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The effect of alpha-lipoic acid on sperm functions in rodent models for male infertility: A systematic review

Nushin Naderi ^a, Zahra Darmishon Nejad ^a, Marziyeh Tavalaei ^a, Mohammad Hossein Nasr-Esfahani ^{a,b *}

^a Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

^b Isfahan Fertility and Infertility Center, Isfahan, Iran

* Corresponding author: Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran. Isfahan Fertility and Infertility Center, Isfahan, Iran. E-mail addresses: mh.nasr-esfahani@royaninstitute.org

Abstract

In this systematic review, we assessed different studies to evaluate the protective effect of alpha-lipoic acid (ALA), as a multifaceted antioxidant, on sperm functions in rodent models. Four databases were searched to find papers reporting the effect of ALA treatment on animal models of male infertility. Up to December 2022, 11,787 articles were identified to explain the ALA protective effects. The included studies were evaluated for eligibility and risk of bias (CRD42022341370). Finally, we identified 23 studies that explain the effect of ALA on sperm functions in rodents. Among them, 15 studies indicated that ALA could restore sperm parameters. Six studies showed a significant reduction in sperm DNA damage by ALA treatment. Seventeen papers displayed the ALA antioxidant ability, and four studies indicated the ALA anti-inflammatory effect. Besides, thirteen studies displayed that ALA could modulate androgenesis. Also, eighteen studies revealed that ALA restored the testicular architecture to normal, and was also effective in restoring reproductive performance in two included studies. This systematic review provided cogent evidence for the protective effect of ALA in rodent models for male infertility by re-establishing spermatogenesis and steroidogenesis and maintaining redox and immune systems homeostasis.

Keywords: Alpha-lipoic acid, Male infertility, Preclinical studies, Rodent models, Systematic review

1. Introduction

Infertility is described as the incapacity to conceive after ordered unprotected sexual intercourse for at least 12 months and influences 10-15% of reproductive-age couples globally [1]. In about 50% of infertile couples, a male factor contributes to infertility [2]. Male infertility is a multifaceted disorder with genetic, epigenetic, environmental, and lifestyle causes [1]. Of the various reasons for male infertility, oxidative stress (OS) and high concentrations of seminal and sperm reactive oxygen species (ROS) may participate to 30–80% of male infertility cases [3]. In normal physiology, ROS are critical for maturation, hyperactivation, capacitation, acrosome reaction, and the fertility capacity of sperm [4]. High levels of ROS underpin sperm pathology through deficient axonemal phosphorylation, lipid peroxidation (LPO), reduced motility and viability, and induced DNA damage, thus reducing the spermatozoa's fertilizing ability [5]. Sperm is very exposed to OS thanks to the lack of part of the cytoplasm and the high amount of membrane polyunsaturated fatty acids (PUFAs) [6]. Moreover, it has been revealed that antioxidant compounds may shield cells and tissues against the harmful effects of ROS, subsequently improving the quality of sperm and male fertility [4]. Amongst a variety of antioxidant compounds, Alpha-lipoic acid (ALA; 1,2, dithiolane-3-pentanoic acid) or thioctic acid, is a natural disulfide byproduct of octanoic acid that exists in different cell types, specifically in the mitochondria acting as an influential cofactor in numerous multienzyme complexes intricate in metabolism of energy [2]. Since ALA is both water and lipid soluble, hence, is extensively distributed in both membranes and cytosol of the cell [7]. ALA as a thiol antioxidant possesses the influential potential to reduce ROS, regenerate the antioxidants including vitamins E, vitamins C, and glutathione (GSH), and chelate transition metals [4, 8]. Current results have also shown that ALA can enter into the Krebs cycle, therefore supporting ATP production, which is needed in viable sperm [9]. Therefore, this systematic review aims to extensively discuss the effect of ALA on decreasing the unfavorable effects of endogenous and exogenous sources of ROS and improving sperm functions in rodent models of male infertility.

2. Material and methods

The current systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [10]. Our systematic review was recorded in the International prospective register of systematic reviews (PROSPERO) on 2 Aug 2022, under the number CRD42022341370.

2.1. Research approach

The search was performed in PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Google Scholar (<https://scholar.google.com/>), Springer Link (<https://link.springer.com/>), and Science Direct (<https://www.sciencedirect.com/>) databases until December 2022. The PICO question was set as follows: (P)opulation: male rodents with induced reproductive toxicity; (I)ntervention: ALA supplementation; (C)omparator: rodents not receiving ALA; (O)utcome: sperm functions. The following combinations of keywords were used: alpha-lipoic acid OR alpha lipoic acid OR lipoic acid OR thiocetic acid AND (male infertility OR semen OR sperm parameters OR sperm functions OR sperm DNA damage). To obtain relevant studies, the screening process was performed by two independent investigators to recognize the eligible publications. If there was a dispute, it would be evaluated by the third researcher.

2.2. Inclusion criteria

In the present study, we included those papers that met the inclusion criteria containing 1) Subjects: all *in vivo* rodent models with induced sperm dysfunction leading to infertility; 2) Intervention: ALA with all doses and duration; 3) Control: similar solvent such as water and saline, and no intervention, etc.; 4) Primary outcomes: sperm parameters, sperm and testicular oxidative status and LPO, sperm DNA damage, testicular histology, and sperm fertility efficacy; 5) Study design: controlled studies with a distinct control group; 6) Language: English. The selection flowchart was created according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [11] then offered in Fig. 1.

2.3. Exclusion criteria

The following studies were excluded: 1) Subjects: non-rodent animals, human, and *in vitro* study designs.; 2) Intervention: complex intervention; 3) Study design: studies without a distinct control group; 4) Not an original article such as randomized clinical trials (RCT),

abstract, book chapters, reviews, letters and comments, theses, and dissertation, 5) irrelevant studies; 6) duplicate publication; 7) papers without full text, 8) non-English studies (Fig. 1).

2.4. Selection of study and data extraction

The screening of papers was carried out in two steps, primary screening based on title and abstract, and after that the full-text screening of the potentially eligible papers for final selection. In each step, two independent investigators extracted the needful data from eligible studies. Any discrepancies were resolved in discussion with a third researcher. The following data was isolated from each paper: 1) citation, 2) animal species, 3) sample size, 4) animal weight range, 5) intervention/exposure inducing sperm dysfunction and male infertility, 6) dose of ALA per each utilization, 7) intervention duration, 8) measurement site, and 9) evaluated main outcomes and results. Finally, all the extracted information was investigated by other authors. This research did not require ethical consent.

2.5. Risk of bias assessment

A systematic evaluation of the included articles was carried out in accordance with the SYStematic Review Center for Laboratory animal Experimentation Risk of Bias (SYRCLE's RoB) tool criteria [12]. "The risk of bias was then assessed using this tool as a judgment (including low, high, and unclear risk) in the following ten domains based on Six types of bias: 1) Sequence generation (selection bias); 2) Baseline characteristics (selection bias); 3) Allocation concealment (selection bias); 4) Random housing (performance bias); 5) Blinding (performance bias); 6) Random outcome assessment (detection bias); 7) Blinding (detection bias); 8) Incomplete outcome data (attrition bias); 9) Selective outcome reporting (reporting bias); 10) Other sources of bias (other)". Two investigators carried out quality evaluations apart, and disputes were tabled with a third researcher. The risk of bias was evaluated by the Cochrane Collaboration risk-of-bias tool in RevMan 5.3 statistical software.

2.6. Methodological quality assessment

The methodological quality of included papers was evaluated according to the CAMARADES checklist [13]. According to this checklist, "the indicators of quality are based on ten criteria: 1) peer-reviewed publication, 2) statement of control of temperature, 3) random allocation to treatment or control, 4) blinded caregiver/investigator, 5) blinded assessment of outcome, 6) use

of co-interventions/co-morbid, 7) appropriate animal model (age, sex, species, strain), 8) sample size calculation, 9) compliance with animal welfare regulations and 10) statement of potential conflict of interests". For each study was determined a quality score out of a possible total of 10 points. Ultimately, the authors estimated the mean score and classified studies into "low quality" for mean scores 1–5 and "high quality" for mean scores 6–10.

