رقم البحث (36)

THE ROLE OF SELENIUM AND VITAMIN C ON CLINICAL OUTCOMES AND OXIDATIVE STRESS STATUS IN DRAFT HORSES WITH INFLAMMATORY AIRWAY DISEASE

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ABSTRACT

The aim of the present study was to evaluate the administration of combine selenium (Se) and vitamin C on the clinical outcomes, antioxidant trace element status and antioxidant enzymes activities in horses with inflammatory airway disease. For this purpose, 40 draft horses with lower airway disease were randomly allocated to 4 groups (10 each). Group 1 and 3 had acute disease; however, group 2 and 4 had chronic disease. For all groups, each horse was administered antibiotic, non-steroidal anti-inflammatory and mucolytic drug. In addition, group 3 and 4 received injectable Se and vitamin C. In supplemented groups compared with non-supplemented; there was a significant increase ($p<0.05$) in the levels of copper (Cu), Zinc (Zn), Se, and iron (Fe) also in the activity of glutathione-S-transferase (GST) and catalase (CAT). Meanwhile, there was a significant decrease ($p<0.05$) in the levels of manganese (Mn), malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and low density lipoprotein (LDL) and in the activity of glutathione reductase (GR). The results of the present study indicate that administration of Se and vitamin C in acute and chronic lower airway diseases in horses may have beneficial effect on clinical outcomes and antioxidant balance.

Key Words: Inflammatory airway disease; Treatment; Antioxidants; Free Radicals; Horses.

INTRODUCTION

Respiratory disease is gratifying to evaluate and treat for equine clinicians. The respiratory system is highly available for diagnostic testing, responds to an extensive armamentarium of drugs, and has a relatively favorable capacity for healing **(Rush and Mair, 2004)**. Respiratory disorders typically respond favorably to appropriate medical therapy, and treatment options for bronchodilation, immunomodulation, antimicrobial activity, and reduction of pulmonary inflammation are well-characterized in horses **(Rush and Mair, 2004)**.

Penicillin G as procaine salt and potentiated sulfonamides is utilized widely in horses with respiratory diseases including inflammatory airway disease (IAD) **(Hodgson and Hodgson, 2002)**. Anti-inflammatory, mucolytic and mucokinetic drugs were also indicated as a definitive therapy for treatment of IAD improving mucokinesis especially when the inflammatory response was exacerbating clinical signs of the disease **(Radostits et al., 2007)**.

Lykkesfeldt and Svendsen (2007) stated that increased oxidative stress was initially counteracted by antioxidant network, as the damaged molecules were either repaired or catabolized. In the light of the pro-inflammatory effects of ROS, antioxidant therapy aimed at restoring the oxidant/antioxidant balance appears to be a promising therapeutic perspective **(De Moffarts et al., 2005)**. Moreover, results of a number of studies by **Schunemann et al. (2001)** have suggested that ascorbic acid may be beneficial in the treatment of asthma, airway obstruction and airway hyper-reactivity in human. Also, **Kirschvink et al. (2002)** reported that bronchoscopic scoring of airway inflammation was reduced after the antioxidant treatment and that the blood antioxidant status tended to be improved.

Kirschvink et al. (2008) stated that antioxidant supplementation (vitamin C, vitamin E and selenium) had been performed in recurrent airway obstruction (RAO) affected horses. There were limited studies on the use of antioxidants in the treatment of respiratory diseases in horses. The previous reports focused mainly in trainer horses **(De Moffarts et al., 2005)**. Based on this speculation, in the present study, we evaluated the effect of injectable Se and vitamin C with antibiotic and anti-inflammatory drugs on selected trace elements status, oxidative stress markers and antioxidant enzymes activity in draft horses with acute and chronic inflammatory airway disease.

MATERIAL AND METHODS

1. Animals

In this study, a total of 40 out of 80 draft horses with signs of lower airway disease were randomly selected. Twenty horses suffered from acute disease and twenty with chronic signs. Selected horses aged between six months to four years old. Horses were classified to have acute or chronic lower respiratory disease.

