Online Journal of Animal and Feed Research

Volume 5, Issue 5: 142-147; Sep 25, 2015 ISSN 2228-7701

OXIDATIVE STRESS IN SHEEP INDUCED BY CADMIUM CHLORIDE TOXICITY, WITH THERAPEUTIC EFFECTS OF ALPHA LIPOIC ACID

Hussien Ali NAJI [1] and Mohammad Mushgil ZENAD[2]

[1] *MSc, Assistance lecturer, Internal and Preventive Medicine Department, College of Veterinary Medicine, University of Basra, Iraq*
[2] *PhD, Assistance professor, Internal and Preventive Medicine Department, College of Veterinary Medicine, University of Baghdad, Iraq*
*Email: zenadaboodi@yahoo.com

ABSTRACT: Cadmium (Cd) is a heavy toxic metal, with harmful effects on animals and public health. Recently the risk of cadmium toxicity is substantially regarded; the environmental pollution is increased due to multi- uses of this element in various industries. This study was performed to clarify the effects of acute cadmium toxicity in sheep with trail of using alpha lipoic acid as an antioxidant therapeutic substance. Fifteen male lambs aged from 5-to-7 months were divided equally in to three groups, they were supplied with ordinary diet and provided with water ad-lib, the first group 1 was administered a single dose of $CdCl_2$ 3 mg/kg.bw subcutaneously (S/C), the second group 2 was injected with the same dose of $CdCl_2$ and by the same route, and then simultaneously administered an alpha lipoic acid 50 mg/kg.bw intramuscularly, the later drug was repeated after 12 hours via the same route. The third group 3 was left as control and given normal saline (S/C). All animals were daily monitored and the clinical signs were recorded. The signs of cadmium toxicity appeared 18 hours post $CdCl_2$ administration in the group 1; the signs were gradually increased in severity and multiple systems were involved included: digestive disturbances, cardiovascular and neurological dysfunctions, and locomotors abnormalities. Significant elevations in the body temperature, respiratory and heart rates were observed, deaths of 2 lambs were recorded 96 hours post $CdCl_2$ injection. The group 2 showed mild clinical signs, and no death was occurred, moreover insignificant variations between clinical parameters in both groups 2 and 3 were recorded. Serum biochemical analysis revealed significant ($P<0.05$) increased of malondialdehyde (5.41 ± 0.282 µmol/L) and glutathione (10.68 ± 0.38 µmol/L) concentrations and marked elevation of serum catalase activity (103.85 ± 3.93 u/L) was also observed in group I, whereas the last three parameters showed no significant differences between groups 2 and 3; these results pointed to the role of alpha lipoic acid in ameliorating the toxic effect of cadmium to great extent.

Keywords: Cadmium Toxicity, Alpha Lipoic Acid, Antioxidant, Sheep.

ORIGINAL ARTICLE
pii: S2228770115000023-5
Received 07 Sep. 2015
Accepted 21 Sep. 2015

INTRODUCTION

Cadmium (Cd) is a heavy toxic metal, has undesirable effects in animals (Bampidis et al., 2013); it had been classified as one of the 126 priority polluters according to (AEPA) American Environmental Protection Agency report (Paul et al., 2014). The sources of environmental pollution with cadmium are mainly including the uses of this metal in different industries as: anticorrosive agent, batteries, glasses, and ceramics, plastic etc. (Miller et al., 2015, Monika et al., 2015) and in the pesticides, and fungicidal drugs (Ognjanovic et al*.,* 2008). The excessive accumulations and poor excretion of cadmium in the organs besides its long biological half-time (15–30 years) were responsible for the damage of tissues, moreover cadmium causes many neoplastic and non neoplastic diseases were reported in animals and human (Lane et al*.,* 2015). The toxic effects of Cd are owing to indirect induction of the oxidative stress (Li et al*.,* 2015). The oxidative stress is occurring as a consequence of an imbalance between pro-oxidant and body's antioxidant defense system (Agarwal and Prabakaran, 2005). The production of free radicals reactive oxygen species (ROS) are attributed to the presence of one unpaired electron even though naturally present in the organism (Wang et al*.,* 2014).