3. Results

3.1. Study inclusion

A total number of 11,787 studies were pinpointed via exploring the databases for systematic review. After that, articles were removed before the start of screening because of the following causes: 1) duplicate records, 2) clinical trials, 3) abstracts, 4) book chapters, 5) reviews, 6) letters and comments, 7) theses and dissertation, 8) non-Related studies, 9) non-English studies, 75 papers remained. In titles and abstract screening, 41 studies were excluded owing to the following reasons: 1) duplicate records ($n = 3$), 2) irrelevance ($n = 3$), 3) reviews ($n = 5$), 4) human studies ($n = 12$), 5) non-rodent studies ($n = 13$), 6) irrelevant population ($n = 5$). After, full-text papers screening of the 34 remaining studies showed that 11 studies were unqualified due to the following reasons: 1) complex intervention ($n = 6$), 2) full article not available ($n = 2$), 3) *in vitro* studies ($n = 2$), and 4) insufficient information ($n = 1$). Finally, 23 eligible articles were involved in this systematic review. The study selection process is revealed in Fig. 1.

3.2. Study characteristics

The features of the 23 eligible articles and their results are summarized in Table 1. The 23 qualified published studies involved 938 animals until December 2022. Of note, 100 % of the experiments in the studies were performed on male rodents, of which the studies used Swiss albino and NMRI mice, and Wistar, Swiss albino, Sprague-Dawley, and Brown Norway rats, 2 studies (8.7%) used mice, and 21 studies (91.3%) used rats. The number of all animals in the experimental group was 735 and that in the control group was 203. In all studies, the mice's weight ranged from 20 to 30 g, and the rat's weight ranged from 120 to 310 g. Various doses of ALA were employed in these studies and the dose varied from 10 to 300 mg/kg, and one study stated the dose of ALA as a percent of the diet. Control groups in the main included similar

solvents, such as normal saline, distilled water, tap water, corn oil, citrate buffer, and no treatment. Ten studies (43.5%) used normal saline as the control group, four studies (17.4%) chosen distilled water, two studies (8.7%) used tap water, three studies (13.0%) used corn oil, one study (4.4%) selected citrate buffer, and the remaining 3 studies (13.0%) had no treatment. The duration of intervention was ≥ 8 weeks in 11 studies (47.8%) and < 8 weeks in 12 studies (52.2%) (from 24 h to 16 weeks). Sperm quality parameters were evaluated in 15 (65.2%), sperm DNA damage in 4 (17.4%), antioxidant status in 17 (73.9%), LPO in 17 (73.9%), 8-OHdG in 2 (8.7%), steroidogenesis and testosterone levels in 13 (56.5%), testicular histopathology in 18 (78.3%), epididymal histology in 3 studies (13.0%), and apoptosis in 1 study (4.4%); only 2 studies (8.7%) evaluated the sperm fertilizing efficacy.

3.3. Risk of bias

A brief of the risk of bias assessment of included articles per the SYRCLE risk of bias tool was provided in Fig. 2. None of the papers reported the random allocation method of animals to the control or treatment groups, allocation concealment, random housing, blinded investigators, random outcome assessment, and blinded outcome assessor except one paper [14]. Nevertheless, 17 studies (73.9%) had an unclear risk of bias about random sequence generation, because we did not find an adequate description for random sequence generation, and the remaining 6 studies did not report the random allocation. Of note, ten (43.5%) studies reported the complete outcome data of animals. Whereas, the risks of reporting bias in included studies were low. Results of this study indicated that all studies did not pay extra attention to the presence of unit-of-analysis errors as other biases.

3.4. Methodological quality

The methodological quality score of included studies was displayed in Table 2. As per the CAMARADES quality score, the score of quality of more than half of the papers (56.5%) in this review was 6 with an average score of 5.87, in summary, these papers possessed poor methodological quality. It is interesting that 21 studies (91.30%) reported papers in peer-reviewed journals. However, no one of these studies explained the random allocation method of animals to the control or treatment groups, blinded caregiver/investigator, blinded assessment of outcome, and sample size calculation. Only one study (4.35%) expressed the blinded assessment of the outcome [15]. 22 studies (95.7%) reported a statement of control of temperature, 20

studies (87%) stated consent with animal welfare regulations, and only 10 studies (43.5%) provided notice of conflict of interest.

4. Discussion

The present study was a systematic review of the effect of ALA on sperm functions in rodent models for male infertility. As far as we know, several studies have been performed to investigate the protective and antioxidant effects of ALA on human and animal sperm functions, and in according to our comprehensive research, no systematic review or meta-analysis studies have been executed to address this subject in rodent models thus far. Former systematic reviews and meta-analysis have evaluated the effects of ALA supplementation on endothelial function [16], glycemic control [17], dyslipidemia improvement [18], human infertility [19], metabolic syndrome [20], central nervous system disorders [21], and obesity [22].

Worldwide infertility impacts nearly 8–12% of couples, with male factors recognized as the principal reason in 50% of cases. Moreover, about 7% of all men are influenced by male infertility all around the world [23]. Male infertility can be thanks to a variety of causes, however, in almost 40% of infertile men, there is no obvious etiology. Several factors dispose to male infertility consist of hormonal deficits, physical problems, sexually transmitted disorders, environmental pollutants like pesticides and chemicals, and lifestyle and genetic factors [24]. Massive evidence proposes the major role of OS in the male infertility etiology, and near 30–80% of infertile men produce high seminal ROS levels [25]. Therefore, OS is one of the central mechanisms involved in mediating the deleterious effects of hypertension, obesity, dyslipidemia, insulin resistance, certain mental disorder, elongated ejaculatory abstinence period [26], varicocele [27], and environmental contaminants [28] which can impair male reproductive functions. Male oxidative stress infertility (MOSI) has recently influenced 37.2 million men with idiopathic infertility [29].

With an enlarging confirmation of the role of OS in male infertility pathophysiology, antioxidant supplementation is suggested as one of the medical options for men with idiopathic infertility [30]. ALA is a critical cofactor for numerous enzymes that intercede energy generation, including synthesis of acetyl-CoA, fatty acid metabolism, tri-carboxylic acid cycle (conversion of α -ketoglutaric acid to succinyl-CoA), and synthesis of nucleotides [14]. Besides, ALA performs the role of an antioxidant through its scavenge free radicals' ability directly and

endogenous antioxidants regeneration, like coenzyme Q10 (CoQ10), vitamin E, vitamin C, and glutathione (GSH), and chelation of transition metal ions (iron and copper) [2, 31].

On account of high lipophilicity and tiny size, ALA suppresses the free radicals in both hydrophilic and lipophilic environments, and both its reduced (di- thiol: dihydro- lipoic acid, DHLA), and oxidized (disulfide) structures act as potent antioxidants [32]. The therapeutic efficacy of antioxidants against induced reprotoxicity is extensively acknowledged. Between a variety of different antioxidants, ALA has been known as a global antioxidant, and based on its extraordinary characteristics, numerous studies described the effectiveness of ALA on the male reproductive system health against various chemicals [2, 6, 8, 14, 32-33] wherein OS is mediated in the male infertility etiology.

4.1. Effect of ALA treatment on sperm quality parameters

Sperm quality parameters like concentration, motility, viability, and normal morphology, as the most important clinical factors, were studied in 15 of the articles we reviewed [2, 6, 8, 14, 32, 33, 35-37, 39, 42, 45, 48-50]. Reviewed papers showed that different chemicals exposure or interventions resulted in a decrease in sperm quality parameters, and instead, sperm concentration, motility, viability, and morphology were reinstated to normal values by ALA administration. Sperm motility mainly depends on three major factors: regulation, structural integrity, and the supply of energy. Sperm motility or movement is regulated at the mid and principal pieces. Each of these pieces controls a unique sperm movement function. The mid piece of sperm flagellum handles the motility activation, whereas the principal piece controls hyperactivation [51]. The unsaturated fatty acids and protein channels in the mid piece of sperm flagellum usually are the first option for the ROS assault. One of the suggested mechanisms for ALA is as follows: it creates a robust shield of the cell membrane and increases the sperm's capability to endure higher volumes of free radical assault, thereby conserving sperm motility and viability [1, 32].