2. Study design

Forty horses (20 with acute and 20 with chronic lower airway) were selected randomly and subjected for treatment. Each group was randomly allocated to two subgroups (10 each). Group one and group two (acute and chronic), respectively treated with procaine penicillin combined with dihydrostreptomycin sulphate (Pen & strep: Norbrook Laboratories), Phenyle-butazone (Buta-phenil: USO VETERINARIO), sulphadimidine-trimethoprim (Borgal 24%: Intervet Egypt for Animal Health) combination and bromhexine (Bisolvon: Chemical Industries Development (CID), Giza-G.C.R.) according to manufacturer for 5 days for acute group and 10 days for chronic ones. Group three and group four (acute and chronic), respectively received the same medications for the same periods as other groups in addition to antioxidant preparations as vitamin E acetate and sodium selenite (Viteselen: ADWIA Co., Egypt) combination and ascorbic acid (Vitamin C) (Cevarol: MEMCo. Memphis Co.) according to manufacturer for 4 weeks.

3. Blood samples collection

Two venous blood samples (ten ml each) were collected from each horse via jugular vein puncture using clean sterile syringes and needles at first time of examination (pre-treatment), 2 weeks and 4 weeks post-treatment. The first blood sample was collected into heparinized sterile syringe to separate blood plasma. Meanwhile, the second blood sample was collected into a clean centrifuge glass tube without anti-coagulant to separate serum. The separated serum and plasma were kept frozen at -80°C for further biochemical analysis.

4. Biochemical Analysis

The serum Se, Cu and Mn levels were determined by established procedures of atomic absorption spectrometry using a Perkin-Elmer 2380 instrument. Serum Zn, Fe, MDA and LDL levels as well as plasma H_2O_2 concentration and activity of plasma GR, GST and CAT

were measured spectrophotometerically (Photometer 5010, Germany) following standard methods using commercially available test kits (Biodiagnostics, Cairo, Egypt).

5. Statistical analysis

Data analyses were performed using a statistical software program (SPSS for windows Version 16, SPSS Inc., Chicago, USA). Data was subjected to repeated measures ANOVA to determine the main effects of treatment and time. Wilks' Lambda test was selected to evaluate the effect of time and evidence of time x treatment interactions.

RESULTS

Clinically, in both acute and chronic disease, there was noticeable improvement of clinical signs in groups treated with Se and vitamin C compared with non-treated groups. Biochemically, in horses with acute illness and treated with Se and vitamin C compared with non-treated group, there were a significant ($p<0.05$) increase in the levels of Cu, Zn, Se and Fe and in the activity of CAT and GST two and four weeks post-treatment **(Tables 1,2,5)**. However, there was a significant ($p<0.05$) decrease of levels of H_2O_2 and MDA and in the activity of GR **(Tables 3,4)** two and four weeks post-treatment. However, levels of Mn and LDL showed a significant ($p<0.05$) decrease four weeks post-treatment only **(Tables 2,3)**. In horses with chronic illness and treated with Se and vitamin C compared with non-treated group, there were a significant ($p<0.05$) increase in the levels of Cu, Zn, Se and Fe and in the activity of CAT and GST two and four weeks post-treatment **(Tables 1,2,5)** and a significant ($p<0.05$) decrease of the levels of Mn, LDL, H_2O_2 and MDA and in the activity of GR **(Tables 3,4)**.

DISCUSSION

All studied groups showed gradual improvement in general health condition post-treatment as those previously reported in horses **(Wood et al. 2005)**. The antioxidant status in acute and chronic groups supplemented with Se and vitamin C was significantly improved compared with non-treated groups. **Kirschvink et al. (2008)** attributed such improvement to increase of Se, vitamin C and vitamin E in the pulmonary epithelial lining fluid with subsequent improvement of lung function by modulating the oxidant-antioxidant balance and reducing oxidative damage.

