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Research Article
Assessment of Cyadox Effects on the Antioxidant Defense System and Hemolysis of Isolated Rabbit Erythrocytes

[1]Mahmoud Alagawany, [2]Mayada Ragab Farag, [1]Mohamed Ezzat Abd El-Hack, [3]Elisabetta Casalino, [4]Vincenzo Tufarelli, [5]Maryam Sayab and [6]Kuldeep Dhama

[1]Department of Poultry, Faculty of Agriculture, Zagazig University, 44519 Zagazig, Egypt
[2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt
[3]Department of Veterinary Medicine, University of Bari 'Aldo Moro', 70010 Valenzano, Bari, Italy
[4]Department of Emergency and Organ Transplantation (DETO), Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', 70010 Valenzano, Bari, Italy
[5]ACE College for Women, Sargodha Road, Faisalabad, Pakistan
[6]Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly, 243122 Uttar Pradesh, India

Abstract

Objective: The current study was conducted to assess the biological *in vitro* impacts of cyadox (CYA) as growth promoter on erythrocytes isolated from rabbits. **Methodology:** Suspensions of erythrocytes were divided into 6 groups (5 replicates each), 1st group served as control; 2nd, 3rd, 4th, 5th and 6th groups were respectively subjected to CYA at 2.5, 5, 10, 20 and 40 μg mL^{-1}, then incubated for 3 and 6 h at 37°C and shaken gently from 3-4 times per hour. **Results:** The data obtained revealed that the low doses of CYA (2.5 and 5 μg mL^{-1}) diminished the reduced glutathione (GSH) content and enhanced the catalase (CAT) and superoxide dismutase (SOD) activities even after incubation with CYA for 6 h. These concentrations also had no linear or quadratic influences on the values of malonaldehyde (MDA) and protein carbonyl (PrC) as well as the hemoglobin (Hb), adenosine triphosphate (ATP) and total protein (TP) levels in erythrocytes. Increasing the concentration of CYA (10, 20 and 40 μg mL^{-1}) and increasing the incubation period resulted in depletion of GSH, inhibited the CAT and SOD activities and decreased the protein content in the treated RBCs while the levels of PrC, MDA, Hb, TP and ATP were increased in response to increasing the dose and incubation time. **Conclusion:** From these results it could be concluded that CYA may be safe at recommended doses (2.5 and 5 μg mL^{-1}) while using at high concentrations revealed pro-oxidant properties which could in turn affect cell survival.

Key words: Cyadox, antioxidants, eryhtrocyes, ATP, hemolysis, rabbit

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Corresponding Author: Mahmoud Alagawany, Department of Poultry, Faculty of Agriculture, Zagazig University, 44519 Zagazig, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Quinoxaline 1, 4-dioxide (QdNOs) derivatives are used widely as antimicrobial agents with useful properties when added to the diets of poultry, pigs and cattle at sub-therapeutic levels[1]. Among QdNOs class, mequindox (MEQ), olaquindox (OLA), quinocetone (QCT), carbadox (CBX) and cyadox (CYA) are the known members[2,3]. Despite their growth promoting effects, the intensive use of QdNOs derivatives in livestock rations in high concentrations for long durations usually produces harmful impacts[4]. The OLA, CBX and MEQ were reported to induce mutagenesis, carcinogenesis and developmental and reproductive toxicities[5,6]. While, CYA (2-formylqui-noxaline-N1,N4-dioxide cyanocetylhydrazone, CAS No. 65884-46-0, C12H9N5O3) has been reported to have beneficial influences on growth, feeding behaviors including feed conversion ratio as well as performance of food-producing animals including goats[7], pigs[8] and poultry[9] with little toxicity. The growth promotion of the antimicrobial CYA is generally believed to be due to the intestinal microflora modifications, including the suppression of intestinal pathogenic microorganisms[10].

The metabolic capacity and rapidity of poultry and swine to produce CYA metabolites by primary hepatocytes, liver microsomes and intestinal microbiota systems has been observed to be greater than experimental animals, with some differences in final metabolites[11]. Increasing feed supplementation of CYA improves growth performance by altering concentrations of peripheral metabolic hormones such as insulin and thyroid hormones as well as epidermal growth factor[12]. The CYA at 50 mg kg^{-1} has been found to decrease the incidence of diarrhea, improve lean percentage, promotes growth efficiently and significantly improving the carcass qualities, whereas increasing its level to 250 mg kg^{-1} reduced the average daily weight gain of animals[7]. The microbiological safety of CYA on human intestinal flora was reported by Hao *et al.*[13].

