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ALPHA-LIPOIC ACID – EFFECTS AND APPLICATIONS

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ABSTRACT

Alpha-lipoic acid is one of the most powerful antioxidants used in both humans and animals. Many of its effects were proven: antioxidant, antidiabetic, anti-inflammatory, immunomodulatory, analgesic, as a growth promoter. Numerous clinical studies showed the effectiveness of alpha-lipoic acid in diseases in which the antioxidant balance was disturbed. Alpha-lipoic acid reduces the complications of diabetes such as neuropathy, nephropathy, retinopathy, and vascular damage. It improves insulin resistance.

In veterinary practice, alpha-lipoic acid is used as a component in supplements for dogs, cats, pigs, and birds. The aim of this review is to present new data about its therapeutic application.

Key words: alpha-lipoic acid, antioxidant, diabetes, immunomodulatory properties.

The free radicals formed under physiological conditions were maintained at a constant level by endogenous and exogenous antioxidants that neutralize the radicals. Oxidative stress occurs when the production of free radicals exceeds the detoxification capacity, leading to biological damage (Georgiev and Gocheva, 2014). Due to their ability to directly oxidize and damage DNA, proteins, and lipids, free radicals play a key role in the pathogenesis of several diseases such as diabetes mellitus, respiratory diseases, cardiovascular diseases (atherosclerosis and hypertension), cataract, neurodegenerative disorders (Alzheimer's disease, Multiple sclerosis, Parkinson's disease), rheumatoid arthritis and in various cancers (breast, colorectal, prostate, etc.) (Phaniendra et al., 2015).

Antioxidants are agents that eliminate reactive oxygen species (ROS), prevent their formation, or repair the damage they cause (Odabasoglu et al., 2010).

Alpha-lipoic acid (ALA) also known as thioctic acid (1,2-dithiolane-3-pentanoic acid) is a universal antioxidant. It is the fat- and water-soluble antioxidant, first identified in 1937 by Snell, Strong, and Peterson as a nutritional factor required for the growth of *Lactobacillus* in potato extract (Snell et al., 1937). In 1951 ALA was isolated from Reed in the form of pale yellow crystals of beef liver. Its first clinical use was in 1959 in Germany in the treatment of liver failure from acute poisoning with *Amanita phalloides* (Gomes et al.,2014).

Alpha-lipoic acid is optically active with two optical isomers: R-LA and S-LA. The natural ALA is in the R (right) configuration. A racemic mixture of R and S forms is used as food supplements for therapeutic purposes (Tibullo et al., 2017).

The largest amounts of ALA were found in red meat (liver, heart, and kidneys) and vegetables (spinach, broccoli, Brussels sprouts, potatoes, tomatoes, peas). It is supposed that ALA was synthesized in human and animal mitochondria (Tilotta et al., 2018). The ALA has two forms: oxidized ALA and reduced form – dihydrolipoic acid (DHLA). Both forms are capable of scavenging various reactive oxygen species (ROS) and exhibiting antioxidant activity (El Barky et al., 2017). The ALA is one of the most powerful antioxidants used in humans and animals. In many experiments its antioxidant, antidiabetic (Singh and Jialal, 2008) anti-inflammatory (Moura et al., 2015). immunomodulatory (Wei et al., 2019), analgesic (Joksimovic et al., 2021), growth-promotors (Guo et al., 2014) effects proved. The ALA had also antitoxic, hepatoprotective (El-Halwagy et al., 2018), and antiaging effects (Patel et al., 2014).

Many clinical studies proved the effectiveness of ALA in diseases in which the antioxidant balance was disturbed. Both ALA and DHLA repaired proteins, lipids, and DNA that were damaged by oxidative stress (Sumathi et al., 1996). Both ALA and DHLA chelate heavy metals: Mn, Zn, Cd, Pb, Co, Ni, Fe, Cu, As, and Hg by increasing glutathione (GSH) in cells and thus eliminate them from the body (Goraca et al., 2011).