Cu, Zn and Se levels showed a significant increase in antioxidant treated groups compared with non-treated ones. Similar finding was documented in adult human with asthma **(Shaheen et al., 1999)**. The author suggested that Se supplementation could be effective in treatment of asthma as Se deficiency cause inhibition of antioxidant activity, which considered as a risk factor for such disease. Moreover, there is a positive correlation between vitamin E and Cu, possibly due to increases of some Cu-dependent enzymes **(Kirschvink et al., 2006)**. Also, vitamin E has critical effects on serum Zn level **(Patlar et al., 2011)**. On the other hand, Mn level was decreased after antioxidant supplementation which may be attributed to decreased lipid peroxidation and to relief of oxidative stress damage **(Coassin et al., 1992)**.

Fe returned to its normal level 4 weeks post-supplementation, suggesting that Fe didn't mediate oxidative stress reactions due to decreased ROS. This suggestion in coincides with previous findings of **Galaris et al. (2008)**. Moreover, the normalization of Fe level may be due to cessation of H_2O_2 formation reactions which is mediated by and exhaust Fe which is supported by the hypothesis of **Galaris et al. (2008)**. Oxidative stress markers; LDL, H_2O_2 and MDA levels were significantly decreased after antioxidant supplementation as Se react with a variety of reactive oxidants contributing to the cellular antioxidant defense and indicating reduction of ROS generation and diminished oxidative stress in concern with previous studies of **Goldfarb et al. (2007)**.

GR activity was significantly decreased after Se and vitamin C supplementation. This result could be attributed to the increased activity of glutathione antioxidant system as indicative of improved antioxidant potential with subsequent increase of the intracellular synthesis of glutathione. These findings were similar to those recorded by **Kirschvink et al. (2008)** and **Marković et al., (2010)**. GST and CAT activity were increased after antioxidant supplementation. This act may be due to increase and modulatation of cellular glutathione redox system due to sparing effect of vitamin E on glutathione utilization for the scavenging of lipid peroxidation reactions **(Johnson, 2006)**. In conclusion, supplementation of Se and vitamin C in horses suffer from inflammatory airway disease may have beneficial effects on clinical outcomes, antioxidant element status and antioxidant enzyme activity.

Table 1. Cu, Zn and Se Levels in Horses with Acute and Chronic Lower Respiratory Tract Diseases Treated with Traditional Treatment (Group 1 and 2) and Those Treated with Traditional Treatment plus Antioxidants (Group 3 and 4)

Groups / Time	Cu (µM /L)			Zn (µM /L)			Se (µM /L)		
	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment
Group 1 *(n = 10)*	13.3 ± 4.3^{a}	12.4 ± 1.4^{a}	17.1 ± 1.1^{a}	1.4 ± 0.4^{ab}	1.6 ± 0.4^{a}	2.3 ± 0.2^{a}	1.1 ± 0.4^{ab}	1.0 ± 0.1^{a}	2.1 ± 0.5^{a}
Group 2 *(n = 10)*	12.7 ± 3.0^{a}	13.7 ± 1.5^{b}	14.8 ± 1.8^{a}	1.1 ± 0.3^{a}	1.8 ± 0.3^{a}	2.0 ± 0.3^{a}	0.9 ± 0.4^{a}	1.1 ± 0.1^{a}	1.6 ± 0.6^{a}
Group 3 *(n = 10)*	13.9 ± 2.1^{a}	16.6 ± 0.6^{c}	22.8 ± 8.6^{b}	1.4 ± 0.4^{b}	2.7 ± 0.2^{b}	4.8 ± 1.6^{b}	1.2 ± 0.4^{b}	1.8 ± 0.4^{b}	4.1 ± 1.3^{b}
Group 4 *(n = 10)*	13.5 ± 1.9^{a}	16.7 ± 0.5^{c}	23.6 ± 8.3^{b}	1.1 ± 0.2^{a}	3.0 ± 0.3^{b}	4.8 ± 1.9^{b}	1.0 ± 0.4^{a}	3.1 ± 0.3^{c}	5.9 ± 1.9^{c}
	Time: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.			**Time**: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.			**Time**: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.		

a, b, c : Variables with different superscript in the same column are significantly different at $P < 0.05$.