The malondialdehyde (MDA) is a convenient biomarker for lipid peroxidation and oxidative stress in biological systems (Nagamani et al., 2015). Antioxidants substances interact with and stabilize the free radicals and consequently they prevent damage of cells caused by these radicals, glutathione (GSH) is an important soluble antioxidant, it is synthesized by cells from their constituent amino acid (Shelly, 2013).

Catalase is an enzymatic scavenger antioxidant (Esra et al*.,* 2012), it is neutralizing reactive oxygen species and removes cellular superoxide and peroxides before their reaction with metal catalysts to form more reactive species, also it catalyzes the reduction of hydroperoxides, thereby protects mammalian cells from oxidative damage (Gill et al., 2015). The alpha lipoic acid is an antioxidant compound discovered in 1988, it is equivalent to antioxidant vitamins (C and E) and coenzyme Q 10 (Silvestri et al*.,* 2015), and it has ability to neutralize the free radicals within lipid and aqueous region in the extra and intra-cellular compartments (Gomes and Negrato, 2014);

To cite this paper: Naji HA and Zenad MM. 2015. Oxidative stress in sheep induced by cadmium chloride toxicity, with therapeutic effects of alpha lipoic acid. *Online J. Anim. Feed Res*., 5(5): 142-147.
Scienceline/Journal homepages: http://www.science-line.com/index/; http://www.ojafr.ir

also it is considered a master antioxidant due to many functional characters: scavenging activity of reactive oxygen species, regeneration of endogenous antioxidants such as glutathione, metal chelating activity and repairing of oxidized proteins (Ahmadi et al., 2013). This study was designed to clarify the effects of the oxidative stress induced by Cd toxicity, with trial to use the alpha lipoic acid as a therapeutic antioxidant agent in sheep.

MATERIAL AND METHODS

Experimental animals

Fifteen males lambs aged from 5-7 months, weight from 17-20 kg, apparently healthy were used in the experiment; they were administered Albendazol (7.5 mg/kg) orally. Lambs were provided twice daily with green fodder (alfa alfa) and concentrate (ordinary diet), water was provided ad-lib. They were kept 20 days for adaptation.

Experimental design

Lambs were allocated equally in to three groups (5 lambs in each group). First group: lambs were administrated subcutaneously (S/C) a single dose of cadmium chloride (sub lethal dose) 3 mg/kg. Second group (II): the lambs also injected (S/C) a single dose of cadmium chloride 3 mg/kg and at the same time administered Alpha lipoic acid 50 mg/kg intramuscularly followed by the same dose (50 mg/kg) and route (I/M) after 12 hours (Harlod et al., 2011). Third group: was left as control group, administered S/C normal saline (milliliters were equivalent to cadmium chloride solution).

Clinical examination

Lambs were examined clinically daily during the period of experiment; the clinical signs were recorded in special card.

Blood samples

Blood was collected aseptically from jugular vein by 10 ml disposable syringe, before administration of $CdCl_2$ and two days post administration of $CdCl_2$ and ALA (after appearance of toxicity signs), serum were separated by centrifugation at 3000 rpm and kept at -20 °C.

Chemicals: Reagents were prepared by using analar grade chemicals obtained from BDH chemical Ltd England Segma.

Determination of malondialdehyde (MDA):

MDA was determined according to Wysocka et al. (1995), thiobarbituric acid (TBA) reacts with MDA to form thiobarbituric acid reactive substance (TBARs) and the absorbance of this resultant was measured by spectrophotometer at 535 nm.

Determination of glutathione concentration

The glutathione concentration of the serum was determined according to the method described by Schafer and Buttner (2001), the method based on the reduction of 5,5-dithio-bis (2- nitrobenzoic acid – DTNB) with glutathione (GSH) to product a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 412 nm wave length.