According to the FDA (U.S. Food and Drug Administration), alpha-lipoic acid is reported to be a safe and effective agent. ALA has no mutagenic or genotoxic effects (Nguyen and Gupta, 2021).

According to Hill et al. (2013), the ALA was 10 times more toxic in cats than in humans, dogs, or rats.

Antioxidant effects

Both ALA and DHLA are powerful antioxidants that directly scavenge the ROS and reactive nitrogen species (RNS) from the body. The alpha-lipoic acid is called an "antioxidant of antioxidants". It is water- and fat-soluble and neutralizes free radicals inside and outside the cells of almost all tissues. The ALA is involved in the regeneration of other antioxidants such as glutathione (GSH), coenzyme Q10, vitamin E, and vitamin C (Reshma et al., 2021).

Alpha-lipoic acid is a cofactor for α-ketoglutarate dehydrogenase and pyruvate dehydrogenase activity, required for the oxidative decarboxylation of pyruvate to acetyl coenzyme A (Serhiyenko et al., 2018). The ALA is a cofactor in the production of adenosine triphosphate (ATP) and the catabolism of α-keto acids and amino acids.

In oxidative stress ALA affects the Keap1/Nrf2 (Kelch-like ECH-associated protein 1/nuclear factor associated with erythroid 2 factor 2) regulatory pathway, inducing Nrf2-mediated antioxidant gene expression and thus it increase the synthesis of glutathione and other detoxifying enzymes and antioxidants in many diseases such as type 2 diabetes, neurodegenerative diseases, cancer, etc. (Deshmukh et al., 2017).

In many *in vivo* and *in vitro* experiments, it was shown that ALA increased intracellular glutathione levels by 30-70%. The GSH is a major intracellular antioxidant with a vital role in protecting tissues against oxidative stress and detoxifying free radicals, produced by mitochondria (Marí, M. et al., 2009).

In many studies, scientists have found that alpha-lipoic acid prevents lipid peroxidation *in vivo* and *in vitro*. The administration of ALA (35 mg/kg body weight and 70 mg/kg b.w.) reduced lipid peroxidation and improved the antioxidant defense system in the blood of insulin-resistant rats (Thirunavukkarasu et al., 2004).

According to Li et al., (2019) the ALA supplementation (300 mg/kg b.w.) in broilers diet significantly reduced the adverse effects of high stocking density-mediated stress by significantly increasing GSH-PX and SOD ($P < 0,05$) serum activity and decreasing serum MDA. The ALA can improve the antioxidant status of broilers and reduce oxidative stress caused by higher stocking densities.

In sows that received ALA (800 ppm), the antioxidant activity of GSH-Px in serum increased ($p < 0.05$), and MDA levels decreased ($p < 0,01$), compared with the control diet at day 21 of lactation (Bai et al. 2012).

Antidiabetic effects

Many data were found concerning the clinical use of ALA in the treatment of diabetes and of diabetes-related chronic complications such as neuropathy, nephropathy, retinopathy, dry eye disease, vascular damage, endothelial dysfunction, wound healing, and diabetic cardiovascular autonomic neuropathy and it improved the insulin resistance (Gomes et al., 2014; Rask-Madsen et al., 2013).

Alpha-lipoic acid has an insulin-mimetic effect. It stimulates glucose uptake by translocation of GLUT1 and GLUT4 glucose transporters to the plasma membrane and increased tyrosine phosphorylation of insulin receptor substrate-1, increased the phosphatidylinositol 3-kinase (PI3K) activity, stimulated Akt (protein kinase B) activity and p38 mitogen-activated protein kinase (p38-MAPK) activity (Rochette et al., 2015).

Alpha-lipoic-acid lowers blood glucose and it has a potential therapeutic effect in diabetic conditions. The ALA increases glucose uptake in insulin-sensitive and insulin-resistant muscle tissues and improves insulin resistance (Lee et al., 2005**).**

The streptozocin (STZ)-diabetic rats received a supplement with ALA (100 mg/kg/day) for 8 weeks. It was found that the alpha-lipoic acid significantly reduced the fasting blood glucose and glycosylated hemoglobin, compared to the control group. The ALA reduced and prevented vascular damage in diabetic rats by improvement of glycemic status, antioxidant activity, and dyslipidemia (Budin et al., 2009).

