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Literature Review

Role of antioxidant to protect leydig cells damage induced by reactive oxygen species: a literature review

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ABSTRACT

The Leydig cells play a crucial role in steroidogenesis and spermatogenesis. Those processes need complex communication in hormonal and testicular to maintain male reproductive function. Abnormal conditions induced by reactive oxygen species reduce cell viability through lipid peroxidation and apoptotic pathway that disrupt specific cells. Antioxidant ameliorates ROS elevation and prevents cell damage. Specifically, Leydig cells are vulnerable to ROS exposure and decline their function in mediating spermatogenesis. The imbalance of antioxidant and ROS level triggers oxidative stress and start damaging the Leydig cells. Loss of Leydig cells' functions in the testicular can lead to severe steroidogenic and spermatogenic impairment, which can contribute to male infertility. Therefore, it is needed to improve Leydig cells viability with antioxidant supplementation. This study aimed to determine the protective effect of antioxidants on Leydig cells induced by reactive oxygen species. This type of study is an integrative literature review. Various studies have been reviewed through critical appraisal tool Olsen-Baisch Scoring for integrated review. The online databases for all articles were found in different journals such as Nature, SAGE Journal, Scopus, and Springer Link. This study retrieved 295 titles, and 17 articles were qualified after the qualitative synthesis. Furthermore, this study highlighted the importance of the mechanism of antioxidants as a protective agent of Leydig cells. Numerous antioxidants can be found naturally, however, there are some factors with the compounds related to its antioxidant activity. Supplementation of antioxidants with the correct administration, dosage, and duration can balance reactive oxygen species level and protect Leydig cells.

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INTRODUCTION

Infertility has become a world issue for reproductive health. Approximately 8-12% of couples of reproductive age experienced the inability to conceive spontaneous pregnancy. Moreover, up to 40% are related to male infertility, and most cases are the reproductive age group (Bisht et al., 2017). In Indonesia, the number of infertility couples of productive ages 20 to 40 years is 12-15%. Moreover, men influenced 40% of the infertility cases (Agustina, Budihastuti and Murti, 2018). Abnormal semen analysis has been reported in many infertile men, but the etiology is still poorly understood. Male infertility factors are genetic, dietary, physiologic, medical, and environmental factors. Recently, reactive oxygen species have been described to be a secret agent of male infertility. A previous study showed that 30-80% of male infertility is caused by reactive oxygen species (Wagner, Cheng and Ko, 2018).

Reactive oxygen species (ROS) is needed in some physiological processes, include capacitation and apoptotic processes. ROS sources in sperm, such as activation of leukocytes in the seminal plasma and the mitochondria in the spermatozoa. The higher level of ROS will cause an imbalance of endogenous antioxidant capacity that is defined as oxidative stress (Redza-dutordoir and Averill-bates, 2016). Oxidative stress is a potential contributor to reproductive cell dysfunction. The elevation of ROS level can damage sperm and testes. Low oxygen tensions in the testes are due to its poor vascularization, and it makes incredibly intense competition for the vital element within the testes. In addition, excessive ROS production in testicular tissue cells stimulated lipid peroxidation then trigger spermatogenesis and steroidogenesis perturbation. Leydig cell plays an important role in testosterone synthesis in male, which can be damaged by higher ROS level in the testicular tissue (Zirkin and Papadopoulos, 2018). Supplementation of exogenous antioxidant is needed to cover the imbalance of ROS level.

Antioxidant capacity can inhibit ROS-induced cell injury by natural or synthetic compounds to protect the Leydig cell. Antioxidant administration has been reported to protect and maintain Leydig cells induced by oxidative stress (Kumar and Neeraja, 2019). However, antioxidants' protective effect to Leydig cells damaged by ROS exposure should be investigated for a better understanding. This literature review aimed to determine the antioxidant's role in protecting Leydig cell induced by reactive oxygen species. This review specifically explores in Leydig cell and its damage through elevation of ROS level. Furthermore, this present study distinguish the important role of antioxidant system in Leydig cells to maintain spermatogenesis and steroidogenesis.