Determination of catalase activity determination

Catalase was determined by colorimetric method according to Aebi (1984). It catalyzes the divalent reduction of hydrogen peroxide (at high concentration) to water and free oxygen.

$$2H_2O_2 + CAT \longrightarrow 2H_2O + O_2$$

Consequently absorbance was decrease due to H_2O_2 consumption (£ = 0.04mmol^{-1} cm^{-1}) (Mueller et al., 1997). The activity determined by reading the initial and final absorbance at 240 nm.

RESULTS

The clinical sings of cadmium toxicity appeared on lambs in the first group 18 hours post administration of $CdCl_2$, the signs were including inappetance, decrease ruminal contraction (1.2 ± 0.2 contraction/ minute), slight increase of the body temperature (40.3 ± 0.2 ºC), increase in respiratory (38 ± 1.095/ minute), and heart rates (110±1.702/ minute). Signs of depression appeared 36 hours after cadmium administration manifested by: segregation of lambs from each other, extended head and neck, sometime lowered down with, general indolence. Seventy two hours post administration of cadmium chloride, some lambs showed sluggish response to external stimuli, anorexia and ruminal stasis with hard feces were obvious, rapid and shallow respiration became more pronounced accompanied with irregular heartbeats (Table 1), congestion of the mucous membrane was observed with moderate to severe dehydration, lateral recumbence with loss of vital response and decrease of the body temperature (subnormal temperature) occurred before death, two lambs were died in this group.

To cite this paper: Naji HA and Zenad MM. 2015. Oxidative stress in sheep induced by cadmium chloride toxicity, with therapeutic effects of alpha lipoic acid. *Online J. Anim. Feed Res*., 5(5): 142-147.
Scienceline/Journal homepages: http://www.science-line.com/index/; http://www.ojafr.ir

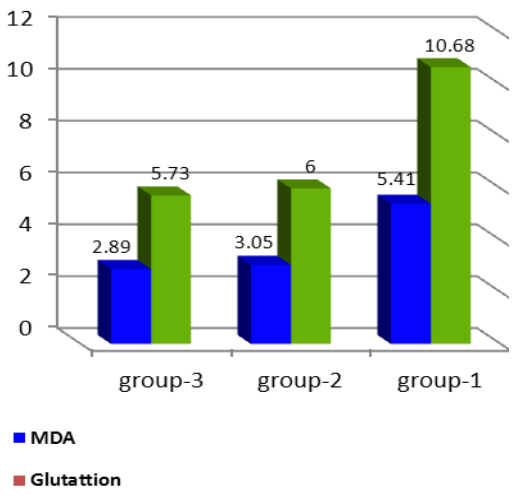
The lambs in the second group 2 showed mild decrease in appetite, normal ruminal contraction (2.4 ± 0.24/ minute) also mild increase in the body temperature (39.9 ± 0.18 ºC), slight increase in respiratory (33 ± 1.953/ minute) and heart (93 ± 1.067/ minute) rates, beside that no death was recorded in this group. No significant variation in the clinical parameters observed between second group and control (3) groups (Table 1).

The malondialdehyde (MDA) concentration in sera of group 1 significantly (P≤ 0.05) increased (5.41 ± 0.282 µmol/L), as compared with group 2 (3.05 ± 0.27 µmol/L) and control 3 groups (2.89 ± 0.167 µmol/L), also non significant variations in the levels of MDA between group 2 and control (3) groups were recorded (Figure 1). A significant (P≤ 0.05) increase of the glutathione concentration in the group 1 was (10.68 ± 0.38 µmol/L) higher than in the group 2 (6.008 ± 0.442 µmol/L) and control (3) group (5.73 ± 0.354 µmol/L). A significant (P≤ 0.05) higher activities of catalase enzyme in the sera of group 1 (103.85 ± 3.93 u/L) than in the group 2 (69.762 ± 2.200 u/L) and control (3) groups (66.46 ± 2.195 u/L) (Figure 2).