In many *in vitro* and *in vivo* animal models alpha-lipoic acid inhibits the progression of vascular damage by improving endothelial function in diabetes, diabetic polyneuropathy, retinopathy, hypertension, peripheral neuropathy in cancer, Alzheimer's disease, atherosclerosis, etc.

The potential mechanisms are 1. The ALA increases energy metabolism regulated by mitochondrial enzymes; 2. it has a powerful antioxidant capacity that can directly scavenge ROS and recycling endogenous antioxidants such as glutathione, coenzyme Q10, and vitamins C and E; and 3. ALA regulates antioxidant and anti-inflammatory genes at the transcriptional level. It increase the synthesis of glutathione and other detoxifying enzymes and antioxidants (Deshmukh et al., 2017).

Kuştepe et al. (2020) were found that ALA had a renoprotective effect and showed the potential to prevent kidney damage from diabetes. The application of ALA (15 days) reduced renal damage in STZ-diabetic rats.

In a 4-year study, the researchers founded that alpha-lipoic acid reduced the neuropathic complications of diabetes by reducing the pain, paresthesias, and formications in patients (Tancova, 2012).

The treatment with ALA (54 mg/kg body weight *i.p*. for 6 weeks) in STZ-diabetic rats lowered the serum glucose, total cholesterol, triglyceride, LDL (low-density lipoprotein) cholesterol, HDL (high-density lipoprotein) concentration, and lipid peroxidation of liver and kidney as well as significantly increased serum vitamin C and liver catalase activity. These results confirm that ALA can improve glycemic status and dyslipidemia and has the potential to reduce cardiovascular complications due to diabetes (Aziza et al., 2012; Mythili et al., 2006). Similar results were obtained by Vasdev et al. (2000; 2005) in hypertensive rats treated with ALA supplements (500 mg/kg feed for 9 weeks), the systolic blood pressure, cytosolic ($Ca^{2+)}$, blood glucose, insulin levels, and tissue aldehyde conjugates were reduced. The ALA also attenuated adverse renal vascular changes.

Thekkuttuparambil (2020) was found that alpha-lipoic acid can prevent dry eye by down-regulating the expression of matrix metalloproteinase-9 in the corneal epithelial cells and activating the

antioxidant status of the ocular surface. The ALA can prevent oxidative stress-induced erosion on the surface of the cornea and lachrymal gland damage. Alpha-lipoic acid can prevent diabetic retinopathy by inhibiting the activity of nuclear factor kappa B and O-linked β-N-acetylglucosamine transferase and reducing oxidative stress.

According to Tao et al. (2016), ALA treatment improved age-related macular degeneration.

The topical application of alpha-lipoic acid on the 7th day leads to an increase in the healing rate of the skin wound (60.7 ± 8.4%), compared with the negative control group (43.0 ± 17.4%), as well as improvement of histological parameters on the skin (Külkamp-Guerreiro et al., 2013).

Potential anti-inflammatory effects

The ALA inhibits the activation of nuclear factor-κappa B (NF-κB) and reduces the release of inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) in the methotrexate-induced oxidative injury of rat kidneys (Çakır et al., 2015). Similar results were obtained by Lee et al. (2015) when ALA is administered to rat mesangial cells (HBZY-1) prior to the addition of lipopolysaccharide (LPS). The LPS-induced expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was inhibited by ALA pretreatment. That's why the secretion levels of PGE2 and nitric oxide (NO) were significantly reduced. The obtained results demonstrate the anti-inflammatory effect of ALA.

In an experiment it was found that ALA reduces inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6) and TNF-α in patients with metabolic syndrome and related disorders **(**Akbari et al., 2018).