In addition, the sperm movement rate is in the main dependent on its energy supply accessibility. Hence, normal active sperm generally possess mitochondria with very active functioning, which in turn produce high free radical amounts [33]. The continuous ATP production is related to the structural integrity maintenance of mitochondria, since the mitochondrial membrane and its different compartments have high lipid substance and are prone

to steadily increasing ROS as a Krebs cycle by-product [32, 52]. Therefore, the effects of ALA administration on sperm concentration and motility may be attributed to assisting in the oxidative decarboxylation metabolism as a co-enzyme. The enhance in oxidative decarboxylation would increase cytochrome C level and therefore improve the mitochondrial membrane potential, function, and biogenesis [1, 33].

4.2. Effect of ALA treatment on sperm DNA damage

As illustrated in six studies [2, 6, 32, 42, 47, 49], different chemical exposures or interventions induced testes and epididymal sperm DNA damage compared to that of the control, and treatment of rodents with ALA, resulting in complete prevention of the enhancement in the levels of DNA damage in the testes and epididymal sperm. Another matter to take into account is that in the six articles included in this systematic review various techniques were used to assess sperm DNA damage including the 8-OHdG measurement by HPLC [47] or ELIAS [2] techniques, measurement of DNA strand breakage by fluorimetric analysis of DNA unwinding (FADU) assay [6, 47], measurement of DNA fragmentation by agarose gel electrophoresis [42], acridine orange (AO) staining [32], and comet assay [49], which measures the susceptibility of sperm nuclear DNA to OS, which generates ROS that can induce DNA denaturation and fragmentation [47], and one of the main factors influencing male infertility is DNA damage of sperm.

One of the suggested possible mechanisms for ALA is that ALA has the potential to protect DNA repairing capability through its ability to upregulate the proliferating cell nuclear antigen (PCNA) as a key DNA repair protein, via the P53 pathway [53], therefore, if sperm DNA-repair capacity, owing to ALA treatment, surpasses the DNA oxidation, then DNA damage will be inhibited, leading to improved genome constancy and normal cell division [42].

4.3. Effect of ALA treatment on sperm antioxidant capacity

Endogenous sources of ROS consist of immature sperm cells and leukocytes in semen and mitochondria in spermatozoa [54]. Exogenous sources of ROS consist of infections and diseases such as varicocele. Moreover, lifestyle and environmental factors have been proposed to contribute to the excessive generation of ROS in semen. These factors include cigarette smoking, alcohol consumption, and radiation or pollution exposure [26]. When the levels of ROS exceed

the physiological levels leading to the alteration of sperm traits by inducing LPO, sperm proteins oxidation, and sperm DNA fragmentation (SDF), leading to male infertility [29]. Seventeen of 23 studies included in our review showed that the testicular toxicants underlie OS to intervene with functions of the male reproductive system [2, 6, 8, 14, 34, 36-44, 46, 48, 49].

In these papers, different important biomarkers are used for the evaluation of OS status. Malondialdehyde (MDA) is one of the end-products of cell LPO. Thus, an enhancement in the level of MDA reflects an overwhelming production of ROS. Protein carbonyl levels are widely analyzed as a measure of protein oxidation. The considerable increase in the levels of LPO and protein carbonyl with a simultaneous decrease in the enzymatic antioxidants activity levels such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), Phospholipid hydroperoxide glutathione peroxidase (PHGPx), and glutathione reductase (GR), and non-enzymatic antioxidants like GSH in the testes and epididymis might be a sign of defeat of defense mechanisms against the oxidative damage stimulated by various toxic conditions. In the included studies, ALA significantly ameliorated OS and reproductive indicators, which could illustrate an efficient approach for preventing male infertility. This was revealed by decreased levels of LPO and protein carbonyl content with normalized enzymatic and non-enzymatic antioxidants and preservation of normal reproductive functions in the ALA-treated rodents. Different from endogenous ALA, which is completely protein-bound and may only function as a co-enzyme for mitochondrial reductases, supplementation of ALA may lead to enhanced unbound ALA and exerts ubiquitously antioxidant activity.

Various antioxidant properties of unbound ALA [32] include 1) the capability to ward off ROS and reactive nitrogen species (RNS) in both aqueous and lipid environments; 2) transition metals chelation; 3) vital antioxidants regeneration like GSH, vitamin C, and E; 4) increased enzymes activity like SOD, GPx, and CAT [49]. DHLA has more antioxidant possessions than ALA, it also scavenges superoxide and peroxy radicals produced in various metabolic processes such as LPO [55], also, DHLA increases cysteine uptake by cells, leading to enhanced synthesis of GSH, and can reduce ascorbyl radicals and dehydroascorbate produced in the ascorbate oxidation by tocopheroxyl radicals, therefore synergistically increasing the tocopherol recycle [6].

ALA treatment by its iron ions chelating and free radicals scavenging activity alleviates the singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\text{OH}\cdot$) [55], also ALA by increasing cells' glucose uptake which performs as a fuel for the oxidative phosphorylation and pentose phosphate pathway, therefore enhancing the cellular NADPH levels [49, 56]. Increased NADPH might seem responsible for a compensation of toxins-induced decrease in the testicular and epididymal SOD and CAT activities [6, 8, 36-38, 41-44, 48, 49], and seems to enhance the GPx activity via stimulation of intracellular GSH synthesis as a substrate for GPx, and also through an increase of GR activity [57] in induced animals.

Furthermore, the nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulator of the antioxidant response. This transcriptional factor regulates the gene heme oxygenase-1 (HO-1) for starting mechanisms of cellular defense [58]. It was found that the antioxidant activity of ALA was mediated by the Nrf2/HO-1 pathway activation, which is also identified to be associated with the expression of other antioxidant proteins, including SOD, GPx, and CAT [59, 60]. Therefore, the included studies confirmed the ALA antioxidant effects against testicular and epididymal oxidative stress induced by various toxins (Fig. 3).

4.4. Effect of ALA treatment on testicular inflammatory capacity

Inflammation in the male reproductive system can be triggered and facilitated by different factors including varicocele, alcohol, obesity/metabolic syndrome, leukocytospermia, bacterial infection (lipopolysaccharide, or LPS), prostatitis, microorganism mutations, viral infections [61], environmental pollutants [34], and radiation [14].

The nuclear factor κB (NF- κB) regulates several genes involved in different processes of the immune functions and inflammatory responses. NF- κB stimulates the expression of different pro-inflammatory genes such as those coding cytokines and chemokines and contributes to inflammasome regulation [62]. Pathogen/microbial-associated molecular patterns (PAMPs/MAMPs), damage-generated molecular patterns (DAMPs), or lifestyle-associated molecular patterns (LAMPs) molecules are recognized by the immune system and released by damaged cells [63].

Pattern recognition receptors (PRRs) have a vital role in the correct innate immune system function and recognize different types of PAMPs and DAMPs [64]. Five families of

PRRs express in mammalian cells including 1) toll-like receptors (TLRs), 2) RIG-I-like receptors, 3), NOD-like receptors (NLRs), 4) C-type lectin-like receptors, and 5) cytosolic DNA sensors [62]. PRRs ligation on sentinel cells causes the generation of proinflammatory cytokines (e.g., tumor necrosis factor (TNF)- α and interleukin (IL)-1), vasoactive amines (e.g., serotonin and histamine), nitric oxide (NO), ROS, neuropeptides, and arachidonic acid (AA) metabolites (e.g., prostaglandins (PGs) and leukotrienes) [65]. The canonical NF- κ B pathway activation is an usual signaling incident of the PRRs, which is responsible for transcriptional induction inflammation development, and progression in different innate immune cells [66].

