hence damaged germ cells and apoptosis [39]. Moreover, industrial pollutants such as 3,1-dinitrobenzene or nonylphenol exerted similar effects [40]. Methoxyethanol, the glycol ether used in colours, brake oils and some other industrial pollutants as well as its main metabolite, methoxyacid, cause increase in testicular oxidative stress and atrohpy [41]. Besides that, being exposed to high concentration of particular metals can cause oxidative stress. For example, a study on rats demonstrated that high concentration of iron increased oxidative stress and in contrast, use of antioxidants relieved oxidative stress in testicular tissue. Cadmium increases testicular oxidative stress, as well [42]. Moreover, exposure to lead causes decline in testicular sperm output, increase in production of sperm ROS, decrease in epididymal sperm motility and increase in lipid peroxidation in mice [43,44]. Finally, it is worth mentioning that adoption of inappropriate lifestyle such as excessive use of alcohol or increased smoking can increase production of free radicals in all tissues and has been frequently associated with or contributed to male infertility [45].

Ionizing radiation: Testicular tissue is highly dependent on X-ray radiation; therefore, being exposed to X-ray can be associated with oxidative stress in testicular tissue [46]. It should be noted that although all testicular cells have the same susceptibility rate to X-ray radiation, Sertoli and Leydig cells seem to be relatively resistant to X-ray, which can be reinforced by use of antioxidants [47,48].

Old age: A study demonstrated that in rats, as age increases, the expression of enzymatic and non-enzymatic antioxidants decreases, which led to increase damage due to oxidative stress. Besides that, the level of glutathione, an antioxidant, decrease in older mice [49]. According to the findings of different studies, aging causes degenerative changes in testicular tissue and decline in sperm quality in rodents. In this regard, certain vacuoles are seen in the germinal cells of testicular tissue in laboratory mice with increase in age. In addition, the number of germinal cells decreased in mice testicles [50]. Despite different studies conducted on the effect of age on increase in testicular oxidative stress, further research should be conducted to establish the association between aging, oxidative stress and testicular function.

Effective Antioxidants in Decreasing Testicular Oxidative Stress

As already mentioned, in addition to relying on the main, free radical-fighting enzymes, testicles are heavily dependent on antioxidant agents with low molecular weight to fight oxidative stress-induced complications. By nature, these factors are considered to be the scavengers or cleansers of free radicals.

Zinc: Zinc is a potent antioxidant agent and a main component of free radical-inhibiting free enzymes such as SOD. Moreover, this element, as a catalyst, can prevent lipid peroxidation through relocating or transferring metals including iron and copper [51]. In a study, the rats fed with zinc-deficient diet exhibited decrease in antioxidant defense potential and concurrent increase in lipid peroxidation in testicular tissue [52].

Vitamins C and E: Vitamin E (α-tocopherol) is a potent, lipophilic antioxidant, which is vital to protect and maintain mammalian sperm. Besides that, this element contributes greatly to the activity of Sertoli cell lines and spermatocytes [53]. Similarly, vitamin C (ascorbic acid) plays an important part in the process of spermatogenesis. As a result, vitamins C or E deficiency leads to induction of testicular oxidative stress and hence disturbs spermatogenesis and production of testosterone [54]. In contrast, a study demonstrated that feeding with ascorbate caused stimulation of spermatogenesis and secretion of testosterone in healthy animals. In addition, use of vitamins C and E is highly effective to fight testicular oxidative stress due to exposure to oxidants such as arsenic, cadmium, endosulfan and alcohol and can considerably decrease the complications due to these substances. A study demonstrated that vitamin E was effective on testicular function through suppressing lipid peroxidation in testicular and mitochondrial microsomes and fighting adverse effects of oxidative stress due to exposure to certain agents such as ozone gas, iron overload, intense exercise, aflatoxin, cyclophosplamide and formaldehyde [55].

Selenium: Selenium is an essential mineral to protect the body against free radicals. This element protects important antioxidants in the body including vitamins C and E and decreases free radical-induced damage. Selenium plays a part in producing thyroid hormones and contributes to the function of this important gland. Selenium contributes greatly to fertility.

