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Role of L-carnitine and Green tea in prevention of Tilmicosin induced cardiotoxicity in Healthy Dogs: An experimental study

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ABSTRACT

Objective: The aim of the present study was to highlight on the effect of cardiotoxicity of Tilmicosin on dogs' health as well as the advantages of protective doses of antioxidants on the incidence of cardiotoxicity.
Design: *Experimental study*
Animals: Twenty adult Mongerl dogs
Procedures: were divided into 4 groups each group consisted of five dogs, the first group was considered to be the control negative, the second group was treated with a single injection of tilmicosin, the third group was treated with L.carnitine followed by single injection of tilmicosin, the fourth group was treated with green tea followed by single injection of tilmicosin.
Results: There was a continuous enlargement of the left ventricle (LVIDs) through the examination time in group 2, while there was transient enlargement in the left ventricle after 20 and 40 minutes in group 3. There was a significant increase in group 2, while there was a transient significant increase in group 3 after 40 minutes then become non-significant after 60 minutes. ESV results in all groups, there was a significant increase in group 2, while there was a transient significant increase in group 3 after 40 minutes then become non- significant after 60 minutes. There was a significant decrease in FS in group 2 more than both groups 3 and 4. Furthermore, there was a significant change by time, as it continued to decrease at 40 and 60 minutes. There was a significant decrease in EF in group 2 more than both groups no 3 and 4. Furthermore, there was a significant change by time, as it continued to decrease at 40 and 60 minutes.
Conclusion and clinical relevance: The present investigation indicates that oxidative stress with alteration of antioxidant enzymes activities are feature of respiratory diseases in draft horses.
Keywords: *Cardiotoxicity; Telmicosin; Antioxidants; Free Radicals; Oxidative stress markers; Dogs*

1. INTRODUCTION

Cardiovascular disorders are common in elderly dogs, one-third of which have been shown to suffer from valvular heart disease (Buchanan, 1999 and Nakagawa et al., 2009). Cardiotoxicity is the occurrence of heart electrophysiology dysfunction or muscle damage in which the heart becomes weaker and is not as efficient in pumping the circulating blood. It may be caused by chemotherapy treatment, complications from anorexia nervosa, adverse effects of heavy metals intake, or an incorrectly administered drug such as bupivacaine (Sishi, 2015).

Cardiovascular toxicities were observed with therapeutic agents used in the treatment of both cardiovascular and non-cardiovascular diseases and affect all components of the CVS. Cardiovascular adverse reactions could be occurred after acute or chronic treatment and could be affected function (e.g. alteration of the mechanical function of the myocardium) and/or structure (e.g. morphological damage or loss of cellular/subcellular components of the heart) or vasculature (Sishi, 2015). Drug-induced structural cardiac damage was found to be associated with changes in multiple cardiac cell types leading to cardiac fibrosis and cardiomyopathy and subsequently, heart failure (Berardi et al., 2013).

Echocardiography is an important, noninvasive tool for evaluating cardiac anatomy and function, as well as surrounding structures. Pericardial fluid, pleural fluid, and mass lesions in or near the heart can also be detected (Ware, 2011). According to the cardiac review committee, drug-induced cardiotoxicity, in terms of cardiomyopathy, includes one or more of the following: (i) a decrease in left ventricular ejection fraction (LVEF), either globally or more severely in the septum; (ii) signs and symptoms of HF, such as tachycardia and/or S3 gallop; (iii) a decrease in LVEF that is equal to or greater than 10% but less than 55% without associated signs and symptoms of HF, or a decrease in LVEF that is less than or

equal to 5% but less than 55% with associated signs and symptoms of HF (Seidman et al., 2002).

Oxidative stress plays a key role in causing various dogs diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder inflammatory disease, muscular dystrophy, liver disorder, and even aging (Andreadis et al., 2003). Besides, there are some antioxidants in the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β-carotene, and vitamin C, and hence these must be supplemented in the normal diet (Birben et al., 2012).

Antioxidants can also act as prooxidants when these are not present at the right place at the right concentration at the right time (Birben et al., 2012). The relative importance of the antioxidant and prooxidant activities is not yet explored fully and needs further research. Green tea is the healthiest beverage on the planet. It is loaded with antioxidants and nutrients that have powerful effects on the body. These include improved brain function, fat loss, a lower risk of cancer and many other impressive benefits. (Chacko et al., 2010). L-carnitine is formed in the liver and kidneys from the amino acids lysine and methionine. However, it is stored elsewhere in the body, primarily in muscle (including the heart), the brain, and even in sperm. In the diet, it mainly comes from meat and other animal products. You can get some from plant products like avocado and soybeans, but as a rule, meat is the best source—and the redder the better. (Brass, 2004 and Cave et al., 2008).

