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A. H. HUSSEIN, K. E. HANAN, O. E. YASMIN

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Oxidative stress indices and pathological changes in cattle with traumatic pericarditis

Hussein A. H.^{1*}, Hanan K. E.¹, Yasmin O. E.²

*Department of Animal Medicine, Faculty of Veterinary Medicine,
Assiut University, Assiut, Egypt*

*Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine,
Assiut University, Assiut, Egypt*

ABSTRACT. Objectives of the study were to provide information on the changes in oxidative stress indices, as well as to describe the possible pathological changes in cattle affected with traumatic pericarditis (TP). Twenty-six cattle with TP were included in the current study. Nine clinically healthy cattle were enrolled as controls. Blood serum samples were collected and stored at -20°C. Measurement of nitric oxide (NO), malondialdehyde (MDA), total antioxidant capacity (TAC) and other biochemical parameters were done within one week from sampling. Postmortem and histopathological examinations were carried for dead or euthanized cases. The mean values of NO, MDA, creatinine, total bilirubin, globulins, blood urea nitrogen, and total proteins were significantly higher in the diseased group ($P<0.05$), while the mean values of TAC, glucose and albumin were significantly lowered in TP group ($P<0.05$). The activities of aspartate aminotransferase (AST) and γ -glutamyl transferase (GGT) were higher in affected cattle than healthy ones. In cattle with TP, a strong significant positive association was found between serum concentrations of NO and MDA. Postmortem examinations of the dead animals revealed severe thickening of the pericardium and accumulation of pericardial effusions either mild or severe in the pericardial sacs. Regarding the recorded histopathological changes; Organized fibrinous pericarditis (constrictive pericarditis), necrosis of the myofiber with congestion and hemorrhage in the myocardium, chronic venous congestion of the liver, and hemosiderosis of the spleen were clearly observed. Furthermore, hemorrhage and interstitial fibrosis of the lung, various degrees of degeneration and hyalinization of the renal tubules were prominently featured. In conclusion, oxidative stress and lipid peroxidation may be involved in the complications of traumatic pericarditis in cattle.

Keywords: Cattle; traumatic pericarditis; Oxidative stress; pathological findings.

Corresponding Author:

Hussein A. Hussein, Department of Animal Medicine, Faculty of Veterinary Medicine,
Assiut University,
Assiut 71526, Egypt .
Email address: hhussein@aun.edu.eg
hussein-vet@lycos.com

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INTRODUCTION

Pericarditis means inflammation of the pericardium with accumulation of fluid or exudate between the visceral and parietal pericardium (Braun, 2009). Pericarditis is the most common pericardial disorder in cattle (Bexiga et al., 2008). It is commonly caused by traumatic foreign bodies (such as needles, wires, and nails), because cattle are lacking the discriminatory dietary habits (Baker and Van Dreumel, 1985). Long, thin, and sharp foreign bodies (such as nails, wires and needles) penetrate the reticulum, diaphragm and pericardial sac, resulting in traumatic pericarditis (TP) (Radostits et al., 2007). This leads to inflammation of the pericardium, with accumulation of serous or fibrinous inflammatory products (Gründer, 2002). Hematogenous pericarditis that due to spread of infectious diseases (such as colibacillosis, pasteurellosis, salmonellosis and anaerobic infections) is much less common and is usually masked by signs of septicemia (Gründer, 2002). Furthermore, idiopathic pericarditis is rare in cattle (Jesty et al., 2005). Traumatic pericarditis is extremely common in developing countries, possibly due to unregulated small-scale farming and unsatisfactory standards of animal management and feeding (Misk et al., 1984).

There are three general forms of pericarditis, effusive, fibrinous and constrictive although combination of three can also occur (Gründer, 2002). Effusive pericarditis characterized by accumulation of a protein rich fluid within the pericardial sac (Athar et al., 2012). Subsequent fibrin deposition will lead to fibrinous pericarditis and if fibrin within the pericardial sac matures to fibrinous tissue and fibrosis of the pericardium or epicardium then constrictive pericarditis will result (Perkins et al., 2004). Constrictive adhesions between epicardium and pericardium with fluid accumulation in pericardial sac impair the ability of the heart to act as a pump and result in congestive heart failure (Radostits et al., 2007; Hussein and Staufenbiel, 2014) or death of animal (Braun et al., 2007).