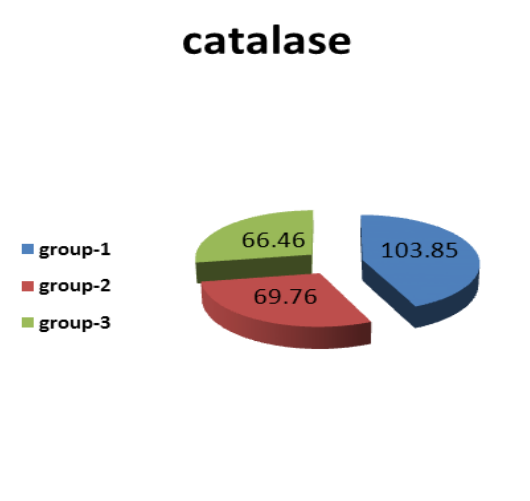
Table 1 - Clinical signs appeared on the three groups

Clinical sings	1st group $CdCl_2$	2nd group $CdCl_2$ & ALA	3rd group Control
Body temperature (ºC)	40.3 ± 0.2[a]*	39.9 ± 0.18[ab]	39.48 ± 0.21[b]
Respiratory rate/minute	38 ± 1.095[a]	33 ± 1.953[b]	31 ± 1.449[b]
Coughing	1(36 h)	0	0
Shallow respiration	4 (48 h)	1 (48 h)	0
Heart rate /minute	110 ± 1.702[a]	93 ± 1.067[b]	91 ± 2.369[b]
Weak and irregular heart beats	5 (48-72 h)	0	0
Congested mucous membrane	5 (18-36 h)	3 (24-48 h)	0
Icteric mucous membrane	2 (72h)	0	0
Mild dehydration	3 (48-72h)	0	0
Severe dehydration	2 (72-96 h)	0	0
Inappetance	5 (18-24 h)	4 (24-36 h)	0
Ruminal contraction	1.2 ± 0.2[a]	2.4±0.422[b]	2.8±0.2[b]
Complete anorexia	5 (36-48 h)	0	0
Constipation	4 (36-72 h)	0	0
Depression	5 (36-72 h)	2 (48 h)	0
Segregation of lambs	4 (24-36 h**)	1 (48 h)	0
Extended head and neck	4 (48-72 h)	1 (48h)	0
Reluctant to move & in coordination	4 (72 h)	0	0
Muscle tremor	2 (72 h)	0	0
Recumbence	3 (72 h)	0	0
Death	2 (96 h)	0	0

*Different small letters referred to significant difference between groups; h: means hours



A



B

Figure 1 – A) Concentrations of (µmol/L) malondialdehyde and **B)** Catalase activity (U/L) in sera of the three groups. Glutathione proxidease (GPx) in sera of lambs in the three groups.

To cite this paper: Naji HA and Zenad MM. 2015. Oxidative stress in sheep induced by cadmium chloride toxicity, with therapeutic effects of alpha lipoic acid. *Online J. Anim. Feed Res.*, 5(5): 142-147.
Scienceline/Journal homepages: http://www.science-line.com/index/; http://www.ojafr.ir

DISCUSSION

The toxic effect of cadmium had been studied in farm ruminant (Phillips et al., 2011; Tomas-Marciniak et al., 2011), The increased body temperature in the first group might be due to aseptic fever, which might be occurred due to damage of vessels and cells specially of liver and kidneys (also other organs) lead to liberation of endogens pyrogen (particularly granulocyte, monocyte and macrophage), the endogenous pyrogen causes releasing of archidonic acid with subsequent synthesis of prostaglandin (Barberà-Cremades et al., 2012). Consequently significant increase in the respiratory and heart rates in the first group were noticed (Table 1), these results were in agreement with others (Zaki and Mohamed, 2012), moreover the accelerated heart and respiratory rates might be attributed to the lesions occurred in the lungs (Roggeman et al., 2014, Lane et al*.,* 2015), beside that an increase demand of the tissues for oxygen because of histotoxic hypoxia or damage of lungs tissues might resulting due to cadmium toxicity leading to shallow and labored respiration (Stoev et al., 2003).