However, CYA showed good safety in some *in vivo* and *in vitro* studies[14] and further investigations on the cellular level are still needed to provide sufficient information about the recommended levels of using CYA as feed additive in poultry and animal diets. There are no reports available on the effect of CYA on the antioxidant status of RBCs which are biological lipid membrane models, highly sensitive to the process of peroxidation owing to high oxygen tension and high content of polyunsaturated fatty acids (PUSFA) in membrane. Therefore, this study was designed with an aim to assess the biological effect of CYA at different concentrations and periods of exposures on superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) of isolated rabbit RBCs as well as its impacts on the content of ATP (adenosine triphosphate) and hemoglobin as markers for hemolysis, besides studying the impact of CYA on protein and lipid peroxidation of rabbit RBCs. The system of SOD-CAT plays a crucial role as the rst line of defense vs peroxidative damage and is indicator to the formation rate of free radical due to the essential function of GSH in cellular transformation[15]. Rabbits were used for this study as they are important to compensate the shortage and very high cost of red meat and maintaining their sound health is considered a critical issue as the oxidative stress could lead to hemolysis of erythrocytes and result in cardiovascular problems and anemia particularly when feed additives were used for long periods.

MATERIALS AND METHODS

Chemicals: Cyadox (CYA, $C_{12}H_9N_5O_3$, molecular weight 271.23, CAS No. 65884-46-0, purity 98%) compound was purchased from Hangzhou Uniwise International Co., Ltd. (Zhejiang, China (Mainland). Antioxidants estimation Kits were purchased from Biodiagnostic, BD and Egypt. All other chemicals were purchased from Sigma (St. Louis, MO, USA). All other reagents used were of analytical grade.

Animals and care: Five male New Zealand white rabbits (3 months of age and initial weight of 2.00±0.05 kg) were used, obtained from Rabbit Farm of Faculty of Agriculture, Zagazig University, Zagazig, Egypt. Animals were individually housed in stainless steel cages at room temperature (25±2°C) with a relative humidity of 50-60% and on a 12 h light-darkness cycle. The animals were provided free access to commercial pellet diet and water *ad libitum*. The care and welfare of the animals conformed to the guidelines of the Animal Use Research Ethics Committee of Zagazig University, Egypt.

Preparation of erythrocytes: Five milliliters of blood was collected aseptically from the ear vein of all animals in a test-tube containing heparin to avoid coagulation. Blood was centrifuged at 3000 rev min^{-1} for 10 min at 4°C and the plasma and buffy coat were carefully removed. The erythrocytes were harvested by centrifugation after washing once with 0.9% NaCl solution and two times with ice-cold phosphate buffered saline (PBS: 145 mM NaCl, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4) and finally re-suspended in PBS to obtain cell suspensions at 10% hematocrit value used for incubations[16].

Treatment of erythrocytes: The CYA was solubilized in a DMSO (0.1%) and further diluted in PBS till required concentrations (0, 2.5, 5, 10, 20 and 40 μg mL^{-1}). Suspensions of RBCs were distributed into 6 treatments with five replications, 1st group was kept as control treated with PBS and DMSO under the same concentration: 2nd, 3rd, 4th, 5th and 6th groups were subjected to the previously mentioned concentrations of cyadox at 2.5, 5, 10, 20 and 40 μg mL^{-1}, respectively. The reaction mixtures of all treatments were incubated at 37°C for 3 and 6 h with gentle shaking every fifteen minutes. For antioxidant indices, lipid and protein oxidation markers, the mixtures were stored after incubation at -20°C and thawed one day later for RBCs lysis by osmotic pressure, then they were centrifuged and supernatants obtained.

Assessment of antioxidant activities of erythrocytes

Antioxidant indices: Superoxide dismutase (SOD) activity was measured according to Misra and Fridovich[17]. Catalase (CAT) activity was determined according to Aebi[18] where the decrease in hydrogen peroxide concentration was measured spectrophotometrically at 240 nm during 1 min. Total GSH contents were measured by Ellman's reaction using 5,5-dithiobis 2-nitrobenzoic acid[19].

Lipid peroxidation: Lipid peroxidation was assessed by determining erythrocyte malonaldehyde (MDA) levels using the thiobarbituric acid method[20] and the reaction product was measured spectrophotometrically at 535 mm.