The effect of alpha-lipoic acid (50, 100, and 200 mg/kg) on the acute phase of inflammation in the carrageenan-induced (CAR) paw edema test in mice was observed (Odabasoglu et al., 2011). Its anti-inflammatory potency was compared to diclofenac (DIC) and indomethacin (IND). All the doses of ALA increased the activity of the antioxidant enzymes such as superoxide dismutase (SOD) ($P < 0.05$), catalase (CAT) ($P < 0.01$), glutathione (GSH) ($P < 0.05$), glutathione peroxidase (GPx) (50 and 100 mg/kg) at ($P < 0.05$ and $P < 0.01$) decreased in the paw tissues of CAR-injected rats. Alpha-lipoic acid reduced the activity of lipid peroxidation (a major measure of oxidative tissue damage), myeloperoxidase (MPx) (an indicator of the degree of inflammation), and inducible NO synthase (iNOS) (an important role in inflammatory models), and thus it had an anti-inflammatory effect.

Many experiments showed that down-regulation of NF-κB with antioxidants leads to a reduction in the amount of NO in the blood, a therapeutic effect attributed to the reduction of iNOS expression (Lingappan, 2018). The decrease in NO levels in the paws may be due to the direct scavenging effect of NO by the sulfhydryl groups of ALA (Biewenga et al., 1997). Oxidative stress has been proven to up-regulate and antioxidants down-regulate NF-κB transcription, which induces cytokine expression and vascular adhesion. These results showed that ALA had an anti-inflammatory effect in acute inflammatory processes by inhibiting the activation of NF-κB and oxidative stress (Serhiyenko et al., 2018).

The scientists suggest that ALA causes down-regulation of NF-κB, which plays a major role in the expression of various genes involved in the inflammatory response and in the processes of cellular apoptosis. Some *in vitro* experiments showed that ALA lowers the expression of vascular cell adhesion molecule-1 (VCAM-1) and endothelial adhesion of human monocytes. Thus, it inhibits

NF-κB-dependent expression of metalloproteinase-9, the enzyme responsible for the degradation of the extracellular matrix (Serhiyenko et al., 2018).

The anti-inflammatory activity of 2,4-bismethylthio-butanoic acid (BMTBA) and tetranor-dihydrolipoic acid (tetranor-DHLA) alpha lipoic acid derivatives in mice was studied using zymosan-induced peritonitis and the carrageenan-induced hind paw edema animal models. The results indicated that the early vascular permeability measured at 30 min of zymosan-induced peritonitis was significantly inhibited in groups receiving BMTBA (10 mg/kg, 30 mg/kg and 50 mg/kg). The early neutrophil infiltration, measured at 4 hours of zymosan-induced peritonitis, was inhibited in the group receiving BMTBA (50 mg/kg) and tetranor-DHLA (50 mg/kg). The increase in paw edema was significantly inhibited in the groups receiving BMTBA (50 mg/kg, 100 mg/kg) and tetranor-DHLA (30 mg/kg, 50 mg/kg). These results demonstrate the anti-inflammatory properties of ALA metabolites (BMTBA, tetranor-DHLA) (Kwiecień et al., 2013).

In the chronic inflammation experiment alpha-lipoic acid (50 mg/kg, 100 mg/kg and 200 mg/kg b.w.) significantly inhibited ($P < 0.01$) granuloma formation surrounding the pellets by 70.6%, 67.7% and 68.9% respectively, compared to the control group. The results confirmed that ALA had an anti-inflammatory effect on cotton pellet-induced chronic inflammation in rats (Odabasoglu et al., 2011).

In an experiment, treatment with indomethacin (5 mg/kg; 30 mg/kg) caused severe gastric damage that was prevented with co-treatment with alpha lipoic acid. These results suggested that the combination of ALA with NSAIDs may simultaneously increase both the anti-inflammatory effect and prevent NSAID-induced gastric damage. The ALA is a promising adjuvant that can reduce the dose and side effects of NSAIDs, especially in cases of long-term high-dose administration (Barut et al., 2021).