Oxidative stress may result from an imbalance

between reactive oxygen species (ROS) and anti-oxidants levels (Lightboy, 2001). It is well known that ROS are produced by several pathological conditions and cause cellular damages such as lipid peroxidation and protein oxidation (Solomons and Fryhle, 2002). These oxidized lipid products are reactive and cause damage to cells and cell membranes (Hennig and Chow, 1988). The biological oxidative effects of free radicals on lipids and proteins are controlled by a spectrum of antioxidants (Serdar et al., 2007).

Clinical, hematological and biochemical alterations in animals with traumatic pericarditis have been widely investigated, comparatively, less research work has been done to explore the role of oxidative stress in the pathogenesis of traumatic pericarditis. The present research, therefore, aimed to study the oxidative stress indices including nitric oxide, malondialdehyde and total antioxidant capacity in blood of cattle suffering from traumatic pericarditis.

MATERIALS AND METHODS

Animals, history and clinical examination

This study was carried out during the daily regular work at the Veterinary Teaching Hospital, Assiut University, Egypt. A total of 35 (diseased, TP; n = 26 and healthy control; n = 9) native breed cows were examined between February 2012 and January 2014. The animals ranged in age from 2 to 5 years. Diseased cattle were referred to the hospital because of brisket edema, anorexia, and sharp drop in milk yield. There was a history of previous medications including antibiotic injections, fluid therapy, and general tonics by the field veterinarians for some diseased cattle, but without obvious improvement. All animals were examined clinically as described according to Jackson and Cockcroft (2002), and tested for foreign bodies in the reticulum or heart included withers pinch technique, strong percussion at the sternum, and mine detector. Based on clinical and radiographic examination, traumatic pericarditis was confirmed in 26 animals. The radiographic examinations were

carried out on the laying posture, where the animals were lied down on the right side, using x-ray device (SUPER 80 CP, PHILIPS, Germany). After complete and thorough physical examinations, 9 cattle were included in this study as a control group. These animals did not have a history of previous disease and their physical examination was within normal ranges. Furthermore, radiographic and mine detection of these animals revealed absence of foreign bodies.

Blood sampling and biochemical analyses

From each cow, a blood sample was collected by puncture of the jugular vein using G 22 needles (International Company, Egypt). The blood samples (10 mL) were collected in plain vacutainer tubes (HI VAC, China). After centrifugation of the blood samples, serum samples were harvested and frozen at -20°C , then biochemical analyses were carried out. The nitric oxide (NO) was estimated by a method depending on the addition of Griess reagent, which converts nitrate into a deep purple azo compound that was measured at 540 nm (Montgomery and Dymock, 1961) using spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea). The total antioxidant capacity (TAC) level (Koracevic et al., 2001). This assay measures the capacity of the serum to inhibit the production of hiobarbituric acid reactive substances from sodium benzoate under the influence of the oxygen-free radicals derived from Fenton's reaction. The reaction was measured spectrophotometrically at 532 nm. The malondialdehyde (MDA) level was measured by the thiobarbituric acid method (Ohkawa et al., 1979). In this method, MDA reacts with thiobarbituric acid to produce a red colored product.

Spectrophotometric measurements of glucose, blood urea nitrogen (BUN), creatinine, total bilirubin, total proteins, albumin, aspartate aminotransferase (AST) and γ -glutamyl transferase (GGT) were done using commercial test kits supplied by Spectrum (Spectrum Company, Egypt) and using a UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea). The biochemical analyses of all

these parameters were spectrophotometrically measured according to the standard protocol of the suppliers and following the manufacturer's instructions. Serum globulin was calculated by subtracting the albumin from the total protein concentration.

The biochemical assays were measured in serum samples of 17 cattle with TP and 8 clinically healthy ones.