The atony of rumen and constipation, beside other signs of weakness and locomotors disturbances as well as neurological signs might be belonged to decrease calcium level in the muscular and nervous tissues, as the calcium ion is an essential for neuromuscular transmitting impulses as well as muscle function, these results corresponded with finding of others (Stoev et al., 2003), the cadmium chloride causes damage of renal tubules, might resulting in high excretion of calcium through the urine (Silvestri et al., 2015), and also occurrence of anorexia in lambs causes excessive reduction in the ingestion and absorbance of calcium, and this also might be contribute in occurrence of dehydration and worsening the condition.

The mild clinical signs in the second group as compared with the first and control groups denoted that alpha lipoic acid has capability to protect tissues from the damage effects produced by the cadmium chloride, in spite of antioxidants role were debated (Basta and Haenen, 2013). The ALA has characteristic functions: metals chelating agent, antioxidant effect, also aids in regeneration of vitamin C and E and plays an important role in the synthesis of glutathione and metallothionine, in addition to decrease the oxidative stress via lowering the free radicals produced by the cadmium toxicity (Park et al*.,* 2014), these characters made it highly efficient antioxidant compound.

The increase of MDA concentration in the sera of first group indicated high oxidative stress occurred due to cadmium toxicity, MDA is the main by–products formed by lipid peroxidation, resulting from high oxidative stress, the oxidative stress leads to excessive production of free radicals which are responsible for impaired cellular functions, furthermore lipid peroxidation causes irreversible damage of cell membrane (Stefania et al., 2013). The increases of glutathione level and catalase activity in the first group due to cadmium chloride toxicity were similar to the findings reported by others (Gills et al., 2015; Kar et al*.,* 2015). It was suggested that the increase of glutathione occurs to offset the free radicals produced by cadmium toxicity and other heavy metals (Jones et al., 2002), beside its role in elimination and detoxification of toxins and carcinogenesis process. Similarly the significant ($P<0.05$) increase of catalase activity in the first group might be belonged to the same reason (Maan and Kataria, 2012).

The non-significant ($P<0.05$) differences of MDA, glutathione concentrations and catalase activity in the second group as compared with control group (Figures 1 and 2) indicated that the alpha lipoic acid ameliorated the toxic effects of cadmium chloride to great extent. Glutathione is considered the main antioxidant enzyme against heavy metals, it was reported that the alpha lipoid acid increases production of cysteine, which is an important amino acid for the synthesis of the glutathione (Suh et al*.,* 2004).

CONCLUSION

The acute cadmium toxicity causes severe clinical signs in sheep involving different organs and multiple systems, gastrointestinal dysfunction, respiratory distress, neurological disorder, and locomotors abnormalities. The alpha lipoic acid has an ameliorating effect on cadmium toxicity as an antioxidant substance.

REFERENCES

Aebi H. (1984). Catalase in vitro. Methods in Enzymology Journal, 5: 121-126. [PMID: 6727660]

Agarwal A and Prabakaran SA (2005). Mechanism, measurement and prevention of oxidative stress in male reproductive physiology. Indian Journal of Experimental Biology, 43: 963-974. [PMID: 16315393]

Ahmadi A, Mazooji N, Roozbeh J, Mazloom Z. and Hasanzade J (2013). Effect of alpha-lipoic acid and vitamin E supplementation on oxidative stress, inflammation, and malnutrition in hemodialysis patients. Iran Journal Kidney Disease, 7(6): 461-7. [PMID: 24241092]

Bampidis AV, Nistor E, and Nitas D (2013). Arsenic, Cadmium, Lead, Mercury as undesirable substances in animal feeds. Journal of Animal Science and Biotechnology, 46 (1): 17-22. http://www.spasb.ro/index.php/spasb/article/viewFile/54/31