LITERATURE REVIEW

This integrative literature review based on four databases included Nature, Sage Journal, Scopus, and Springer Link. Based on the original research article keywords used in this study were "Leydig cells AND antioxidant AND reactive oxygen species AND protective". The articles have been selected by inclusion and exclusion criteria. In this study, the inclusion criteria were articles published in 2014 till 2019 range, written in English, available and accessible to the entire article. Only original article with true experimental study and laboratory study design will be included. Article without of involvement of Leydig cell will be excluded. Quality of the article defined by critical appraisal tool Olsen-Baisch Scoring for integrated review. The literature tracing scheme can be seen in Figure 1.

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Antioxidant as protective agent evidence

This literature review selected 17 research articles. The article must be an original article with true experiment or laboratory research. This present review explored and analyzed the type of antioxidant, doses, and antioxidant effect to counter ROS as Leydig cells protection. The details of the evidence can be seen in Table 1.

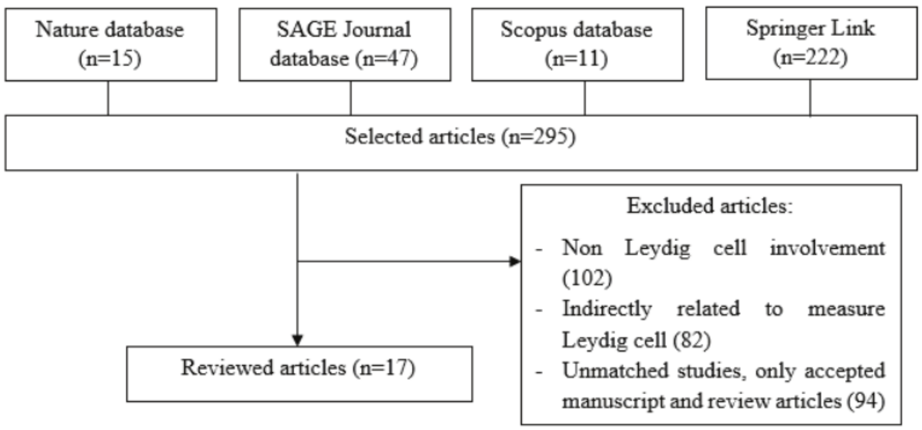

Figure 1. Literature review tracing scheme

Table 1. List of antioxidant protect Leydig cells induced by ROS evidence

No	Title and Author	Type of Antioxidant	Result
1	Adrenomedullin protects Leydig cells against lipopolysaccharide - induced oxidative stress and inflammatory reaction via MAPK/ NF-κB signalling pathways (Hu et al., 2017)	Adrenomedullin (ADM)	Addition of 100 nM ADM were significantly increased Leydig cell viability and advanced cell proliferation compare to control, 10 nM ADM, 50 nM ADM, and 300 nM ADM.
2	Vitamin D alleviates lead induced renal and testicular injuries by immunomodulatory and antioxidant mechanisms in rats (Basalamah, Abdelghany and El-boshy, 2018)	Vitamin D (VD_3)	Administration of VD_3 1,000 IU/Kg in 3 days per week decreased apoptotic index of Leydig cell and maintain cell viability due to lead toxicity.

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3	Protective role of Nigella sativa oil against reproductive toxicity, hormonal alterations, and oxidative damage induced by chlorpyrifos in male rats (Mosbah et al., 2016)	Nigella sativa oil (NSO)	Administration of 1 ml/kg/day NSO increased diameter of Leydig cells and decreased ROS level in testicular.
4	Prevention of carbon tetrachloride (CCl_4)-induced toxicity in testes of rats treated with Physalis peruviana L. fruit (Moneim, 2016)	Physalis peruviana L.	P. peruviana juice supplementation significantly increased the testicular glutathione and significantly decreased the level of lipid peroxidation and the nitric oxide production compared with the CCl_4 group. P. peruviana juice also prevented the degeneration of germ and Leydig cells along with deformities in spermatogenesis induced after CCl_4.
5	Vitamin C ameliorates the adverse effects of dexamethasone on sperm motility, testosterone level, and spermatogenesis indexes in mice (Sadeghzadeh, Mehranjani and Mahmoodi, 2019)	Vitamin C	Supplementation of 100 mg/kg/day Vitamin C showed significant differences between control and DEX group. Vitamin C reduce testicular toxicity induced by ROS and protect Leydig cells number.
6	Zinc sulphate and vitamin E alleviate reproductive toxicity caused by aluminium sulphate in male albino rats (Rawi, Seif and Nassr, 2015)	Zinc sulphate and vitamin E	Administration of 50 mg/kg/day Zinc sulphate and Vitamin E 15 mg/kg/day individually or combination have been proven to maintain Leydig cells due to ROS caused by aluminium sulphate.
7	Cerium oxide nanoparticles protect Cyclophosphamide-induced testicular toxicity in mice (Hamzeh et al., 2019)	Cerium oxide nanoparticles (NC)	Supplementation of 100 µg/kg NC prevented Leydig cells destruction induced by ROS generated by Cyclophosphamide.