Histopathology

Specimens from pericardium, myocardium, liver, lungs, spleen and kidneys were taken from dead or euthanized animals ($n=17$). Unfortunately, the rest of diseased animals ($n=9$) were discharged from the hospital without information about their fate. The tissue samples were fixed in 10% neutral buffered formalin, dehydrated in a graded alcohol series, cleared with methyl benzoate and embedded in paraffin wax. Sections of $4\ \mu\text{m}$ were cut and stained with haematoxylin and eosin (H&E) (Bancroft et al., 1996). Stained sections were examined by light microscope and photographed using digital camera.

Statistical analysis

The data were analyzed by means of the program SPSS/PC (SPSS for Windows Version 16; SPSS GmbH, Munich, Germany). The normal distribution of all variables was tested using Shapiro-Wilk normality test. Means and standard deviations of the variables were calculated. All parameters were compared using the Student t-test. Probability values of less than 0.05 ($P < 0.05$) were considered significant. Values are presented as the mean \pm standard deviation (SD).

RESULTS

Serum concentrations of nitric oxide and MDA were significantly higher in cattle with traumatic pericarditis when compared with concentrations in healthy control group. In contrast, serum concentration of TAC was significantly lower in the group of cattle with traumatic pericarditis when compared with concentrations in healthy control group (Table

Table 1. Serum concentrations of nitric oxide, malondialdehyde and total antioxidant capacity in cattle with traumatic pericarditis and control ones

Parameters	Traumatic Pericarditis (n = 17)	Control (n = 8)
Nitric oxide ($\mu\text{mol/L}$)	$17.8 \pm 4.6^{**}$	6.8 ± 2.4
Malondialdehyde (nmol/mL)	$7.9 \pm 1.6^*$	6.4 ± 0.5
Total antioxidant capacity (mmol/L)	$0.8 \pm 0.4^{**}$	1.3 ± 0.4

* $P < 0.05$ ** $P < 0.01$

1, $P < 0.05$). In addition, serum concentrations of glucose and albumin were significantly lower in TP group in comparison with control one (Table 2, $P < 0.01$). Serum concentrations of creatinine, total bilirubin, and globulins were significantly higher in TP group than the healthy control one (Table 2, $P < 0.01$). Furthermore, blood urea nitrogen and total proteins were significantly increased in cattle with TP ($P < 0.05$). In contrast, the albumin: globulin ratio was significantly lowered in animals with TP ($P < 0.01$). The activities of AST and GGT were higher in affected cattle than healthy control ones (Table 2, $P < 0.01$).

In cattle with TP, a strong significant positive correlation was found between serum concentrations of NO and MDA ($r=0.65$; $P=0.01$). A significant negative correlation was noticed between TAC concentration and NO concentration ($r=-0.43$; $P=0.04$), and MDA concentration ($r=-0.52$; $P=0.02$).

Postmortem examinations of the animals, revealed severe thickening of the pericardium (fibrinous pericarditis) and accumulation of pericardial effusions either mild or severe in the pericardial sac. The hearts were covered with yellow fibrin threads (Fig. 1a). In some cases, the foreign body could be seen penetrating the heart (Fig. 1b). Severe enlargement of the liver could be seen in some animals (Fig. 1c).

Organized fibrinous pericarditis (constrictive pericarditis) was the most prominent feature in which there was severe thickening of the pericardium with formation of newly formed blood capillary perpendicular to the fibroblast cells (Fig. 1a). Necrosis of the myofiber, accompanied with congestion and

hemorrhage were pronounced in the myocardium (Fig. 1b). In association with organized fibrinous pericarditis, chronic venous congestion of the liver was seen (Fig. 1c). The lesion characterized by fibrosis of central vein, dilatation of sinusoid, necrosis of the hepatocytes around the central vein and fatty change of the hepatocytes surrounding the portal area (Fig. 1d). Hemosiderosis of spleen was a characteristic feature in most animals (Fig. 2 a). There was hemorrhage and interstitial fibrosis of the lung. The renal tubules undergo varying degree of degeneration and hyalinization associated with congestion (Fig. 2c). Connective tissue proliferation in the interstitium of kidney was prominent (Fig. 2d).