Arsenian, 1997 and Ruggenenti et al., 2009 demonstrated a potential for reducing blood pressure and the inflammatory process associated with heart disease. L-carnitine is also linked to improvements in patients with severe heart disorders, such as coronary heart disease and chronic heart failure. Moreover, L-carnitine was shown to be beneficial against the cardiotoxic effects of several chemicals such as in doxorubicin-induced cardiotoxicity and adriamycin-induced cardiomyopathy (Abdel-Aleem et al., 1997and Ferrari et al., 2004). Therefore, this study was planned to highlight on the effect of cardiotoxicity of Tilmicosin on dogs' health as well as the advantages of protective doses of antioxidants on the incidence of cardiotoxicity.

2. MATERIALS AND METHODS

2.1. Animals and medical records

A total number of twenty adult Morgel dogs had been selected in this study, their weights were ranged from 15-25 kg body weight. All animals were clinically healthy with no evidence of heart diseases based on physical examination according to (Forfia, 2013), as well as, complete blood count (CBC) and echocardiography using the method adopted by (Thomas et al., 1993). These dogs were housed in individual cages. The present study was carried out between November 2018 and December 2018 at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Mansoura University. Data concerned with competent history, clinical findings, and medical records for each animal were recorded.

2.2. Experimental groups

All animals under experimentation were divided into 4 groups each group consisted of five dogs, the first group was considered to be the control negative, the second group was treated with a single injection of tilmicosin in a dose rate of 5 mg/kg body weight i/v according to (Main et al., 1996) , the third group was treated with L.carnitine for 5 days in a dose rate of 50 mg/kg body weight P/O three time daily according to (Hall and Jewell, 2012) followed by single injection of tilmicosin in a dose rate of 5 mg/kg body weight i/v while, the fourth group was treated with green tea for 5 days 7 mg/kg body weight P/O twice daily according to (Wynn and Fougere,2007) followed by single injection of tilmicosin in a dose rate of 5 mg/kg body weight i/v.

2.3. Echocardiographic examination

Transcutaneous echocardiographic examination of the heart was carried out according to the method described by (Singh et al., 2014). All echocardiographic procedures and precautions were followed according to the recommendation of the American Society of Echocardiography. The B and M-mode were performed using a 3.5 MHz phased array transducer. with a CHION Digital Color Doppler Ultrasound System, iVis60 EXPERT VET, CHISON Medical Imaging Co., Ltd, China. For guided B and M-mode measurements, the animal was restrained in standing position, the area of examination was shaved, coupling gel was applied, the transducer was placed over the precordial impulses between the second to fourth intercostal space, the left parasternal short axis view was obtained at the level of the papillary muscle, once the heart was located , the transducer was angled or rotated optimal visualization was generally achieved for B and M-mode studies when the ultrasound beam is perpendicular to the cardiac structures. Measurement of echocardiographic parameters, including interventricular septal thickness at the end-systole (IVSTs) and at end-diastole (IVSTd), left ventricular internal diameter at end-systole (LVIDs) and at end-diastole (LVIDd),left ventricular posterior wall thickness at end-systole (LVPWs) and at end-diastole (LVPWd) were performed through Cube Method according to the method described by (Singh et al., 2014; Thomas et al.,1993 and Yadav, 2011). Standardized image planes were obtained by B-mode which was used to guide M-mode views for measurements. Through Teicholz Method, left ventricular volume at end –systole(EVS) and at end-diastole (EDV) were calculated (Thomas, 1984 and

Crippa et al., 1992) then stroke volume was calculated from the following equation, Stroke volume= EDV-ESV. The left ventricular M-mode measurements were used to calculate the fractional shortening (FS%) and ejection fraction (EF %) (Bonagura 1983 and Crippa et al., 1992)

2.4.Statistical analysis

Data were subjected to statistical analysis using a statistical software program (SPSS for Windows, version 15, USA). Repeated measure MANOVA to examine the effect of time and treatment. Means and standard deviation for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. MANOVA fit for a group and for treatment x time interaction to confirm the significant changes. Differences between means at $P < 0.05$ were considered significant. (Watkins et al., 2005).

3. RESULTS

All dogs were clinically healthy before the experimental technique, while just starting the injection of tilmicosin, all dogs suffered from shaking and scratching, fatigue and lethargy, pale gums, panting, tachycardia and dyspnea. With regard to the echocardiographic examination, normal features with normal echocardiographic parameters of the heart were recorded in control negative group. There was a continuous enlargement of the left ventricle (LVIDs) through the examination time in group 2, while there was transient enlargement in the left ventricle after 20 and 40 minutes in group 3, then this enlargement become slight (non-significant). Moreover, there was a slight enlargement of the left ventricle after 20 minutes, which become nearly normal after 40 and 60 minutes. (table.1)