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8	Effect of Cistanche Tubulosa extracts on male reproductive function in streptozotocin–nicotinamide-induced diabetic rats (Kong et al., 2018)	Cistanche tubulosa extracts (CTE)	Administration of 160 mg/kg CTE decreased ROS level induced by diabetes mellitus in rats and it can maintain Leydig cells due to testicular toxicity.
9	Anti-apoptotic and anti-oxidant effects of caffeic acid phenethyl ester on cadmium-induced testicular toxicity in rats (Erboga et al., 2016)	Caffeic acid phenethyl ester (CAPE)	Therapy with 10 μmol/kg b.w. CAPE had significantly prevented oxidative stress due to ROS level and increased the number of Leydig cells viability.
10	Ameliorative effect of VE, IGF-I, and hCG on the fluoride-induced testosterone release suppression in mice Leydig cells (Yu et al., 2018)	Vitamin E (VE), IGF-1, and hCG	Leydig cells viability were maintained through ameliorative effect of VE, IGF-1, and hCG due to ROS induced testicular toxicity with the most powerful antioxidant showed in VE.
11	Malathion induced testicular toxicity and oxidative damage in male mice: the protective effect of curcumin (Ali and Ibrahim, 2018)	Curcumin	Administration of 200 mg/kg/day of curcumin showed normal histological structure, no Leydig cell hyperplasia, and decreased ROS level induced by malathion.
12	Testicular antioxidant mechanism of cultivated wild ginseng extracts (Ok, Kang and Kim, 2016)	Wild ginseng extracts	Supplementation of 50 mg/kg/day wild ginseng extracts increased Leydig cells viability and eliminated ROS level cause by Bisphenol-A.
13	Antioxidant activity of Spirulina platensis alleviates doxorubicin-induced oxidative stress and reprotoxicity in male rats (Eleiwa et al., 2018)	Spirulina platensis	Prevention effect of Spirulina platensis at a dose 300 mg/kg showed improvement in Leydig cells number and ameliorated ROS caused by doxorubicin.
14	Ameliorative effect of taurine-chloramine in azathioprine-induced testicular damage; a deeper insight into the mechanism of protection (Schaalan, Ramadan and Elwahab, 2018)	Taurine-chloramine (TAU-CL)	Supplementation of TAU-CL increased Leydig cells viability, decreased distortion in interstitial area, and prevented cell damage induced by ROS level.

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15	Testicular toxicity and sperm quality following copper exposure in Wistar albino rats: ameliorative potentials of L-carnitine (Khushboo et al., 2018)	L-carnitine	Supplementation of 100 mg/kg L-carnitine showed significantly restored Leydig cells number induced by copper exposure.
16	Comparative analysis of the protective effects of curcumin and N-acetyl cysteine against paracetamol-induced hepatic, renal, and testicular toxicity in Wistar rats (El-Maddawy and El-Sayed, 2018)	Curcumin (CUR) and N-acetyl cysteine (NAC)	Administration of 200 mg/kg b.w. CUR and 150 mg/kg b.w. NAC increased the integrity of cellular membrane and stimulated regeneration of damaged cells. It showed that CUR has stronger ability to protect Leydig cells rather than NAC to improve reproductive function.
17	Bisphenol A exposure and healing effects of Adiantum capillus-veneris L. plant extract (APE) in bisphenol A-induced reproductive toxicity in albino rats (Yousaf et al., 2016)	Adiantum capillus-veneris L. plant extract (APE)	Administration of 25 mg/kg/day APE prevented Leydig cells damaged induced by Bisphenol-A.