Barberà-Cremades M, Baroja-Mazo A, Gomez AI, Machado F, Di Virgilio F and Pelegrín P. (2012). P2X7 receptor-stimulation causes fever via PGE2 and IL-1β release. The Journal of the Federation of American Societies for Experimental Biology (FASEB J), 26(7): 2951-62. [PMID: 22490780]

Bast A and Haenen GR (2013). Ten misconceptions about antioxidants. Trends in Pharmacological Science, 34(8): 982-99. Doi: 10.1016/j.tips.2013.05.010

Esra B, Umit MS, Cansin S, Erzurum S and Kalayci O (2012). Oxidative Stress and Antioxidant Defense World Allergy Organization (WAO) Journal, 5(1): 9–19. [PMID: 23268465]

Gill KK, Sandhu HS and Kaur R (2015). Evaluation of lipid peroxidation and antioxidant status on fenvalerate, nitrate and their co-exposure in Bubalus bubalis. Pesticide Biochemistry and Physiology, 123:19-23. [PMID: 26267048]

Gomes MB and Negrato CA (2014). Alpha-lipoic acid as a pleiotropic compound with potential therapeutic use in diabetes and other chronic diseases. Diabetology & Metabolic Syndrome, 6:80. doi:10.1186/1758-5996-6-80. http://www.dmsjournal.com/content/6/1/80

Harlod E, James A, Doglas C, Cherly L, Franklin M and Glenn H (2011). The merck veterinary manual, Merck & CO., Inc. Rahaway. N.J., U.S.A. 11 ed.

Jones W, Li X, Qu ZC, Perriott L, Whitesell RR, and May JM (2002). Uptake, recycling, and antioxidant actions of alpha-lipoic acid in endothelial cells. Free Radical Biology and Medicine, 33(1): 83-93. [PMID: 12086686].

Kar R, Garg S, Halder S, Galav H, Chandra N and Mehndiratt N (2015). Cadmium Exposure Induces Oxidative Stress by Decreasing Expression of Antioxidant Enzymes in Mice Liver. International Journal of Clinical Biochemistry and Research, 2(2): 89-96.

Lane EA, Canty MJ and More Sj (2015). Cadmium exposure and consequence for the health and productivity of farmed ruminants. Research in Veterinary Science, 101:132-9. [PMID: 26267103]

Li R, Luo X, Li L, Peng Q, Yang Y, Zhao L, Ma M and Hou Z (2015). The Protective Effects of Melatonin Against Oxidative Stress and Inflammation Induced by Acute Cadmium Exposure in Mice Testis. Biological Trace Elements Research, First online 30 July. Doi:10.1007/s12011-015-0449-6. [PMID: 26224376] supplied by Springer publisher.

Maan R and Kataria N (2012). Evaluation of oxidative stress during adverse environmental conditions in Marwari sheep from arid tracts in India. Animal Biology and Animal Husbandry Bioflux, 4(2): 38-42.

Miller GZ and Harris ZE, (2015). Hazardous metals in vintage plastic toys measured by a handheld X-ray fluorescence spectrometer. Journal of Environmental Health, 77(6): 8-13. [PMID:25619030].

Monika J, Binkowski LJ, Blaszczyk M, Plauch J, Wojtas W, Massanyi P and Stawarz R (2015). Cadmium, Lead and Mercury concentration and their influence on morphology parameters in blood donors from differed age groups from souther Poland. Journal of Trace Elements in Medicine and Biology, 29: 342-346. Doi:10.1016/j.jtemb.2014.10.002.