Four of 23 of the included papers studied inflammation and found that inflammatory markers including IL2 and NO [2], prostaglandins (PG) E2 and F2 α [34], interleukin-1 β (IL-1 β), and TNF- α [37], and interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), IL-10, NF- κ B, transforming growth factor- β (TGF- β), and peroxisome proliferator-activated receptor- γ (PPAR- γ) [14] were markedly stimulated in toxic conditions compared to the controls, and treatment with ALA could ameliorate testicular inflammation induced by toxic conditions when compared with those not receiving ALA.

It is found that NF- κ B activators such as ROS can also provoke priming signals of NLR family pyrin domain containing 3 (NLRP3) inflammasome and pro-IL-1 β activation in response to different PRR ligands and cytokines [62]. The NLRP3 inflammasome is a key component of the innate immune system that intercedes caspase-1 activation and the pro-inflammatory cytokines IL-1 β /IL-18 secretion in response to microbial infection and cellular damage [67]. Several processes of NLRP3 inflammasome activation induced by ROS include mitochondrial dysfunction, apoptosis, and endogenous thioredoxin inhibition [68].

Moreover, it has been revealed that alteration of DNA base as a result of ROS attack can induce inflammation. A signaling cascade is triggered by the base excision repair of oxidatively damaged DNA base (8-hydroxy-2'-deoxyguanine, 8-OHdG) by the 8-oxoguanine-DNA glyoxalase-1, which results in the NF- κ B pathway activation, stimulating expression of pro-inflammatory genes, and the inflammatory cells' accumulation [69]. It has been found that the 8-iso-prostaglandin F2 α (8-iso-PGF2 α), an end-product of AA and a marker of OS, can enhance the expression of the pro-inflammatory chemokine IL-8 via the mitogen-activated protein kinases (MAPK) activation [70]. In OS, NF- κ B up-regulates iNOS, leading to an enhancement of

NO production. Besides, a high NO level can cause NF- κ B up-regulation, thus inducing an inflammatory signaling cascade [71]. Therefore, these findings explain how inflammation and OS interlink in male infertility pathogenesis.

One of the targets involved in the shielding effects of ALA for toxic conditions is via quelling NF- κ B-mediated inflammation [72-74]. The anti-inflammatory effect of ALA is shown by the NF- κ B downregulation, which consequently controls the downregulation of IL-6 and COX-2 expression together with a considerable enhance in testicular IL-10 level [14]. Furthermore, the previous reports indicate that inhibitor kappa B kinase 2 (IKK2) inhibition may be a critical mechanism for ALA inhibiting NF- κ B [75].

Besides, ALA via down-regulating NF- κ B is credited to the decrease in the expression of iNOS and resulted in decreases in the testicular NO level. A different description for the NO levels reduction might be owing to the direct scavenging effect of NO by the ALA sulphydryl groups. Moreover, treatment of ALA produced an effective decline in the PGE2 and PGF2a production and their catabolic rate, resulting in a favorable anti-inflammatory state imaged at a systemic level. The ALA anti-inflammatory effect probably exists in the fact that COX-2 and phospholipase A2 (PLA2) – the main substrate sources for the biosynthesis of prostaglandin – are both repressed by ALA [34]. In brief, it seems ALA by inhibition of the NF- κ B pathway leads to suppressing the activation of the NLRP3 inflammasome, subsequently reducing cytokines production and inflammatory responses [76] in rodent models for male infertility (Fig. 4).

4.5. Effect of ALA treatment on steroidogenic enzymes and testosterone levels

The androgenic events are associated with the activity of the main enzymes embroiled in testosterone biosynthesis, 3 β -hydroxy steroid dehydrogenase (3 β -HSD) and 17 β -hydroxy steroid dehydrogenase (17 β -HSD). Both enzymes catalyze the main steps in the androgenic pathway and are affected by toxic conditions [34]. Thirteen of the 23 articles studied testicular steroidogenic enzymes activities and serum testosterone levels, in the included studies, the activities of 3 β -HSD [34, 42, 47-49] and 17 β -HSD [34, 47-49] steroidogenic enzymes, and serum testosterone levels [8, 14, 33, 34, 36, 37, 39, 40, 42, 43, 47-49] were significantly decreased in induced animals, and ALA treatment along with all toxic conditions could modulate the alterations in androgenesis when compared to the toxins-treated animals alone.

As is well-known, testosterone is the major androgen in males, and Leydig cells are the primary supply of testosterone in the testes. The biosynthesis of testosterone from cholesterol consists of several steps of enzymatic reactions, including cholesterol channeling and cholesterol-to-testosterone conversion. Steroidogenic acute regulatory protein (StAR) is a mitochondrial protein that moves cholesterol from the outer to the inner mitochondrial membrane, while the cholesterol-to-testosterone conversion is catalyzed by 3β - and 17β -HSDs that is dependent on NADPH [77]. A decline in the 3β - and 17β -HSDs activities [34, 47-49] and the level of StAR mRNA expression [49] in the testes of toxins-exposed animals may associate with the accumulation of cholesterol, and appear that the HSDs and StAR proteins could be vulnerable targets of toxins-induced reprotoxicity. These occurrences afterward lead to improper channeling of cholesterol and reduced steroidogenesis thereby diminishing serum testosterone levels.

Glucose-6-phosphate dehydrogenase (G6PD) is another testicular important enzyme and plays a key role in the metabolism of glucose, accompanied by NADPH production which is supplied by the G6PD in the pentose phosphate pathway [78]. G6PD is also directly associated with GSH metabolism and the stabilization of catalase. GR catalyzes the reduction of GSSG to GSH, and this reduction needs NADPH. GSH and catalase have a main role in the detoxification and neutralization of ROS [79]. Therefore, the decreased activity of G6PD diminishes the NADPH availability, which in turn origins a reduction in the GR activity that can cause increased OS in the testicular tissues and then sperm death [44].

The protective effects of ALA in the restoration of testicular steroidogenic enzymes (3β - and 17β -HSDs), levels of serum testosterone, expression of StAR mRNA, testicular cholesterol, and G6PD activity against a range of testicular toxicant-induced oxidative damage, are through increased cell glucose uptake as a fuel for the pentose phosphate pathway, this might be the possible reason for increased testicular G6PD activities and thus enhancing the NADPH cellular levels in induced animals treated with ALA [33, 44]. Thus, these results confirm the antioxidant and/or steroidogenic effects of ALA at the Leydig cells level in induced rodent models.

4.6. Effect of ALA treatment on testicular architecture

Eighteen of 23 articles that studied testicular architecture found that toxic conditions can alter the histological structure of the testes. Histological studies demonstrated seminiferous

tubules atrophy [8, 14, 36, 39-41], hypospermatogenesis [2, 8, 34, 36, 37, 40, 45, 49], seminiferous tubule hyalinization [40], sloughed epithelial cells [37, 40, 41], dilation of interstitial blood vessels [2], damage to the germinal cell series and interstitial (Leydig) cells [34, 40, 41], vacuolization of germinal epithelium and Sertoli cells [8, 14, 38-41, 46], coagulative necrosis [40], irregularities in the arrangement of epithelium [8, 14, 37, 43, 48, 49], separation germinal epithelium from the basement membrane [14, 39, 49], enhancement of the volume of interstitial spaces accompanied by edematous regions [8, 14, 41], congestion of blood vessels [2, 14, 41], and hemorrhage [14] in induced animals. On the other hand, treatment with ALA restored the architecture of the testes to normal in animals exposed to toxic conditions.

ROS-induced LPO plays a vital role in cell signaling, including apoptosis, autophagy, and ferroptosis [80]. Ferroptosis is an oxidative, iron-dependent, and nonapoptotic form of regulated cell death, and is mainly distinguished by intracellular accretion of lipid hydroperoxides arising from an iron-dependent ROS accumulation [81], and it is initiated by the loss of the lipid repair enzyme activity, GPx4, and/or cysteine uptake inhibition, leading to GSH reduction and lethal ROS accumulation [82]. Pathways for abnormal iron metabolism may initiate ferroptosis in at least two ways involving iron-mediated ROS production through the Fenton reaction [83] and the other is included the activation of iron-containing enzymes, such as lipoxygenase (ALOX). Finally, the accretion of iron causes LPO, leading to subsequent cell membrane damage and rupture [84]. Multiple chemicals and drugs have been recognized that target the process of LPO to induce ferroptosis [85].