Leydig cells

The Leydig or interstitial cells lie in the connective tissue between the seminiferous tubules. This cell-synthesized male sexual hormone testosterone makes Leydig cell the endocrine cells of the testes (Ji et al., 2015). Testosterone secreted by Leydig cells has various functions in men's reproductive health system. Those functions were needed before birth, such as testosterone secretion by the Leydig cells of the fetal testes masculinizes the reproductive tract and external genitalia and promotes the descent of the testes into the scrotum. After birth, when initiated at puberty, testosterone secretion and spermatogenesis occur continuously throughout the male's life. Ongoing testosterone secretion is fundamental for spermatogenesis, maintaining a mature male reproductive tract, and influencing fertility (Sherwood, 2010). Biosynthesize testosterone as a steroid hormone-regulated by the mitochondria inside the Leydig cells. The abnormal condition can change its function and damage the cells (Ye et al., 2017).

Reactive oxygen species

Free radicals are a state of an unstable number of cell electrons so that it disrupts the balance of other electrons and takes place in a chain. Reactive oxygen species (ROS) have the destructive properties of free radicals. Some examples of ROS such as superoxide anion ($O2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($HO^{\bullet}$), all of which contain oxygen radical and non-radical species formed by the reduction of some oxygen (Phaniendra, Jestadi

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and Periyasamy, 2015). Endogenous cellular production of ROS results from oxidative phosphorylation in mitochondria, or the compound is produced from the interaction between exogenous factors and xenobiotic components (Sisein, 2014).

Antioxidant

Antioxidants are compounds that can eliminate, cleanse, and resist the formation of ROS effects. Antioxidants as compounds that can inhibit the process of auto-oxidation of all ingredients that contain lipids (Rajendran and Devi, 2018). The antioxidant process inhibits the formation of free radicals by acting as an H donor against free radicals, transforming into a more stable form. Antioxidants by source are classified in two groups, namely endogenous antioxidants and exogenous antioxidants. Endogenous antioxidants are antioxidants naturally present in body cells, namely SOD, catalase (CAT), and glutathione peroxidase (GPx) (Ighodaro and Akinloye, 2019). Exogenous antioxidants are antioxidants that come from outside the body, can come from everyday foods that contain vitamins (vitamin C, vitamin E beta-carotene) and phytochemical compounds (carotenoids, isoflavones, saponins, flavonoids, polyphenols) (Asih *et al.,* 2018). Exogenous antioxidants also consist of two major groups: the natural antioxidant group obtained from natural ingredients and synthetic antioxidants obtained from chemically synthesized ingredients.

Antioxidant protective effect on Leydig cells induced by ROS

Leydig cells secreted androgen, which plays a pivotal role in male sexual differentiation and sexual behavior, and it is crucial for initiating, maintaining, and regulating the process of spermatogenesis (Zhou et al., 2019). The various study has been reported that Leydig cells as the main source of androgens. The Leydig cells help to regulate spermatogenesis through its function in steroidogenesis. Testosterone as the primary androgen synthesize by Leydig cells is required for meiosis and sperm formation, mediating normal spermatogenesis. Significantly, Leydig cells have the important role in maintaining male fertility. Leydig cells are vulnerable to toxicants especially induced by reactive oxygen species (ROS).

Endogenous ROS production mainly occurs in mitochondria as the electron transport processes; other sources are in the endoplasmic reticulum, cytoplasm, peroxisomes, lysosomes, and plasma membrane (Kurutas, 2016). Exogenous sources of ROS are inappropriate diet, lifestyle, and environmental factors. Despite ROS's function in cell signaling mediation, increasing ROS level can trigger an imbalance endogenous antioxidant capacity. The effect of imbalance ROS and antioxidant level become oxidative stress. Oxidative stress as the mediator of damage to cell structures and an inductor for apoptotic cells. A previous study stated that Leydig cell dysfunction and lipid peroxidation of Leydig cell membrane stimulated by oxidative stress (Asadi *et al.,* 2017). Associated with testicular toxicity, it has been noted that ROS elevation induces Leydig cell damage and influence abnormal spermatogenesis.