Protein oxidation: The content of protein carbonyl was determined as an index of protein oxidation according to Uchida and Stadtman[21].

Hemoglobin and total protein: The process of RBCs hemolysis was monitored by hemoglobin (Hb) release. Directly after the incubation periods, 50 mL of cell suspension was added to 1 mL of PBS and centrifuged (1000 rev min^{-1}, 7 min). The Hb content of supernatants was determined by absorbance at 540 nm using the Varian Cary 50 UV-vis spectrophotometer. While, total protein (TP) concentrations in the hemolysates of the thawed erythrocytes was determined by standard kits spectrophotometrically based on the colorimetric biuret method according to Bradford[22].

Measurement of extracellular ATP content: The technique for measurement of the ATP level in the reaction mixture was based on the reactions according to Adams[23].

Statistical analysis: All data of antioxidant and other parameters were subjected to one-way ANOVA procedures for completely randomized design, using the GLM procedure (SAS[24], version 8) at the pre-set level of 5% and orthogonal polynomial contrast (linear and quadratic) when significant.

RESULTS

Impact of CYA on antioxidant indices: The influence CYA on antioxidant indices of the normal rabbit erythrocytes incubated with indicated concentrations for different times is shown in Table 1 and 2. The CYA at low doses (2.5 and 5 μg mL^{-1}) enhanced the activities of SOD and CAT and increased GSH concentration compared to the control group. CAT activity was linearly ($p<0.001$) decreased with gradual increase in the concentration of CYA from 10-40 μg mL^{-1} and the incubation time. The lowest value was recorded at 40 μg mL^{-1} (1372 IU g^{-1} Hb) after 6 h of incubation compared with control group (1465 IU g^{-1} Hb). The SOD activity was linearly and quadratically ($p<0.001$) affected by the higher

Table 1: Effect of cyadox (CYA) concentrations on catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) activities of rabbit erythrocyte exposed to CYA for 3 h

Treatments	Antioxidant parameters		
(μg mL^{-1})	CAT (IU g^{-1} Hb)	SOD (IU g^{-1} Hb)	GSH (μmol g^{-1} Hb)
0 CYA	1465	879	3.95
2.5 CYA	1471	880	4.05
5 CYA	1463	877	3.85
10 CYA	1458	864	3.75
20 CYA	1414	765	3.07
40 CYA	1386	734	2.14
SEM[1]	9.30	10.78	0.19
p-value[2]			
Linear	<0.001	<0.001	<0.001
Quadratic	0.627	<0.001	0.002

[1]SEM: Standard error means, [2]Linear and quadratic effects of cyadox levels

Table 2: Effect of cyadox (CYA) concentrations on catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) activities of rabbit erythrocyte exposed to CYA for 6 h

Treatments	Antioxidant parameters		
(μg mL^{-1})	CAT (IU g^{-1} Hb)	SOD (IU g^{-1} Hb)	GSH (μmol g^{-1} Hb)
0 CYA	1461	857	3.94
2.5 CYA	1461	875	3.81
5 CYA	1464	865	3.63
10 CYA	1440	855	3.31
20 CYA	1401	750	2.99
40 CYA	1372	729	1.87
SEM[1]	9.30	10.78	0.19
p-value[2]			
Linear	<0.001	<0.001	<0.001
Quadratic	0.287	0.176	0.402

[1]SEM: Standard error means, [2]Linear and quadratic effects of cyadox levels

concentrations of CYA for 3 h, 40 μg mL^{-1} decreased SOD activity to 734 IU g^{-1} Hb as compared to control value (879 IU g^{-1} Hb). However, incubation with CYA for 6 h has no quadratic effect (0.176) on SOD activity, this incubation period linearly ($p<0.001$) decreased its activity by increasing CYA concentration at 40 μg mL^{-1} (729 IU g^{-1} Hb) compared to control (857 IU g^{-1} Hb). The same effects of concentration and time response on SOD activity were observed for GSH concentration.