Mueller S, Riedel HD and Stremmel W. (1997). Determination of catalase activity at physiological hydrogen peroxide concentration. Anaylytical Biochemistry, 245(1): 55-60. [PMID: 9025968]

Nagamani M, Parhaladu P, Vijayababu PVSS, Ashalata K, Kusuma KP and kumari LK (2015). IOSR Journal of Dental and Medical Sciences 14(5) Version II: 18-20. Doi: 10.9790/0853-14521820

Ognjanovic BI, Markovic SD, Pavlovic SZ, Zikic RV, Stajn AS and Saicic ZS (2008). Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: Protective effect of selenium. Physiological Research, 57(3): 403–411. [PMID: 17465690]

Park S, Karunakaran U, Jeoung NH, Jeon JH and Lee IK (2014). Physiological effect and therapeutic application of alpha lipoic acid. Current Medicinal Chemistry. 21(32): 36-45. [PMID: 25005184]

Paul BT, Clement GY, Anita KP and Dwayne JS (2014). Heavy Metals Toxicity and the Environment. Experientia Supplementum, 101: 133–164. Doi: 10.1007/978-3-7643-8340-46. [PMCID: PMC4144270]

Phillips CJ and Tudoreanu L (2011). A model of cadmium accumulation in liver and kidney of sheep derived from soil and dietary characteristics. Journal of the Science of Food and Agriculture 91: 370-376. [PMID: 20981736]

Rogeman S, de Boeck G, De Cock H, Blust R and Bervoets L (2014). Accumulation and detoxification of metals and arsenic in tissues of cattle (Bos Taurus) and the risk for human consumption. Science of the Total Environment, 466-467: 174-184. Doi: 10.1016/j.scitotenv.2013.07.007 [PMID: 23906855]

Schafer F and Buettner GR (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/ glutathione couple. Free Radical Biology and Medicine, 30 (11): 1191-1212. Doi: 10.1016/S0891-5849(01)00480-4

Shelly CU (2013). Review: Glutathion synthesis. Biochemica et Biophysica Acta, 830(5): 3143-3153. Doi: 10.1016/jbbagen.2012.09.008. www.siencedirect.com/science/article/pii/s0304416512002632

Silvestri S, Orlando P, Armeni T, Padella L, Brugè F, Seddaiu G, Littarru GP and Tiano L (2015). Coenzyme Q10 and α-lipoic acid: antioxidant and pro-oxidant effects in plasma and peripheral blood lymphocytes of supplemented subjects. Journal of Clinical Biochemistry and Nutrition, 57(1): 21-26. [PMID: 2623609]

Stefania P, Eric C, Martina D, Piergiorgio P, Alessia A, Gianpaolo C, Rosalba M, Chiara D, Alessio L, Fabrizio G and Giuseppina B (2013). Interaction of aldehydes derived from lipid peroxidation and membrane proteins. Frontier in Physiology, 4: 242. Doi: 10.3389/fphys.2013.00242 [PMCID: PMC3761222]

Stoev SD, Grozeva N, Simeanov R and Borisov I (2003). Experimental cadmium poisoning in sheep. Experimental and Toxicologic Pathology, 55(4): 309-314. [PMID: 14703778]

Suh JH, Shenvi SV, Dixon BM, Liu H, Jaiswal AK, Liu RM and Hagen TM (2004). Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. Proceeding of the National Academy of Sciences of the United State of America, 101(10): 3381-3386. [PMID: 14985508]

Tomaz-Marciniak A, Pilarczyk B, Bakowaska M, Pilarczyk R, Wojcik J, Marciniak A and Herdzel D (2011). Relationship between Selenium and selected heavy metal concentration in serum of cattle from a non polluted area. Biological Trace Element Research, 144: 517-524. [PMID: 21603865]

Wang J, Zhu H, Liu X and Liu Z (2014). Oxidative stress and Ca (2+) signals involved on cadmium-induced apoptosis in rat hepatocyte. Biological Trace Element Research, 161(2): 180-9. Doi: 10.1007/s12011-014-0105-6. [PMID: 25123461]

Wysocka M, Kubin M, Vieira LQ (1995). Interleukin-12 is required for interferon production and lethality in LPS-induced shock in mice. European Journal of Immunology, 25: 672-676. DOI: 10.1002/eji.1830250307 [PMID: 7705395]

Zaki MS and Mohamed MI (2012). Some studies in Baraka sheep intoxicated with cadmium. Life Sciences Journal, 9(3): 786-790.