In a separate study, arsenite as a male reproductive toxicant significantly activated ferroptosis-related signal pathways in the mouse testes, revealed by the accumulation of iron, ROS production, and LPO products [86]. A previous study has stated that the toxic effect of cadmium on testes can be adjusted by iron tissue concentration [87], which may induce ferroptosis in the testes. Fine particulate matter (PM_{2.5}) as one of the most common pollutants can cause dysfunction of Sertoli cells exposed to PM_{2.5} through excessive ROS and ferroptosis through the overload of iron and LPO [88]. Lately, it has been found that the mechanism of di-(2-ethylhexyl) phthalate to motive male infertility is ferroptosis in mouse testes [89]. These findings reveal that inhibition of ferroptosis may be a potential strategy for reproductive toxicity treatment. Therefore, ALA in the role of a potent free radical scavenger, an iron chelator, a

regenerator for GSH, vitamin C and, vitamin E, an agent for cystine uptake, an activator of antioxidant enzymes such as SOD, GPx, and CAT, and an inhibitor of inflammation is a very promising antioxidant that can combat ferroptosis [90] by reducing ROS or reversing oxidative damage (Fig. 5).

4.7. *Effect of ALA treatment on male fertility efficacy*

Two of the 23 articles that studied rodents' reproductive performance found that chemical-exposed rodents took more mating trials to impregnate the females compared with the controls [48, 49]. The mating index and the number of live pups [48, 49] were decreased in female mice cohabiting with chemical-exposed males. But, treatment with ALA reduced the number of mating trials and improved the mating index and number of live pups in induced males as compared with the chemical-exposed males alone. Therefore, chemicals suppressed fertility efficacy in male rodents due to the inhibited testicular steroidogenesis, diminished sperm quality parameters, induced inflammation, and also due to testicular and cauda epididymal OS. Taken together, ALA through its antioxidant [2, 6, 8, 14, 34, 36-44, 46, 48, 49] anti-inflammatory [2, 14, 34, 37], anti-ferroptosis [90], metals chelation [2, 31], and steroidogenic properties [8, 14, 33, 34, 36, 37, 39, 40, 42, 43, 47-49] can restore male reproductive performance by improvement of testicular architecture, sperm concentration and motility, testicular steroidogenic enzymes and testosterone levels against different types of testicular toxicants which induce oxidative damages in rodent models.

5. Conclusion

This systematic review presents persuasive evidence for the protective effect of ALA in rodent models for male infertility by re- establishment of spermatogenesis and steroidogenesis, and also by maintaining redox and immune systems homeostasis. The protective effect of ALA was not different in low dosage (<50 mg/kg) and high dosage ($\geq$ 50 mg/kg); thus, this study can suggest only the beneficial antioxidant effects of ALA in rodents with reproductive toxicity. It should be noted that based on SYRCLE's risk of bias tool for animal studies, the rodent studies of male infertility published within the last eighteen years, contain a high risk of bias, and we could not suggest the best dosage and duration for both animals and humans. Therefore, more double- blind placebo- controlled randomized clinical trials are necessitated to assess the protective effect of ALA and to clarify the effective dose and duration in health or disease. This

conclusion must be expounded in beam of the strong high risk of bias and poor methodological quality of animal studies. To improve clinical generalizability, later investigations should carry out preclinical studies designed with random allocation, blinding of investigators and assessors, and power sample size calculation.

Author contributions

M. H. Nasr-Esfahani, M. Tavalaei, N. Naderi, and Z. Darmishon Nejad provided literature searches, reviewed and prepared the main outline of the manuscript, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Fig. 1. PRISMA flow diagram of the study selection

Fig. 2. Quality Assessment. A: Risk of bias summary, review authors' judgments about each risk of bias item for each included study (green for low risk of bias, yellow for unclear risk of bias, and red for high risk of bias). B: Risk of bias graph: Review authors' judgments about each risk of bias item presented as percentages across all included studies.

Fig. 3. Schematic description of antioxidant mechanisms of ALA and its derivatives through the activation of the Nrf2/ARE signaling pathway, ROS/RSN quenching activities, chelation of transition metals, and regeneration of important antioxidants such as GSH, CoQ10, vitamin C, and vitamin E. ALA: Alpha-lipoic acid; DHLA: Dihydrolipoic acid; Vit. E: Vitamin E; Vit. C: Vitamin C; GSSG: Glutathione disulfide; GSH: Glutathione; GPx: Glutathione peroxidase; GR: Glutathione reductase; NADPH: Nicotinamide adenine dinucleotide phosphate; CAT: Catalase; SOD: Superoxide dismutase; HO-1: Heme oxygenase-1; ROS: Reactive oxygen species; mtROS: mitochondrial reactive oxygen species; KGDH: Alpha-ketoglutarate dehydrogenase; NFR2: nuclear factor erythroid 2-related factor 2; ARE: antioxidant response element; CoQ10: Coenzyme Q10; NO: Nitric oxide; H₂O₂: Hydrogen peroxide; O₂⁻: Superoxide R: reduced; O: oxidized.

Fig. 4. Schematic description of anti-inflammatory mechanisms of ALA and its derivatives by down-regulating of NF-κB attributed to inhibition of the NLRP3 inflammasome activation, reduction of iNOS expression, inhibition of the COX-2 and PLA2, and subsequently reducing cytokine production. ALA: Alpha-lipoic acid; DHLA: Dihydrolipoic acid; NO: Nitric oxide; PGE2: Prostaglandin E2; AA: Arachidonic acid; iNOS: Inducible nitric oxide synthase; COX-2: Cyclooxygenase-2; PLA2: Phospholipases A2; IL-1β: Interleukin 1 beta; IL-18: Interleukin-18; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; NF-κβ: Nuclear factor kappa beta; IKK2: Inhibitor kappa B kinase 2; MAPK: Mitogen-activated protein kinases; PRRs: Pattern recognition receptors; PAMPs: Pathogen-associated molecular patterns; MAMPs: Microbe-associated molecular patterns; DAMPs: Damage-associated molecular patterns; LAMPs: lifestyle-associated molecular patterns.

Fig. 5. Schematic description of anti-lipid peroxidation and anti-ferroptosis mechanisms of ALA and its derivatives by GSH-dependent GPx4 pathway, chelation of iron, enhancement of cystine uptake, and regeneration of GSH and vitamin E. ALA: Alpha-lipoic acid; DHLA: Dihydrolipoic acid; Vit. E: Vitamin E; Vit. C: Vitamin C; GSSG: Glutathione disulfide; GSH: Glutathione; GPx4: Glutathione peroxidase 4; NADPH: Nicotinamide adenine dinucleotide phosphate; ROS: Reactive oxygen species; mtROS: mitochondrial reactive oxygen species; KGDH: MDA: Malondialdehyde; LOX: Lipoxygenases; PL-PUFA-OH: Phospholipid polyunsaturated fatty acid alcohols; PL-PUFA-OOH: Phospholipid polyunsaturated fatty acid peroxides; H₂O₂: Hydrogen peroxide; O₂⁻: Superoxide; R: reduced; O: oxidized.