The germ cells in the testes undergo complex proliferation and maturation processes, from diploid spermatogonia through meiosis to mature haploid spermatozoa, which are highly dependent on oxygen metabolism (Oliveira and Alves, 2015). Cells that are oxidized in the testes become a source of ROS for the testicular organs themselves, thus causing an increase in oxidative stress. If oxidative stress is excessive, communication between cells in the testes, such as Sertoli cells, Leydig cells, and other spermatogenic cells can be damaged (Hai et al., 2014). Oxidative damage occurs in many types of molecules such as lipids, proteins, nucleic acids, and sugars. Every cell, nucleus, and mitochondrial membrane, structural and

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cytoplasmic protein, complex carbohydrates, ribonucleic acid (RNA) and DNA, all have the potential to cause oxidative stress. Tissues such as testes with high metabolic rates and cell replication make oxidative stress increase and surpass the endogenous antioxidant level (Guerriero et al., 2014). Oxidative stress due to increased ROS can cause damage to cells, tissues, to organs. This results in disruption of the process of sperm formation in the seminiferous tubules due to damage to cells that play a role in spermatogenesis so that the number of spermatogenic cells decreases and ultimately leads to male factor infertility.

According to ROS's deleterious effect oxidative stress-induced, it has been reported to administer exogenous antioxidants. Antioxidant sources are varied and can be a natural or synthetic antioxidants. Recently, natural antioxidants have been chosen for ameliorating cell damaged caused by ROS. As we have known, Leydig cells need to be protected, and numerous studies have been reported that ROS elimination through antioxidant supplementation. Many experimental studies showed that medicinal plants rich in vitamin C and vitamin E have a strong antioxidant capacity to eliminate ROS. Moreover, some minerals like Zinc also have the antioxidant capacity to encounter cell damage by ROS (Ko et al., 2014). The mechanism of action of antioxidant to protect cells are complex and need better understanding. Studies show that antioxidant plays a key role in achieving balance or cellular redox homeostasis (He et al., 2017). Supplementation of antioxidants is needed to protect Leydig cells damage from testicular toxicity induced by ROS. However, a misleading dose of administration of antioxidants can cause severe cell damage (Shahidi and Zhong, 2015). The antioxidant dose variation can give different effects, which can be an inverse relationship between antioxidant activity and antioxidant concentration. The greater concentration of antioxidants results in smaller antioxidant activity. Antioxidants are chemical compounds that can contribute one or more electrons to free radicals, so that free radicals can be suppressed. The higher the concentration of antioxidants the more dense the molecules so that the electrons of the antioxidant become unable to react with free radicals (Apak et al., 2016). The rate of oxidation can be affected by increasing antioxidant concentration. Another previous study also mentioned that giving concentrations of antioxidants with high concentrations can be a pro-oxidant. Essentially, the importance is balanced the ROS and antioxidants level to protect Leydig cells.

Antioxidants' protective effect on the Leydig cells has been reported to raise male fertility. The exposure of ROS increased Leydig cells damage and decline its function. Administration of antioxidants as pretreatment increased Leydig cells viability and regenerated its function on steroidogenesis and spermatogenesis (Darbandi et al., 2018). Antioxidants' activities to protect Leydig cells regarding to its administration, dosage, and duration (Haw et al., 2012). The administration of antioxidants through oral or injection can give slightly different effects. Various dosage of antioxidant supplementation has been studied to find the best-chosen dosage that does not harm the cells. Moreover, antioxidant supplementation duration is crucial to prevent the accumulation of exogenous antioxidants that can inverse into pro-oxidant and damage the cells. Long term of ROS exposure will decrease anti-oxidative defense system of the Leydig cells. Therefore, antioxidant supplementation is needed to be a protective agent of the Leydig cells within rational recommendation, so that can maintain and enhance male fertility.

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CONCLUSION

This literature review has been shown that antioxidants as a potential agent to protect Leydig cells induced by ROS. The importance is to maintain the cellular redox state of ROS and antioxidant level. Several studies have proven that supplementation of antioxidants within the correct dosage and duration can enhance Leydig cells viability and generate its function to influence normal spermatogenesis. Genetic factors originate in Leydig cells, and the mechanism of survival with an antioxidant defense system need to get a better understanding to identify innovation for treating male infertility.

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