Table 2. Mn, Fe and LDL Levels in Horses with Acute and Chronic Lower Respiratory Tract Diseases Treated with Traditional Treatment (Group 1 and 2) and Those Treated with Traditional Treatment plus Antioxidants (Group 3 and 4)

Groups / Time	Mn (µM /L)			Fe (µM /L)			LDL (mg/dL)		
	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment
Group 1 *(n = 10)*	2.3 ± 0.5^{a}	2.2 ± 0.2^{a}	2.1 ± 1.0^{a}	32.7 ± 3.7^{a}	25.7 ± 1.6^{a}	29.7 ± 1.5^{a}	93 ± 11.1^{a}	98 ± 15.3^{ab}	54.6 ± 19.1^{a}
Group 2 *(n = 10)*	2.7 ± 0.4^{b}	2.1 ± 0.2^{a}	1.9 ± 0.1^{a}	28.3 ± 4.5^{b}	28.6 ± 2.3^{b}	31.2 ± 1.4^{a}	155 ± 19.5^{b}	108 ± 14.8^{a}	90.9 ± 10.7^{b}
Group 3 *(n = 10)*	2.4 ± 0.3^{a}	1.9 ± 0.3^{ab}	1.4 ± 0.3^{b}	33.1 ± 3.6^{a}	29.9 ± 1.5^{b}	37.9 ± 2.6^{b}	93.3 ± 11.5^{a}	92.1 ± 15.4^{bc}	17.9 ± 10.6^{c}
Group 4 *(n = 10)*	2.7 ± 0.4^{b}	1.7 ± 0.6^{b}	1.4 ± 0.4^{b}	28.8 ± 4.1^{b}	32.6 ± 1.9^{c}	41.7 ± 4.7^{c}	154 ± 19^{b}	83.1 ± 11.3^{c}	23.3 ± 10.1^{c}
	Time: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.			**Time**: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.			**Time**: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.		

a, b, c : Variables with different superscript in the same column are significantly different at $P < 0.05$.

Table 3. H_2O_2 and MDA Levels and GR activity in Horses with Acute and Chronic Lower Respiratory Tract Diseases Treated with Traditional Treatment (Group 1 and 2) and Those Treated with Traditional Treatment plus Antioxidants (Group 3 and 4)

Groups / Time	H_2O_2 (µM/L)			MDA (nmol/mL)			GR (U/L)		
	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment
Group 1 *(n = 10)*	19.2 ± 4.3^{a}	18.4 ± 3.4^{a}	13.6 ± 1.8^{a}	15.1 ± 2.8^{a}	13.2 ± 1.0^{a}	7.6 ± 1.7^{a}	1694 ± 99^{a}	1758 ± 29^{a}	1554 ± 25^{ab}
Group 2 *(n = 10)*	23 ± 7.8^{a}	19.6 ± 1.8^{a}	15.7 ± 1.6^{a}	29.9 ± 3.0^{b}	23.8 ± 1.5^{b}	20.6 ± 2.7^{b}	1636 ± 107^{a}	1669 ± 55^{b}	1574 ± 26^{b}
Group 3 *(n = 10)*	19.7 ± 3.7^{ab}	12.7 ± 2.1^{b}	8.3 ± 2.3^{b}	15.5 ± 2.4^{a}	7.0 ± 2.0^{c}	7.6 ± 2.3^{a}	1685 ± 104^{a}	1576 ± 16^{c}	1381 ± 146^{c}
Group 4 *(n = 10)*	23.5 ± 7.4^{b}	13.1 ± 1.8^{b}	8.2 ± 3.3^{b}	30.9 ± 2.8^{b}	18.6 ± 2.1^{d}	9.3 ± 2.8^{a}	1619 ± 104^{a}	1551 ± 24^{c}	1476 ± 100^{a}
	Time: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.			**Time**: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.			**Time**: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group**: Wilks' Lambda test is significant at $P < 0.05$.		