Table 1. Characteristics of the included studies

N o.	Citation	Species	SS Int/Ctrl	Weight (g)	Int/Exp induced SDF	ALA dose	Duration	Ctrl	SOM	Measured outcomes and Results
1	Prahalathan et al., 2004	Wistar rat	6/6	120±10	Adriamycin	35	10 W	Normal saline	Testis	↑ AOC ↓ LPO
2	Prahalathan et al., 2005	Wistar rat	6/6	140±10	Adriamycin	35	10 W	Normal saline	Testis and epididymis	↑ SQA ↓ TES.Ab
3	Prahalathan et al., 2006	Wistar rat	6/6	140±10	Adriamycin	35	10 W	Normal saline	Testis	↑ AOC ↓ TES.Ab
4	Prahalathan et al., 2006	Wistar rat	6/6	140±10	Adriamycin	35	10 W	Normal saline	Testis, epididymis, and serum	↓ DNAD ↑ STG
5	Selvakumar et al., 2006	Wistar rat	6/6	140±20	Cyclophosphamide	35	10 W	Normal saline	Testis and epididymis	↑ SQA ↓ DNA.D ↑ AOC ↓ LPO
6	Ashour et al., 2011	Swiss albino rat	10/10	200–250	Lipopolysaccharide	200	24 h	Normal saline	Testis, epididymis, and serum	↑ SQA ↓ DNA.D ↑ AOC ↓ LPO ↓ INF ↓ TES.Ab
7	Othman et al., 2012	Wistar rat	8/8	120±20	4-tert-octylphenol	50	5 W	Any treatment	Testis, epididymis, and serum	↑ SQA ↓ DNA.D ↑ AOC ↓ LPO ↑ STG ↓ TES.Ab
8	Yeni et al., 2012	Sprague-Dawley rat	8/8	260–310	Thinner	100	8 W	Distilled water	Testis and epididymis	↑ SQA
9	Astiz et al., 2013	Wistar rat	6/6	190±20	Pesticides	25, 50 and 100	5 W	Normal saline	Testis, epididymis, and plasma	↑ AOC ↓ LPO ↓ INF ↑ STG ↓ TES.Ab

10	El-Beshbishy et al., 2014	Wistar rat	6/6	170±10	Bin-Butyl Phthalate	20	2 W	Corn oil	Testis, epididymis and serum	↑ SQA ↑ AOC ↓ LPO ↑ STG ↓ TES.Ab
11	Lebda et al., 2014	Rat	10/10	210 ± 7	Acrylamide	1%	4 W	Any treatment	Testis, epididymis and serum	↑ AQC ↓ LPO ↑ STG ↓ TES.Ab
12	Güleş And Eren., 2016	Wistar rat	8/8	180–200	polychlorobiphenyl	25 mg/kg	4 W	Any treatment	Testis, epididymis and serum	↑ AOC ↓ TES.Ab
13	Al-Azawi and Asker., 2017	Swiss albino mice	5/5	28-30	Nitrofurantoin or Methotrexate	10 mg/kg	6 W	Distilled water	Epididymis and serum	↑ SQA ↓ LPO ↑ STG
14	EL-Maddawy and Abd El Naby., 2018	Brown Norway rat	16/16	-	Ivermectin	50 µg/kg	8 W	Distilled water	Testis, epididymis and serum	↑ SQA ↑ AOC ↓ LPO ↑ STG ↓ TES.Ab
15	Pinar et al., 2018	Wistar rat	10/10	250-300	Methotrexate	100mg/kg	10 days	Normal saline	Testis and serum	↑ AOC ↑ STG ↓ TES.Ab
16	Prathima et al., 2018	Wistar rat	10/10	180–210	Sodium arsenite	70 mg/kg	10 W	Tap water	Testis, epididymis and serum	↑ SQA ↑ AOC ↓ LPO ↑ STG ↓ TES.Ab ↑ FERT
17	Shayganinia et al., 2018	Wistar rat	10/10	241.4±8.9	Varicocele induction	300mg/kg	16 W	Tap water	Testis and epididymis	↑ SQA ↓ DNA.D ↓ LPO ↓ TES.Ab
18	Corrêa et al., 2019	Wistar rat	8/8	250	Streptozotocin	300mg/kg	8 W	Citrate buffers	Testis and epididymis	↑ SQA ↓ TES.Ab
19	Goudarzi et al., 2020	NMRI mouse	10/10	20-30	Di-2-ethylhexyl phthalate	20mg/kg	2 W	Normal saline	Testis, epididymis and serum	↑ SQA ↑ AOC ↓ LPO ↓ INF ↑ STG ↓ TES.Ab

20	Moustafa et al., 2020	Albino rat	8/8	140-170	Cyclophosphamide	25 mg/kg	4 W	Normal saline	Testis	↑ AOC ↓ LPO ↓ TES.Ab
21	Prathima et al., 2020	Wistar rat	10/10	185±10	linuron	70 mg/kg	8 W	Corn oil	Testis, epididymis and serum	↑ SQA ↑ AOC ↓ LPO ↓ DNA.D ↑ STG ↓ TES.Ab ↑ FERT
22	Said et al., 2020	Sprague-Dawley rat	10/10	120–150	Ionizing radiation	50mg/kg	1 W	Corn oil	Testis, epididymis and serum	↑ SQA ↑ AOC ↓ LPO ↓ INF ↑ STG ↓ TES.Ab
23	Ibrahim et al., 2021	Wistar rat	10/10	200-250	Carbimazole	60 mg/kg	4 W	Distilled water	Testis, epididymis and serum	↑ SQA ↑ AOC ↓ LPO ↑ STG ↓ TES.Ab

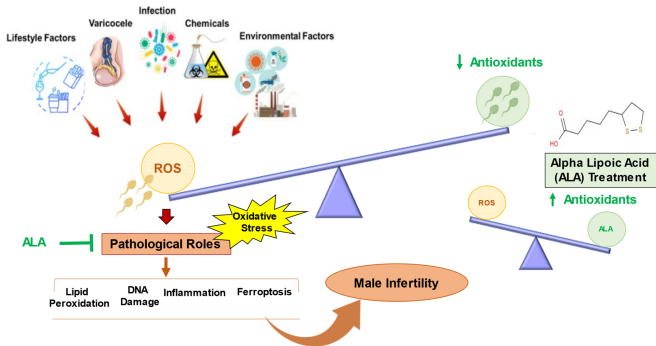
Sampler size: SS; Int: intervention; Ctrl: Control; Exp: Exposure; SDF: Sperm dysfunction; SQA: Sperm quality; DNA.D: DNA damage; AOC: Antioxidant capacity; LPO: Lipid peroxidation; INF: Inflammation; STG: Steroidogenesis; TES.Ab: Testicular abnormality; FERT: Fertility efficacy; SOM: Site Of measurement.

Table 2. Methodological quality of included studies

No.	Citation	1	2	3	4	5	6	7	8	9	10	Total score
1	Prahalathan et al., 2004	*	*	*			*	*		*		6
2	Prahalathan et al., 2005	*	*	*			*	*		*		6
3	Prahalathan et al., 2006a	*	*	*			*	*		*		6
4	Prahalathan et al., 2006b	*	*	*			*	*		*		6
5	Selvakumar et al., 2006	*	*	*			*	*		*		6
6	Ashour et al., 2011	*	*	*			*	*			*	6
7	Othman et al., 2012	*	*	*			*	*		*		6
8	Yeni et al., 2012	*	*	*			*	*		*		6
9	Astiz et al., 2013	*	*	*			*			*		6
10	El-Beshbishy et al., 2014	*	*	*			*			*		6
11	Lebda et al., 2014	*	*	*			*			*	*	6
12	Güleş And Eren., 2016	*	*	*			*	*		*	*	7
13	Al-Azawi and Asker., 2017	*	*				*	*				4
14	EL-Maddawy and Abd El Naby., 2018	*	*				*			*		4
15	Pınar et al., 2018	*	*				*	*		*	*	6
16	Prathima et al., 2018	*	*	*			*	*		*		6
17	Shaygannia et al., 2018	*	*				*	*			*	5
18	Corrêa et al., 2019	*	*	*			*	*		*	*	7
19	Goudarzi et al., 2020	*	*	*			*	*		*	*	7
20	Moustafa et al., 2020						*	*		*	*	4
21	Prathima et al., 2020	*	*	*			*	*		*	*	7
22	Said et al., 2020	*	*			*	*	*		*	*	7
23	Ibrahim et al., 2021		*				*	*		*		4