Rats injected intraperitoneally (*i.p.*) with alpha-lipoic acid (60 mg/kg and 120 mg/kg) preventively or postoperatively significantly reduced the evoked mechanical hyperalgesia induced after surgical incision of the rat's paw (Joksimovic et al., 2021).

Immunomodulatory effects

In a study, alpha-lipoic acid showed immunomodulatory effects on the differentiation and proliferation of T cells. It was found that ALA reduced the number of Th_{17} and Th_1 cells in CNS and increased the number of splenic Treg cells in mice with experimental encephalomyelitis (Wang et al., 2013).

According to Kim et al. (2011) ALA reduced the production of IFN-γ and IL-4 by activated CD4 (+) T cells. The ALA significantly reduced T-cell migration and infiltration into the atherosclerotic lesion in association with decreasing expression of intercellular adhesion molecule (ICAM) and CD62L (L-Selectin) (Ying et al., 2010).

Alpha-lipoic acid plays a regulatory role in B cell proliferation, apoptosis, and function. It increased spleen B cells in endotoxemic mice (Wessner et al., 2006) and decreased total serum IgE levels in the atopic dermatitis model (Kim et al., 2011).

The ALA reduces the function of NK (natural killer) cells. It regulates the activation, phagocytosis, and migration of macrophages in direct or indirect ways. The ALA inhibited phagocytosis of myelin by macrophages and reduced the production of monocyte chemotactic protein-1 (MCP-1) and TNF-α induced by lipopolysaccharide (LPS) of macrophages (Kiemer et al., 2002).

The alpha-lipoic acid improves the immune function, antioxidant status and decreases oxidative damage, and revitalizing antioxidants in the blood in adult rats.

According to Palaniyappan et al. (2011), the increase in total leucocyte count and the absolute lymphocyte count in adult rats treated with ALA for 14 days was observed. The treatment with ALA significantly ($p < 0{,}001$) decreased and improved the phagocytic index in rats, compared to the untreated control group. There was a significant increase ($p < 0.001$) in NBT (nitroblue tetrazolium reduction test) positive cells, a significant increase ($p < 0.001$) in IgG, IgA and IgM concentrations and the concentration of complement 3 (C3) ($p < 0.001$) in aged rats, compared, to the respective control aged group. Alpha-lipoic acid supplement significantly inhibited the increase of LPO and glutathione disulfide (GSSG) and the decrease of total antioxidant capacity (T-AOC) in the plasma of adult rats, compared to the untreated control group. Alpha-lipoic acid treatment significantly increases ($p < 0.001$) the levels of GSH, GSH/GSSG molar ratio and adenosine triphosphate (ATP) in aged rats, compared to the respective control rats. The administration of ALA significantly inhibited the decrease in SOD, CAT, GPx and glutathione reductase (GR) activities in adult rats.

According to Li et al., (2019) the addition of ALA (300 mg/kg b.w. for 3 weeks) to the broilers diet inhibited the high, density-mediated reduction in serum IgA and IgG titers ($p < 0.05$) and improved the humoral immune response.

Alpha-lipoic acid as a growth promoter

In an experiment, chickens receiving supplements with ALA (500 mg/kg) during the starter, grower and whole period improved the average feed intake (AFI), increased their body weight gain, and reduced their abdominal fat. The alpha-lipoic acid improved antioxidant enzyme activities of GSH, T-SOD, CAT, and GSH-Px in the plasma, liver, and muscles of birds, as well as in the meat of broiler chickens. The ALA applied to chickens during the whole period improved the meat quality and carcass characteristics (Guo et al., 2014). Similar results were obtained by Murali et al. (2014). The application of ALA supplements leads to an increase in the oxidative stability of poultry meat and meat-based products by reducing the rate of lipid peroxidation leading towards the decreased incidence of pale, soft, exudative behavior in processed meat products during storage (Shen and Du, 2005). The use of ALA as a feed supplement is a pragmatic approach to enhance shelf life and the quality of chicken meat and meat products (Sohaib et al., 2018).