DISCUSSION

Excessive production of free radicals and ROS, and/or a decrease in body antioxidant defense, lead to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan et al., 2001). When ROS are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results. Therefore, an imbalance between increased production of ROS and reduced availability of antioxidant defense as a result of infection increases oxidative stress and may aggravate the inflammatory process (Gitto et al., 2002).

The higher NO levels detected in the cattle with TP in the present study might be due to stimulation of the pericardium by trauma caused by foreign bodies and possibly by entry of bacteria into the pericardial sac. Previous studies have claimed that

Table 2. Serum concentrations of glucose, urea, creatinine, total proteins, albumin, globulins, and total bilirubin and activities of AST and GGT in cattle with traumatic pericarditis and control one

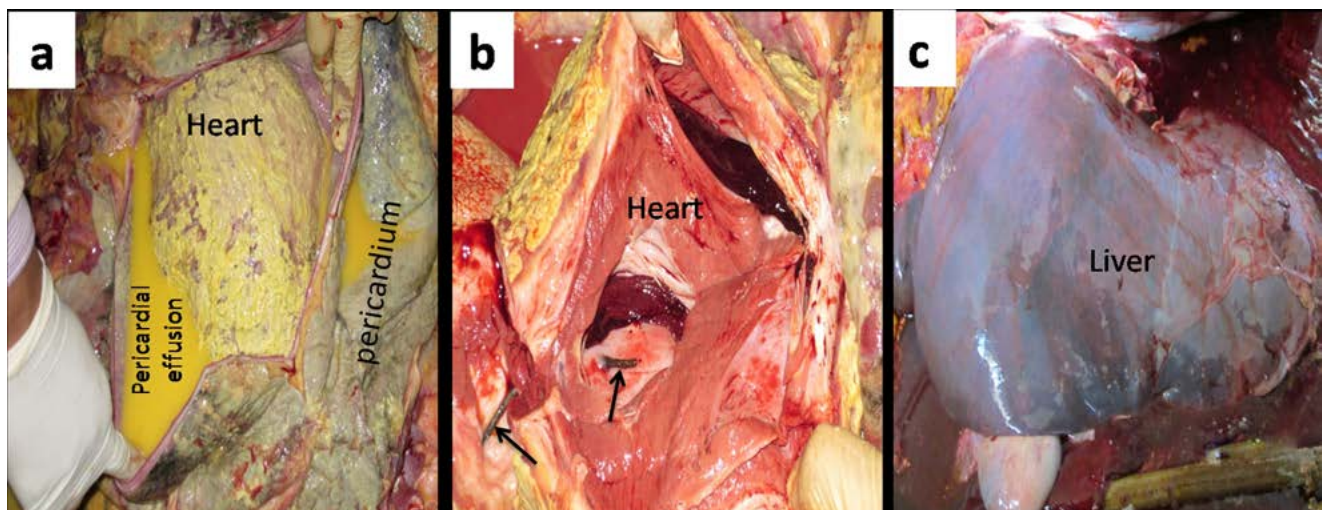
	Glucose (mmol/L)	BUN (mmol/L)	Creatinine (μ mol/L)	Total bilirubin (mmol/L)	Total proteins (g/L)	Albumin (g/L)	Globulins (g/L)	A/G	GGT (U/L)	AST (U/L)
Control (n = 8)	4.9 \pm 0.7	23 \pm 2	125 \pm 2	5.6 \pm 1.4	64 \pm 4	32 \pm 1	32 \pm 5	1.0 \pm 0.2	21 \pm 2.8	55 \pm 10
Traumatic Pericarditis (n = 17)	3.0 \pm 0.4**	33 \pm 11*	152 \pm 9**	20.1 \pm 4.4**	73 \pm 11*	27 \pm 4**	46 \pm 10**	0.6 \pm 0.1**	36 \pm 6.7**	90 \pm 20**

* $P < 0.05$ ** $P < 0.01$ BUN: Blood urea nitrogen, A/G: Albumin to globulins ratio, GGT: γ -glutamyl transferase, AST: Aspartate aminotransferase

NO act as a free radical (Atakisi et al., 2009). The increase in serum concentration of MDA found in the present study suggests there is significant lipid peroxidation in cattle with TP compared with healthy control animals. Malondialdehyde is the final product of lipid peroxidation and therefore is used as index of this process (Yang et al., 2011; Hanan et al., 2013). In addition, increased lipid peroxidation may be caused by disturbed redox balance (Gutteridge, 1995). The decrease in serum concentration of total antioxidant capacity in the TP group in this study might have been due to

over-usage of antioxidant capacity to eliminate free radicals produced in the disease process of TP. The drop in antioxidant activity may be associated an imbalance between lipid peroxidation and antioxidant capacity (Heidarpour et al., 2013).