(1) peer-reviewed publication, (2) statement of control of temperature, (3) random allocation to treatment or control, (4) blinded caregiver/investigator, (5) blinded assessment of outcome, (6) use of co-interventions/co-morbid, (7) appropriate animal model (age, sex, species, strain), (8) sample size calculation, (9) compliance with animal welfare regulations and (10) statement of potential conflict of interests

Graphical abstract



Graphics Abstract

Identification of studies via databases and registers

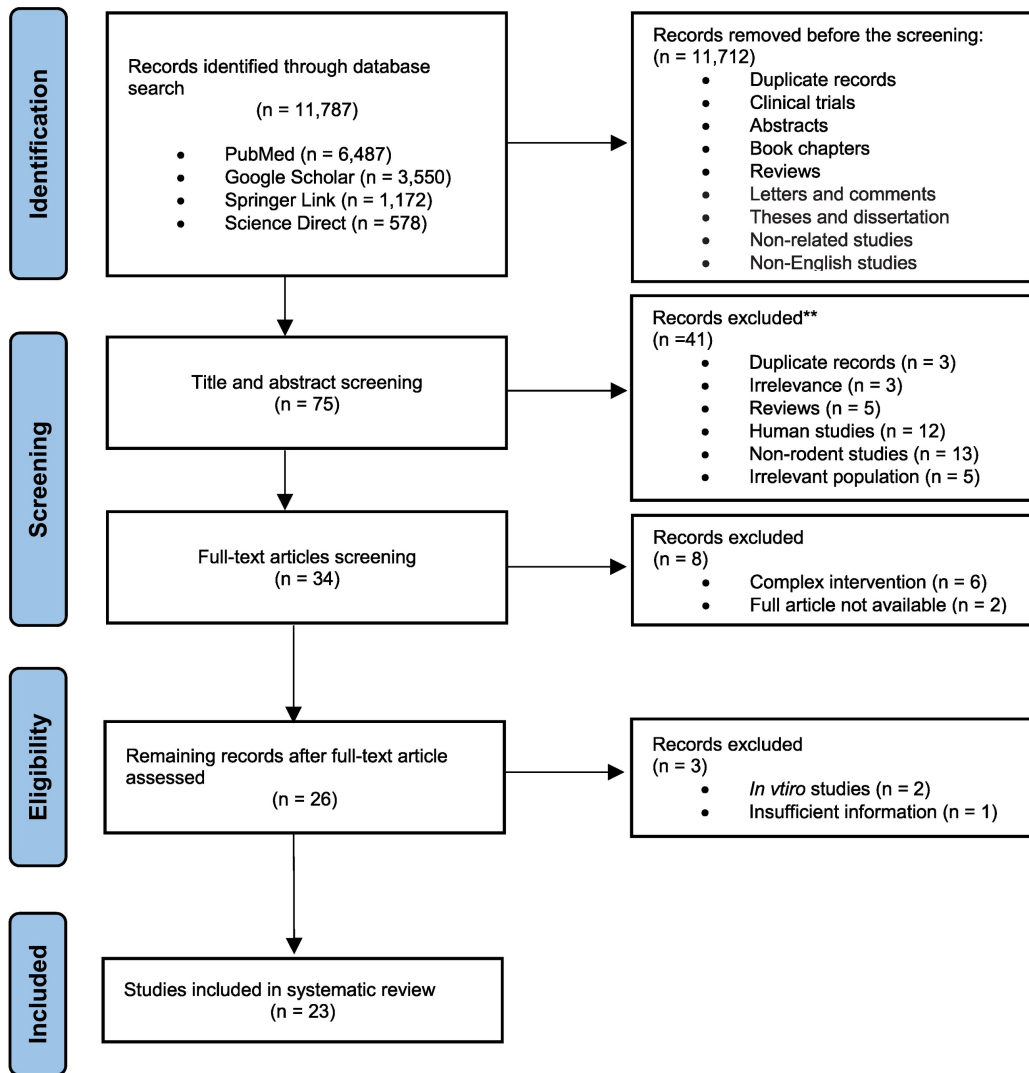


Figure 1

(A)

	Random sequence generation (selection bias)	Baseline characteristics (selection bias)	Allocation concealment (selection bias)	Random housing (performance bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Blinded outcome assessor (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Al-Azawi and Asker., 2017	●	●	●	●	●	●	●	●	●	●
Ashour et al., 2011	?	●	●	●	●	●	●	●	●	●
Astiz et al., 2013	?	●	●	●	●	●	●	●	●	●
Corrêa et al., 2019	?	●	●	●	●	●	●	●	●	●
El-Beshbishy et al., 2014	?	●	●	●	●	●	●	●	●	●
EL-Maddawy and Abd El Naby., 2018	●	●	●	●	●	●	●	●	●	●
Goudarzi et al., 2020	?	●	●	●	●	●	●	●	●	●
Güleş And Eren., 2016	?	●	●	●	●	●	●	●	●	●
Ibrahim et al., 2021	●	●	●	●	●	●	●	●	●	●
Lebda et al., 2014	?	●	●	●	●	●	●	●	●	●
Moustafa et al., 2020	?	●	●	●	●	●	●	●	●	●
Othman et al., 2012	?	●	●	●	●	●	●	●	●	●
Prahalathan et al., 2004	?	●	●	●	●	●	●	●	●	●
Prahalathan et al., 2005	?	●	●	●	●	●	●	●	●	●
Prahalathan et al., 2006 a	?	●	●	●	●	●	●	●	●	●
Prahalathan et al., 2006 b	?	●	●	●	●	●	●	●	●	●
Prathima et al., 2018	?	●	●	●	●	●	●	●	●	●
Prathima et al., 2020	?	●	●	●	●	●	●	●	●	●
Pinar et al., 2018	●	●	●	●	●	●	●	●	●	●
Said et al., 2020	●	●	●	●	●	●	●	●	●	●
Selvakumar et al., 2006	?	●	●	●	●	●	●	●	●	●
Shayannia et al., 2018	●	●	●	●	●	●	●	●	●	●
Yeni et al., 2012	?	●	●	●	●	●	●	●	●	●

(B)