In a study, goats receiving a supplement containing ALA (600 mg/kg b.w.) for 70 days had a significantly ($p < 0.05$) higher average daily gain and a better feed conversion rate, compared to goats without alpha-lipoic acid. It significantly increased the serum levels of the antioxidant enzymes such as GSH-Px, SOD, CAT, and total antioxidant capacity. Thus ALA improved the carcass characteristics, antioxidant capability, and meat quality in Hainan black goats (Wang et al., 2016).

According to Li et al., (2019) the ALA supplementation (300 mg/kg) in broilers diet significantly reduced the adverse effects of high stocking density-mediated stress by significantly increasing GSH-PX and SOD serum activity and decreasing serum MDA. The ALA improved broiler growth performance, feed utilization, and carcass characteristics.

The sows fed with ALA supplements (800 ppm) from the 85th day of pregnancy to the 21st day of lactation increased the birth weight and weaning weight of piglets (between days 1 and 21) ($p < 0.01$), compared with the control group. In sows that received alpha-lipoic acid, the antioxidant activity of GSH-Px in serum increased and MDA levels decreased, compared with the control diet at

day 21 of lactation. These results showed that the addition of ALA to the diet of sows in late pregnancy and lactation improves the antioxidant activity of sows and can improve the growth efficiency of piglets (Bai et al., 2012).

Potential anticancer effects

Both ALA and DHLA decreased the activity of protein tyrosine phosphatases PTP1B and SHP2-overexpressed in breast cancer cells and had inhibitory effects on the viability and proliferation of breast cancer cells (MCF-7) after 72 h incubation time (Kuban-Jankowska et al., 2017).

Berkson et al. (2006) described a clinical experiment for the long-term survival of a man with pancreatic cancer and metastases to the liver, treated intravenously with ALA (300 to 600 mg, 2 days a week) and oral low-dose naltrexone (4.5 mg) without any adverse effects. He was alive and well 78 months after the initial treatment and felt normal. His computed tomography scan displayed a reduction of the pancreatic tumors and the hepatic metastases. The same protocol was administered to another patient with the same diagnosis. He was alive and well 39 months after the therapy. He had no signs of cancer. For the next patient with the same diagnosis and treatment after 5 months of therapy, the positron emission tomography (PET) scan showed no evidence of disease. The fourth patient in addition to pancreatic cancer with liver and retroperitoneal metastases had a history of B-cell lymphoma and adenocarcinoma of the prostate. After 4 months with the same protocol, his PET scan showed no signs of cancer. According to the authors, ALA reduces oxidative stress, stabilizes NF-kB and it has the ability to stimulate pro-oxidant apoptotic activity and ability to inhibit the proliferation of malignant cells. In addition, the ability of low-dose naltrexone to modulate an endogenous immune response is considered (Berkson et al., 2009).

According to Novotny et al. (2008), the ALA prevented or reduced toxicity from cancer chemotherapy is due to its ability to scavenged free radicals formed during chemotherapy. The scientists are discussing the inclusion of ALA as a chemoprophylactic agent in chemotherapy protocols.

The cultivation of HT-29 cells (colon cancer) with alpha-lipoic acid resulted in a dose-dependent increase in caspase-3-like activity, DNA fragmentation, and apoptosis in cancer cells by a prooxidant mechanism that is initiated by an increased uptake of oxidizable substrates into mitochondria. This apoptotic effect of ALA was not observed in normal human colonocytes (Wenzel et al., 2005).

In a study, ALA showed a reduction of malathion-induced mammary tumor incidence and reversed intra-tumor histopathological alterations. These results indicate that ALA exhibits a chemoprophylactic effect in breast hyperplastic and malignant changes by suppressing abnormal cell proliferation and inducing apoptosis with an oncostatin effects during early-stage breast cancer (Omran et al. 2015).

In this review, we described the main effects of alpha-lipoic acid such as antioxidant, antidiabetic, anti-inflammatory, immunomodulatory, analgesic, as a growth promoter, and its potential applications.

Further research is needed to prove the need for use of ALA in human and veterinary medicine.

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