Impact of CYA on lipid and protein oxidation: The LPO (Lipid peroxidation) as represented by the MDA level and protein oxidation measured by PrC content was not significantly affected by incubation with low doses of CYA (2.5 and 5 μg mL^{-1}) for 3 or 6 h (Table 3, 4). Meanwhile, MDA and PrC content were both linearly and quadratically ($p<0.001$) increased with the high concentrations of CYA in a dose and time dependent manner, where the highest MDA and PrC concentrations were obtained after incubation with 40 μg mL^{-1} of CYA for 6 h (3.74 and 7.72 μmol g^{-1} Hb) compared to untreated group (2 and 5.60 μmol g^{-1} Hb), respectively and compared with the all other treated groups.

Table 3: Effect of cyadox (CYA) concentrations on malondialdehyde (MDA) and protein carbonyl (PRC) concentrations of rabbit erythrocyte exposed to CYA for 3 h

	Lipid and protein peroxidation	
Treatments (μg mL^{-1})	MDA (μmol g^{-1} Hb)	PRC (μmol g^{-1} Hb)
0 CYA	2.10	5.70
2.5 CYA	2.00	5.72
5 CYA	2.07	5.68
10 CYA	2.88	6.11
20 CYA	3.11	6.59
40 CYA	3.21	7.04
SEM[1]	0.04	0.04
p-value[2]		
Linear	<0.001	<0.001
Quadratic	<0.001	<0.001

[1]SEM: Standard error means, [2]Linear and quadratic effects of cyadox levels

Table 4: Effect of cyadox (CYA) concentrations on malondialdehyde (MDA) and protein carbonyl (PRC) concentrations of rabbit erythrocyte exposed to CYA for 6 h

	Lipid and protein peroxidation	
Treatments (μg mL^{-1})	MDA (μmol g^{-1} Hb)	PRC (μmol g^{-1} Hb)
0 CYA	2.00	5.60
2.5 CYA	2.10	5.73
5 CYA	2.18	5.78
10 CYA	2.94	5.94
20 CYA	3.32	6.20
40 CYA	3.74	7.72
SEM[1]	0.04	0.04
p-value[2]		
Linear	<0.001	<0.001
Quadratic	<0.001	<0.001

[1]SEM: Standard error means, [2]Linear and quadratic effects of cyadox levels

Impact of CYA on TP content: In Table 5 and 6, the incubation of erythrocytes with low doses of CYA (2.5 and 5 μg mL^{-1}) for the two indicated periods showed the same effects on the protein content of the hemolysate (i.e., did not alter the protein content). While, protein content in hemolysate was linearly ($p<0.001$) but not quadratically (0.244 at 3 h and 0.0684 at 6 h) decreased with increasing the concentration of CYA and the incubation time to record the lowest value (3.65 g dL^{-1} hemolysate) at 40 μg mL^{-1} after 6 h of incubation comparing with control group (7.14 g dL^{-1} hemolysate).

Impact of CYA on Hb content: The impact of CYA at different doses and incubation periods on content of Hb in the hemolysate of rabbit RBCs are represented in Table 5 and 6.

Table 5: Effect of cyadox (CYA) concentrations on on hemoglobin (HB) and total protein (TP) and adenosine triphosphate (ATP) concentrations of rabbit erythrocyte exposed to CYA for 3 h

	Parameters		
Treatments (μg mL^{-1})	Hb (g dL^{-1} hemolysate)	TP (g dL^{-1} hemolysate)	ATP (μmol g^{-1} Hb)
0 CYA	12.83	7.21	4.62
2.5 CYA	12.77	7.15	4.62
5 CYA	13.27	7.22	4.57
10 CYA	14.11	6.78	5.01
20 CYA	14.63	6.07	5.56
40 CYA	14.96	5.53	6.11
SEM[1]	0.22	0.16	0.14
p-value[2]			
Linear	<0.001	<0.001	<0.001
Quadratic	0.009	0.244	0.023

[1]SEM: Standard error means, [2]Linear and quadratic effects of cyadox levels

Table 6: Effect of cyadox concentrations on hemoglobin (HB) and total protein (TP) and adenosine triphosphate (ATP) concentrations of rabbit erythrocyte exposed to CYA for 6 h

	Parameters		
Treatments (μg mL^{-1})	Hb (g dL^{-1} hemolysate)	TP (g dL^{-1} hemolysate)	ATP (μmol g^{-1} Hb)
0 CYA	12.78	7.14	4.73
2.5 CYA	13.40	6.88	4.76
5 CYA	13.71	6.42	4.85
10 CYA	14.84	5.91	5.31
20 CYA	15.44	5.71	6.27
40 CYA	16.25	3.65	7.60
SEM[1]	0.30	0.28	0.25
p-value[2]			
Linear	<0.001	<0.001	<0.001
Quadratic	<0.001	0.684	0.480