There were non-significant changes in LVIDd, IVSd, IVSs, LVPWd, LVPWs, EDV and SV. Concerning to LVIDs, there was a significant increase in group 2, while there was a transient significant increase in group 3 after 40 minutes then become non- significant after 60 minutes ($p<0.004$). ESV results in all groups, there was a significant increase in group 2, while there was a transient significant increase in group 3 after 40 minutes then become non- significant after 60 minutes ($p<0.0001$) (table.2). There was a significant decrease in FS in group 2 more than both groups 3 and 4 ($p<0.0001$). Furthermore, there was a significant change by time ($p<0.0001$), as it continued to decrease at 40 and 60 minutes (table.3). There was a significant decrease in EF in group 2 more than both groups no 3 and 4 ($p<0.0001$). Furthermore, there was a significant change by time ($p<0.0001$), as it continued to decrease at 40 and 60 minutes. (table .4)

Table 1. LVIDs in all groups pre and post-tilmicosin injection in dogs with experimentally induced cardiotoxicity

Group	B.mode Time of examination(minutes) 20	40	60
1	19.99 ± 11.98^{b}	19.99 ± 11.98^{b}	19.99 ± 11.98^{b}
2	37.95 ± 4.91^{a}	36.15 ± 10.7^{ab}	43.54 ±2.8^{a}
3	37.71 ± 7.47^{a}	44.77 ± 4.92^{a}	37.43 ±7.41^{ab}
4	29.86 ± 8.89^{ab}	25.33 ±13.26^{b}	20.91 ±10.28^{b}
Group	**M.mode Time of examination(minutes) 20**	**40**	**60**
1	2.12 ± 0.17^{b}	2.12 ± 0.17^{b}	2.12 ± 0.17^{b}
2	2.71 ± 0.5^{a}	2.88 ± 0.59^{ab}	3.03 ±0.57^{a}
3	3.15 ± 0.51^{a}	3.37 ± 0.32^{a}	3.42 ±0.91^{ab}
4	2.93 ± 0.89^{ab}	2.79 ±0.68^{b}	2.77 ±1.05^{b}

Manova fit: P<0.004 Time P<0.0001
Time /Group P<0.004
Means with different superscript letters in the same column are significantly different at $P < 0.05$.

Table 2.ESV in all groups pre and post-tilmicosin injection in dogs with experimentally induced cardiotoxicity.

Group	B.mode Time of examination(minutes) 20	40	60
1	21.07 ± 9.29^{b}	21.07 ± 9.29^{b}	21.07 ± 9.29^{b}
2	56.71 ± 24.62^{a}	60.68 ± 33.3^{ab}	83.37 ±16.11^{a}
3	58.71 ± 39.99^{a}	92.33 ± 29.45^{a}	57.06 ±24.89^{ab}
4	32.08 ± 22.5^{ab}	40.20 ±17.29^{b}	44.98 ±27.66^{b}
Group	**M.mode Time of examination(minutes) 20**	**40**	**60**
1	9.67 ± 2.92^{b}	9.67 ± 2.92^{b}	9.67 ± 2.92^{b}
2	21.54 ± 12.33^{a}	26.52 ± 16.4^{ab}	30.35 ±18.16^{a}
3	33.35 ± 16.03^{a}	39.26 ± 10.68^{a}	47.65 ±45.4^{ab}
4	20.93 ± 15.6^{ab}	18.18 ±18.85^{b}	18.17 ±29.8^{b}

Manova fit: P<0.003 Time P<0.0001
Time /Group P<0.003
Means with different superscript letters in the same column are significantly different at $P < 0.05$.

Table 3. FS in all groups pre and post-tilmicosin injection in dogs with experimentally induced cardiotoxicity.

Group	B.mode Time of examination(minutes) 20	40	60
1	36.8 ± 2.94^{a}	36.8 ± 2.94^{a}	36.8 ± 2.94^{a}
2	12.8 ± 6.45^{c}	9.4 ± 3.64^{c}	10.6 ±2.8^{c}
3	15.8 ± 9.78^{bc}	16.4 ± 6.49^{bc}	16.8 ±7.46^{b}
4	25.8 ± 4.69^{b}	20.6 ±2.6^{b}	16.8 ±3.5^{b}
Group	**M.mode Time of examination(minutes) 20**	**40**	**60**
1	39.0 ± 9.92^{a}	39.0 ± 9.92^{a}	39.0 ± 9.92^{a}
2	14.4 ± 4.5^{c}	12.8 ± 4.5^{c}	13.4 ±4.26^{c}
3	18.4 ± 6.64^{bc}	19.4 ± 4.48^{bc}	19.4 ±3.51^{b}
4	28.4 ± 6.9^{b}	23.4 ±5.4^{b}	20.7 ±5.4^{b}

Manova fit: P<0.0001 Time P<0.0001

Time /Group P<0.0001

Means with different superscript letters in the same column are significantly different at $P < 0.05$.