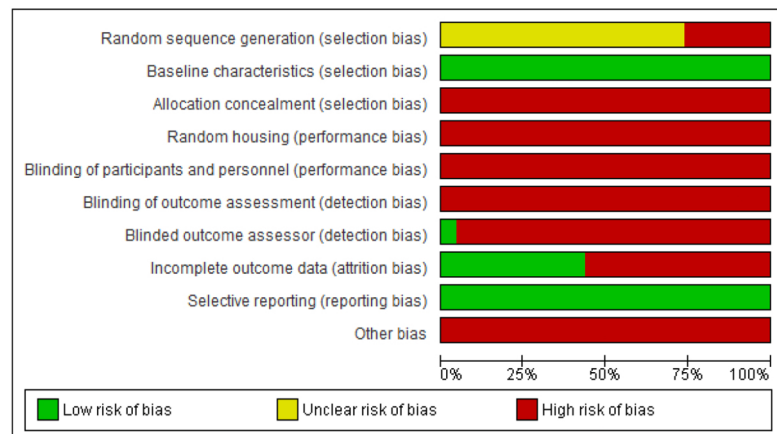


Figure 2

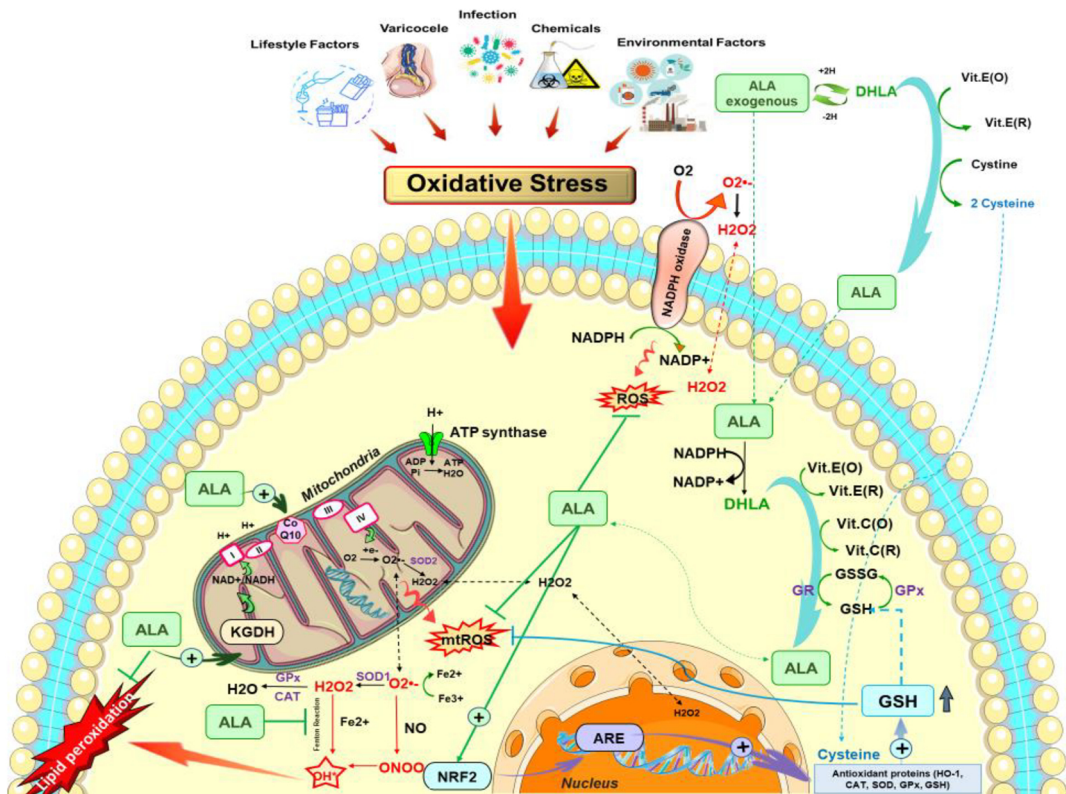


Figure 3

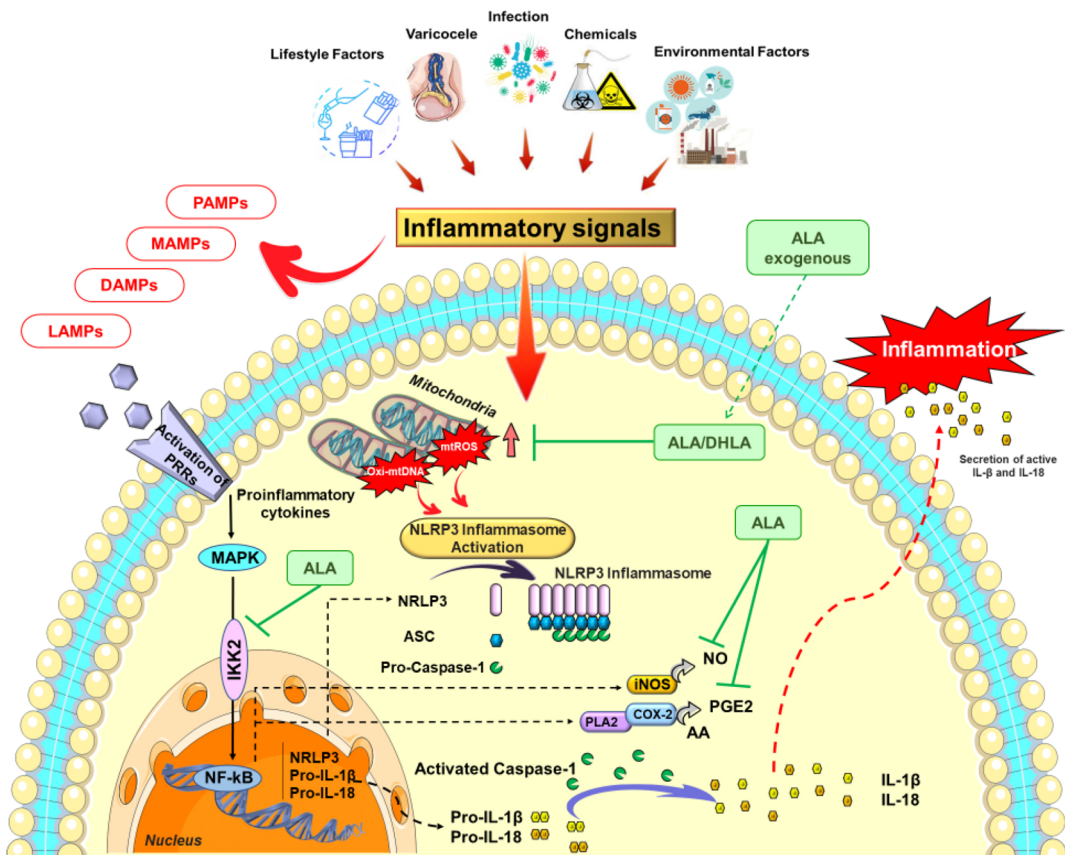


Figure 4

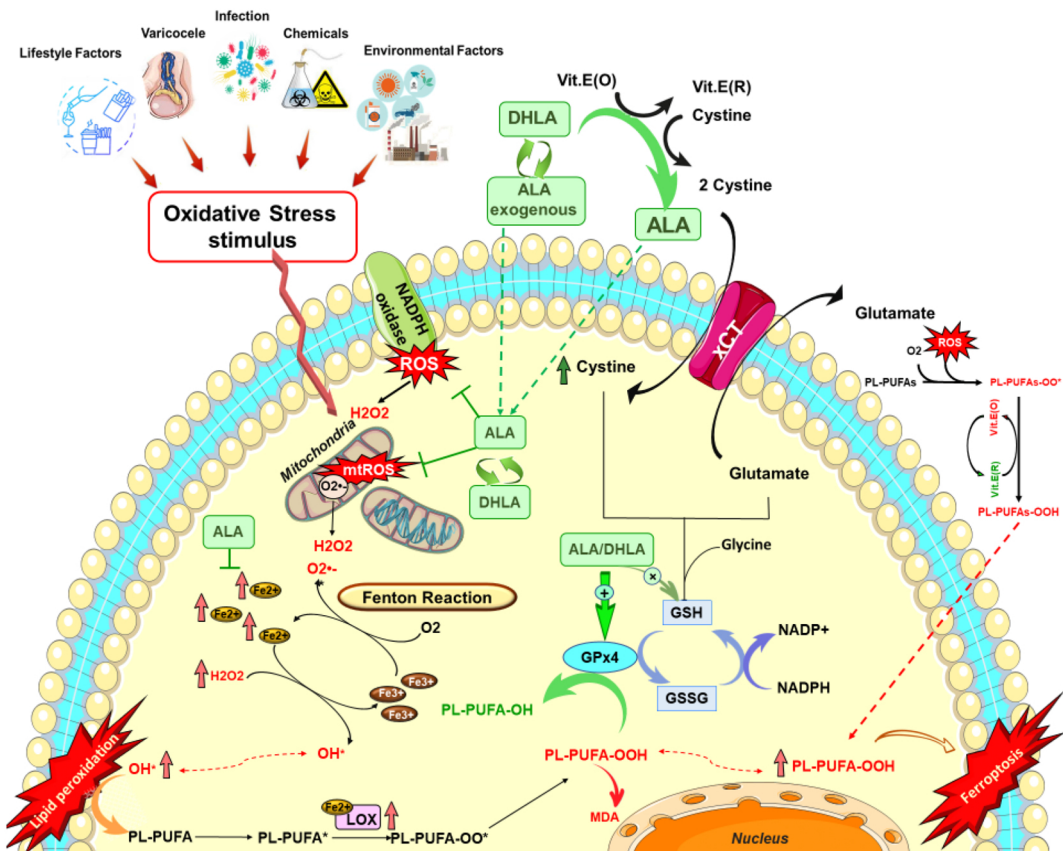


Figure 5