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

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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 CdCl2 3 mg/kg.bw subcutaneously (S/C), the second group 2 was injected with the same dose of CdCl2 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 CdCl2 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 CdCl2 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 (MDA) and glutathione (GSH) concentrations and marked elevation of serum catalase activity (103.85 ± 3.93 µmol/L) was also observed in group 1, 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.

INTRODUCTION

Cadmium (Cd) is a heavy toxic metal, with undesirable effects in animals (Bampidis et al., 2013); it has 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);
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 administrated 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, administrated 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 CdCl2 and two days post administration of CdCl2 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.

\[
2H_2O_2 + \text{CAT} \rightarrow 2H_2O + O_2
\]

Consequently absorbance was decrease due to H2O2 consumption (\(\varepsilon = 0.04\)mmol-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 CdCl2, the signs were including inappetance, decrease ruminal contraction (1.2 ± 0.2 contraction/ minute), slight increase of the body temperature (40.3 ± 0.2 °C), 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.
The lambs in the second group showed mild decrease in appetite, normal ruminal contraction (2.4 ± 0.24/minute) also mild increase in the body temperature (39.9 ± 0.18 °C), 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

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>1st group CdCl₂</th>
<th>2nd group CdCl₂ &amp; ALA</th>
<th>3rd group Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature (°C)</td>
<td>40.3 ± 0.2**</td>
<td>39.9 ± 0.18b</td>
<td>39.48 ± 0.21b</td>
</tr>
<tr>
<td>Respiratory rate/minute</td>
<td>38 ± 1.095a</td>
<td>33 ± 1.953b</td>
<td>31 ± 1.449b</td>
</tr>
<tr>
<td>Coughing</td>
<td>1(36 h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shallow respiration</td>
<td>4(48 h)</td>
<td>1(48 h)</td>
<td>0</td>
</tr>
<tr>
<td>Heart rate /minute</td>
<td>110 ± 1.702a</td>
<td>93 ± 1.067b</td>
<td>91 ± 2.369b</td>
</tr>
<tr>
<td>Weak and irregular heart beats</td>
<td>5(48-72 h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Congested mucous membrane</td>
<td>5(18-36 h)</td>
<td>3(24-48 h)</td>
<td>0</td>
</tr>
<tr>
<td>Icteric mucous membrane</td>
<td>2(72h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mild dehydration</td>
<td>3(48-72h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Severe dehydration</td>
<td>2(72-96 h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inappetance</td>
<td>5(18-24h)</td>
<td>4(24-36 h)</td>
<td>0</td>
</tr>
<tr>
<td>Ruminal contraction</td>
<td>1.2 ± 0.2a</td>
<td>2.4±0.422b</td>
<td>2.8±0.2b</td>
</tr>
<tr>
<td>Complete anorexia</td>
<td>5(36-48 h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td>4(36-72 h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Depression</td>
<td>5(36-72 h)</td>
<td>2(48 h)</td>
<td>0</td>
</tr>
<tr>
<td>Segregation of lambs</td>
<td>4(24-36 h ***)</td>
<td>1(48 h)</td>
<td>0</td>
</tr>
<tr>
<td>Extended head and neck</td>
<td>4(48-72 h)</td>
<td>1(48h)</td>
<td>0</td>
</tr>
<tr>
<td>Reluctant to move &amp; in coordination</td>
<td>4(72 h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Muscle tremor</td>
<td>2(72 h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Recumbence</td>
<td>3(72 h)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Death</td>
<td>2(96 h)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

![Figure 1](http://www.science-line.com/index/) - A) Concentrations of (μmol/L) malondialdehyde and B) Catalase activity (U/L) in sera of the three groups. Glutathione peroxidase (GPx) in sera of lambs in the three groups.
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 locomoters 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 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


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