Table 4.EFV in all groups pre and post-tilmicosin injection in dogs with experimentally induced cardiotoxicity

B.mode			
Group	Time of examination(minutes)		
	20	40	60
1	74.4 ± 3.43[a]	74.4 ± 3.43[a]	74.4 ± 3.43[a]
2	34.0 ± 13.45[c]	26.0 ± 9.13[c]	28.6 ±12.05[c]
3	39.2 ± 18.45[bc]	41.0 ± 12.63[b]	38.4 ±11.05[b]
4	57.4 ± 8.08[ab]	50.0 ±5.29[b]	36.98 ±7.36[b]
M.mode			
Group	Time of examination(minutes)		
	20	40	60
1	68.4 ± 18.76[a]	68.4 ± 18.76[a]	68.4 ± 18.76[a]
2	28.4 ± 9.7[c]	20.52 ± 12.4[c]	23.35 ±18.16[c]
3	32.3 ± 9.03[bc]	34.2 ± 10.48[b]	31.65 ±17.4[b]
4	50.93 ± 15.6[ab]	43.18 ±12.15[b]	30.17 ±9.8[b]

Manova fit: P<0.0001 Time P<0.0001
Time /Group P<0.0001
Means with different superscript letters in the same column are significantly different at P < 0.05

4. DISCUSSION

Concerning to clinical picture, shaking and scratching, panting, fatigue, lethargy, pale gums, tachycardia, and dyspnea were the common clinical signs of cardiotoxicity. These findings were in agreement with those reported by DeFrancesco (2013); Klüser et al., (2018); Tidholm and Jönsson (1996), while there was an enhancement in clinical effect in groups 3 and 4. This finding is similar to that reported by Lango et al., (2001) and Ferrari et al., (2004) .

Concerning echocardiography, there were non-significant changes in LVIDd, IVSd, IVSs, LVPWd, LVPWs, EDV and SV. These findings were in accordance with the finding observed by Ahmed et al.,(2013). Concerning to LVIDs, there was a significant increase in group 2, while there was a transient significant increase in group 3 after 40 minutes then become non- significant after 60 minutes. This elevation indicate presence of systolic dysfunction, these findings were in agreement with those reported by Ahmed et al.,(2013); Dukes et al.,(2003); Mausberg et al.,(2011); Meurs (2010) and Mehlman et al.,(2013) who recorded that dilated cardiomyopathy was minimally defined as increased LV internal dimension at end-systole (LVIDs) with concomitant reduced fractional shortening (FS), they also added that dilated cardiomyopathy is a disease of the cardiac muscle characterized by reduced systolic function, dilatation and impaired contraction of the left ventricle or both ventricle. It manifests as progressive dilation of the cardiac chambers due to volume overload.

In regard to ESV, there was a significant increase in group2, while there was a transient significant increase in group 3 after 40 minutes then become non- significant after 60 minutes, these elevations may be due to left ventricular dysfunction. These findings were similar to those reported by Dujardin et al.,(1997) and Talwar et al.,(2000); who recorded that, the correlation between simultaneously measured LV diameters and volumes was good overall, suggesting that diameter can be used to diagnose enlargement of the LV. Significant correlations were found between end-systolic diameter and end-systolic volume small increase in diameter may correspond to a large increase in volume in enlarged ventricles. Gaudron et al.,(1993); Pfeffer and Braunwald (1990) and Turakhia et al.,(2009) also stated that left ventricular end-systolic volume is a marker of ventricular contractility that is relatively insensitive to loading conditions, they also added that end-systolic volume is also an indicator of remodeling after myocardial necrosis.

On the other hand, there was a significant decrease in FS in group 2 more than both groups 3 and 4 that indicate ventricular dysfunction. These findings were similar to what was reported by Cornell et al.,(2004); Dukes et al.,(2003); Nelson and Couto (1998); Nelson and Couto (2008); Singh et al.,(2014); Voros et al.,(2009) who reported that FS is the most common echocardiography parameter performed to see the myocardium contractility and to estimate the capability of the left ventricles. FS is widely used as an indicator of left ventricular systolic function and the value ≤ 0.25 is usually associated with heart diseases

Moreover, there was a significant decrease in EF in group 2 more than both groups 3 and 4 that indicate ventricular dysfunction. These findings were similar to what was reported by Dukes et al.,(2003); Gaasch (1994); Smith et al.,(2003) who prove that, in dogs, the authors consider that an ejection fraction less than 40% is abnormally low.

Shaking and scratching**,** fatigue and lethargy**,** pale gums, panting, tachycardia, and dyspnea were the most common clinical signs in case of cardiotoxicity. There was a non-significant difference between both groups in all echocardiographic parameters LVIDs, ESV, FS, and EF. Both regimes had a positive effect on cardiotoxicity but green tea had a better effect on some results.

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