$^{a, b, c}$: Variables with different superscript in the same column are significantly different at $P < 0.05$.

Table 4. CAT and GST activities in Horses with Acute and Chronic Lower Respiratory Tract Diseases Treated with Traditional Treatment (Group 1 and 2) and Those Treated with Traditional Treatment plus Antioxidants (Group 3 and 4)

Groups / Time	CAT (U/L)			GST (U/L)		
	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment	Pre-treatment	2 weeks post-treatment	4 weeks post-treatment
Group 1 *(n = 10)*	995 ± 271 [a]	968 ± 126 [a]	1155 ± 198 [a]	170 ± 20 [a]	153 ± 7 [a]	209 ± 13 [a]
Group 2 *(n = 10)*	596 ± 102 [b]	988 ± 62 [a]	912 ± 122 [a]	183 ± 45 [a]	169 ± 9 [b]	216 ± 12 [ab]
Group 3 *(n = 10)*	1031 ± 234 [a]	1097 ± 69 [b]	1542 ± 387 [b]	172 ± 16 [a]	199 ± 5.3 [c]	265 ± 69 [c]
Group 4 *(n = 10)*	596 ± 101 [b]	1162 ± 31 [b]	1620 ± 176 [b]	183 ± 45 [a]	206 ± 7 [d]	257 ± 70 [bc]
	Time: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group:** Wilks' Lambda test is significant at $P < 0.05$.			**Time**: Wilks' Lambda test is significant at $P < 0.05$. **Time * Group:** Wilks' Lambda test $P = 0.088$.		

[a, b, c, d] : Variables with different superscript in the same column are significantly different at $P < 0.05$.

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الملخص العربى

دور السيلينيوم وفيتامين ج على النتائج السريرية ومؤشرات الاكسدة في الخيول المصابة بالتهاب الشعب الهوائية

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قسم الأمراض الباطنة والأمراض المعدية والأسماك

كلية الطب البيطرى – جامعة المنصورة - المنصورة ٣٥٥١٦ - جمهورية مصر العربية

كان الهدف من هذه الدراسة تقييم تأثير اعطاء السيلينيوم و فيتامين (ج) على النتائج السريرية ، ومضادات الأكسدة ووضع العناصر النادرة وأنشطة الإنزيمات المضادة للأكسدة في الخيول المصابة بالتهابات الجهاز التنفسى. لهذا الغرض ، تم اختيار عدد ٤٠ من الخيول المصابة بالتهابات الجهاز التنفسى وتخصيصها بشكل عشوائي إلى ٤ مجموعات (١٠ لكل منهما) . مجموعة ١ و ٣ كان المرض الحادة؛ ولكن كانت مجموعة ٢ و ٤ الأمراض المزمنة . لجميع الفئات ، كان كل حصان يأخذ المضادات الحيوية ومضاد للالتهابات و مذيب للبلغم وبالإضافة إلى ذلك، مجموعة ٣ و ٤ تلقت حقن السيلينيوم و فيتامين (ج). تم الحصول على عينات من الدم الوريدي من الخيول المريضة في ثلاث مرات ؛ الساعة الصفر، ٢ أسابيع و ٤ أسابيع بعد المعالجة . تحسنت النتائج السريرية وحالة مضادات الأكسدة في الحالات الحادة والمزمنة على حد سواء . كانت هناك زيادة معنوية في مستويات النحاس، و الزنك والسيلينيوم و الحديد وأيضا في نشاط الجلوتاثيون ـاس- ترانسفيراز و الكاتاليز. وفي الوقت نفسه، كان هناك انخفاض معنوي في مستويات المنجنيز والمالوندای الدهيد وفوق أكسيد الهيدروجين و البروتين الدهني منخفض الكثافة و في نشاط انزيم الجلوتاثيون المختزل. تشير نتائج هذه الدراسة إلى أن إعطاء السيلينيوم وفيتامين ج في التهاب الشعب الهوائية الحادة والمزمنة في الخيول قد يكون لها تأثير مفيد على النتائج السريرية وتوازن مضادات للأكسدة.