[1]SEM: Standard error means, [2]Linear and quadratic effects of cyadox levels

Incubation of erythrocytes with low doses of CYA (2.5 and 5 μg mL^{-1}) for 3 or 6 h did not linearly or quadratically affect the Hb content. On the other hand, Hb content was both linearly and quadratically ($p<0.001$) increased by incubation with higher CYA concentrations in a dose and time dependent manner. The highest Hb content (16.25 g Hb dL^{-1} hemolysate) was obtained after incubation with 40 μg mL^{-1} for 6 h compared to the control and all other treated groups.

Impact of CYA on ATP release: The ATP level in the control group erythrocytes was 4.62 and 4.73 μmol g^{-1} Hb at 3 and 6 h, respectively (Table 5, 6). Supplementation of 2.5 and 5 μg mL^{-1} of CYA to rabbit erythrocyte for 3 or 6 h did not statistically affect the ATP content. While, incubation with the higher concentrations of CYA for 3 h produced linear ($p<0.001$) and quadratic ($p = 0.023$) increase of the ATP content to be 6.11 μmol g^{-1} Hb compared to control (4.62 μmol g^{-1} Hb) at 40 μg mL^{-1}. On the other hand, incubation with CYA for 6 h had no quadratic effect ($p = 0.480$) on the ATP level, however this incubation period linearly ($p<0.001$) increased ATP level by increasing CYA concentration to achieve the highest level (7.60 μmol g^{-1} Hb) at 40 μg mL^{-1} compared to the control treatment (4.73 μmol g^{-1} Hb).

DISCUSSION

Studies on QdNOs derivatives, particularly CYA gave some information on their growth promoting influences and hazardous impacts but on the other hand could not give a sufficient characterization of the biological safety limit of these kinds of feed additives[3,25]. Therefore, in the present experiment we tried to assess the permissible limits of CYA that could be helpful in improving the function of the body cells without causing deleterious impacts. The most important ndings of the present experiment are that the low doses of CYA (2.5 and 5 μg mL^{-1}) displayed a positive effect on enhancing the activities of SOD and CAT and GSH concentration even after incubation with CYA for 6 h. These concentrations also did not influence the MDA and PrC concentrations as well as the Hb, ATP and total protein contents of erythrocytes suggesting the safety of these concentrations on the biological lipid membrane of erythrocytes.

The in vitro effects of QdNOs including CYA have been characterized earlier by Huang *et al.*[25], their results suggested that CYA displayed much less toxicity in adrenocortical cells. Moreover, Ihsan *et al.*[14] reported very weak mutagenic activities of CYA on bacterial cells on Ames test comparing with other QdNOs. Similar results on the microbiological safety of cyadox, on human intestinal flora at 0, 16, 32 and 128 μg mL^{-1} were reported by Hao *et al.*[13] where they observed no change in short-chain fatty acids (SCFAs) after exposure to different concentrations. Lower concentration of cyadox (16 μg mL^{-1}) had no adverse effect on human microflora. In this regard, Wang *et al.*[26] and Huang *et al.*[27] speculated that quinoxaline derivatives including CYA have been widely used as antibacterial factors and growth enhancers. In addition, QdNOs have manifold beneficial applications and biological characteristics, including antioxidant, antitumoral, anti-inflammatory, antimicrobial, antibacterial and antitrypanosomal activities[28,29]. Zhao *et al.*[30] studied the in vitro evaluation of QdNOs including cyadox, quinocetone, mequindox and their metabolites for antiviral, antimycoplasma, antituberculosis, antifungal and activities. However, higher concentrations at 32 and 128 μg mL^{-1} could change population of bacteria and increased the proportion of resistant *Enterococcus* and *E. coli* and this might be agreed with us in that, increasing the concentration of CYA (10, 20 and 40 μg mL^{-1}) as well as increasing the period of incubation resulted in decreased level of GSH and TP as well as the activities of CAT and SOD in rabbit RBCs while increasing the markers of hemolysis (Hb and ATP) and the concentrations of MDA and PrC in a dose and time dependent manner. Furthermore, Farag *et al.*[31] pointed out that incubation of rabbit RBCs with CYA (40 mg mL^{-1}) increased MDA and PrC contents while it decreased the TP, HB and antioxidant enzymes.