In the light of biologically important role of selenium especially in male reproductive system, people with high oxidative stress such as chronic disease (diabetes, cardiovascular disease and HIV), elderly people, alcoholics and smokers are recommended to use a selenium-rich meal. In this regard, an inverse correlation has been observed between seminal selenium level and sperm motility. In addition, selenium levels were significantly higher in fertile men's semen than in infertile men's [56]. Use of vitamin E supplement and selenium considerably reduced Malondialdehyde (MDA) and improved sperm motility. Furthermore, selenium is useful to protect sperm DNA against oxidative stress and hence increase in motility and viability of sperm. According to these findings, selenium deficiency in rat's diet caused damage to seminiferous tubules and decrease in sperm motility and count [57].

Melatonin and cytochrome C: Melatonin has two important characteristics that differentiate it from other oxidants. First, melatonin, as an antioxidant, shares one electron, rather than two electrons, in oxidation reaction. Therefore, free radicals are more likely to target melatonin. As a result, melatonin causes decrease

in free radicals and oxidation rate. Secondly, melatonin is both water- and fat-soluble and can easily pass through testicular blood barrier to protect germinal epithelium. The melatonin level in seminal plasma is associated with weak sperm motility, leukocytospermia, varicocele and non-obstructive azoospermia in infertile men and consequently oxidative stress in male reproductive system [58]. In addition, intraperitoneally administered melatonin has caused decrease in testicular oxidative stress after experimental induction of varicocele of the left side [59]. Cytochrome C is another antioxidant that can effectively fight free radicals and special role has recently been confirmed in reducing testicular H_2O_2. Cytochrome C is a small protein, which is similar to coenzyme Q (ubiquinone) in terms of motility. The cytochrome C isoform is considered a potent apoptosis activator and plays a part in increasing the protective capacity of testicular tissue through eliminating damaged germ cells [60].

The Mechanism of Semen Antioxidant Protection

Semen contains antioxidant compounds that protect spermatozoa against oxidative stress and therefore, offset deficiency of sperm cytoplasmic enzymes. Semen contains a group of enzymatic antioxidants such as SOD, catalase, GPX and certain non-enzymatic antioxidants such as taurine, pyruvate, ureate, ascorbate, and α-tocopherol. Fertile men's semen has a higher antioxidant capacity than infertile men's [61]. Seminal plasma contains three enzymatic antioxidants, SOD, catalase and GPX that prevent damage to cell structure and also the reduction of hydrogen peroxide into water and alcohol. Meanwhile, SOD with a comparably higher amount than other antioxidant enzymes leads to conversion of superoxide (NO_2) anion into O_2 and H_2O_2. Furthermore, this enzyme protects spermatozoa against O_2 toxicity and lipid membrane against peroxidation. Catalase decomposes H_2O_2 into O_2 and H_2O. This enzyme also separates the superoxide anion produced by NADPH oxidase from neutrophils and protects spermatozoids against oxidative damage. In addition to containing enzymatic antioxidants, semen has a number of non-enzymatic antioxidants such as vitamins E and A, ascorbate, pyruvate, ubiquinole, glutathione, albumin, ureate, taurine, and hypotaurine, each of which plays a vital part in fighting oxidative stress [62]. In this regard, certain amount (mM) of glutathione has been found in a number of cells. Glutathione reacts directly with ROS. Glutathione is a cofactor for GPX and protects mammalian cells against oxidative stress while reducing H_2O_2 and other peroxides. A study showed that 5 mM of glutathione exerts protective effects on sperm against freezing and is associated with enhanced sperm motility after freezing. Furthermore, this compound increases enzymatic activity in sheep semen [63]. Vitamin E is the most abundant fat-soluble antioxidant that contributes greatly to seminal antioxidant system. Therefore, the activity of vitamin E, as a coenzyme for enzymatic reactions, is vital and helps to neutralize free radicals and ultimately reduce seminal oxidative stress. In addition, vitamin E or tocopherol protects intracellular and cell membrane unsaturated fatty acids against oxidative damage. Moreover, vitamin E activity is complementary to GPX activity, which facilitates the peroxides reduction in seminal cytoplasm.

CONCLUSION

Free radicals' life-threatening attacks to the body's different organs can cause arterial occlusion and induction of oxidative stress and subsequently causing serious damage to tissues. Meanwhile, testicular tissue is highly predisposed to activity of free radicals and oxidative stress due to several reasons including high cell division rate, cell competition for oxygen rate, low oxygen pressure due to weakened vessels as well as high levels of unsaturated fatty acids. Furthermore, since the body's antioxidant system, including antioxidant enzymes such as SOD, catalase and GPX produced in the body is not able to neutralize all free radicals, the use of antioxidant supplements is recommended to fight adverse effects of oxidative stress, enhance spermatogenesis and increase enhance fertility.

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