The antioxidant enzyme SOD is widely distributed in all cells and high amount of it is present in erythrocytes[32]. The SOD protects cell against oxidative damage by converting the highly reactive superoxide anion to O_2 and to a less reactive[33] species, H_2O_2. The SOD contains both copper and zinc. Zinc keeps the stability of the enzyme, while copper maintains its activity[34]. So the decreased activity of SOD obtained in this study may be returned to the consumptions of this enzyme in converting the O_2 to H_2O. The CAT present in erythrocytes in the form of soluble protein that protect hemoglobin from peroxidation as catalase is important in eliminating the potentially dangerous formation of H_2O_2 in cells[35]. Additionally, the antioxidant enzymes including CAT and SOD could eradicate ROS acting as defense ways against intracellular oxidative stress and protecting cell by their free radical scavenging activities[36]. So decreasing their activities is suggestive for the proxidant effect of higher doses of CYA and its ability to produce an unstable release of ROS which was the key mediator of QdNOs-induced apoptosis via mitochondrial dependent pathway in adrenocortical cells as reported by

Huang *et al.*[25]. The ROS can react with macromolecules like nucleic acids, proteins and lipids resulting in alterations in the structure and activity of biologically relevant molecules causing cell damage[37] and had important roles in lipid peroxidation, ageing and the peroxidative hemolysis[38].

The CYA also induced fall of the GSH level that is known to provide the primary antioxidant defense for the stored erythrocytes, protect membrane lipids and proteins and keep the stability of the membrane skeleton and survival of erythrocytes against oxidative damage[39]. It is also well established that, in RBCs, GSH protects important proteins against oxidation and that it is a vital sulfhydryl buffer, maintaining SH groups in Hb and enzymes in the reduced state[36]. The decreased content of GSH after incubation with high concentrations of CYA could be returned to its oxidation that can be induced directly due to the attack of free radicals or indirectly through consumption of GSH during repair processes such as the reduction of oxidized membrane-protein thiol groups as suggested by Bartosz[20]. Moreover, reduced glutathione could bind with MDA and other deleterious endogenous substances beside its important role in cellular transformation[15]. This came parallel to the observation that, MDA increased in the presence of high doses of CYA (20 and 40 μg mL^{-1}), hence, indicating a state of considerable erythrocytic oxidative injury. The MDA, the marker of LPO of erythrocytes, is a highly reactive bifunctional molecule, that cross-link erythrocyte phospholipids and proteins to alter the functions of cell membrane leading to decreased erythrocytic survival and has been proposed as a general mechanism for cell injury and death (i.e., induce hemolysis)[40]. This comes on line with our results concerning the effect of high doses of CYA on Hb and ATP content of the hemolysate. The ATP is used by erythrocytes to maintain membrane shape, control deformation and maintain osmotic stability as well as sub-membrane skeletal-network proteins[41]. The basal stimulated ATP-release in the present study found to be correlated tightly with extracellular hemoglobin, a marker of hemolysis. This is in agreement with Sikora *et al.*[42] who demonstrated that the only source of extracellular ATP was cell lysis. These closely related effects could be explained by the opinion of Banerjee *et al.*[43] who stated that as a result of LPO, the erythrocyte membrane becomes weak and loses its integrity rapidly leading to Hb release. The present findings supported those of May[44] that cell membrane is often the initial site of damage and peroxidation of membrane lipids causes hemolysis and alter the protein and lipid content to different extents. The increased damage as a result of LPO is indicative for the insufficiency of enzymes in reacting with ROS generated in the membrane[45].

Erythrocytes SOD contains copper and zinc (CuZn-SOD). The interaction between MDA and CuZn-SOD leads to the modi cation of histidine amino acid residues and the production of protein-protein cross-linked derivatives as a result each type of ROS gives a different protein oxidation pattern[46]. This could explain the generation of protein carbonyl derivatives which may be the main reason of the decreased protein content of the rabbit erythrocytes after incubation with CYA on a dose-time-dependent way. The present findings give some information for the food safety assessment of CYA in rabbit nutrition and will improve the understanding of the healthical, pharmacological and toxicological aspects of CYA in rabbits.

CONCLUSION

In conclusion, low concentrations of CYA could be helpful in improving the function of the body cells without causing hazardous effects. However, the higher concentrations could result in serious health hazards indicating the possibility of CYA to generate superoxide radicals and a state of oxidative injury.

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