Serum biochemistry results showed decreased concentration of blood glucose, which may be due to low feed intake and anorexia in diseased animals. In addition, blood serum levels of BUN and creatinine were significantly higher in diseased groups, indicating impaired kidney function. Increased total proteins, globulins, liver enzymes

Figure 1. (a) Postmortem image of the heart and pericardial sac, which has been opened. Note severe thickening of the pericardium, accumulation of yellow pericardial effusion in the pericardial sac and the heart is covered with yellow fibrin. (b) Postmortem image of a thin black wire penetrating the myocardium of the left ventricle (arrow). (c) Postmortem image revealing severe enlargement of the liver.

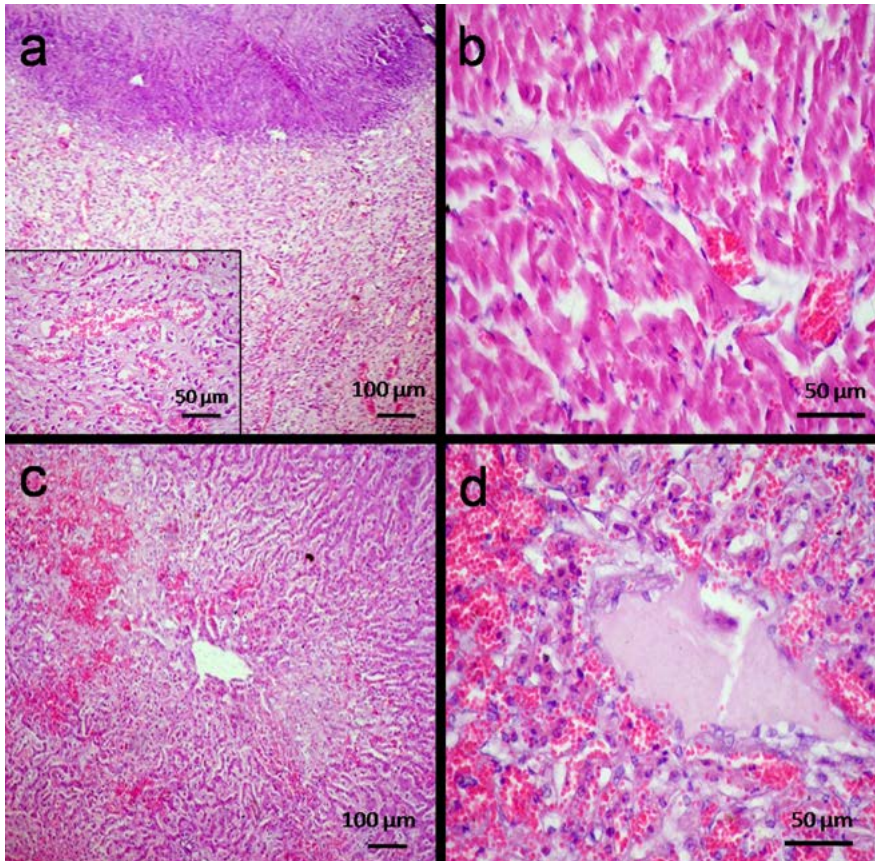


Figure 2. (a) Organized fibrinous pericarditis, severe thickening of the pericardium, newly formed blood capillary perpendicular to the fibroblast cells. (b) Necrosis of the myofiber, hemorrhage and congestion. (c) Chronic venous congestion of liver. (d) Fibrosis of the dilated central vein, necrosis of the surrounding hepatocytes and hemorrhage. H&E.

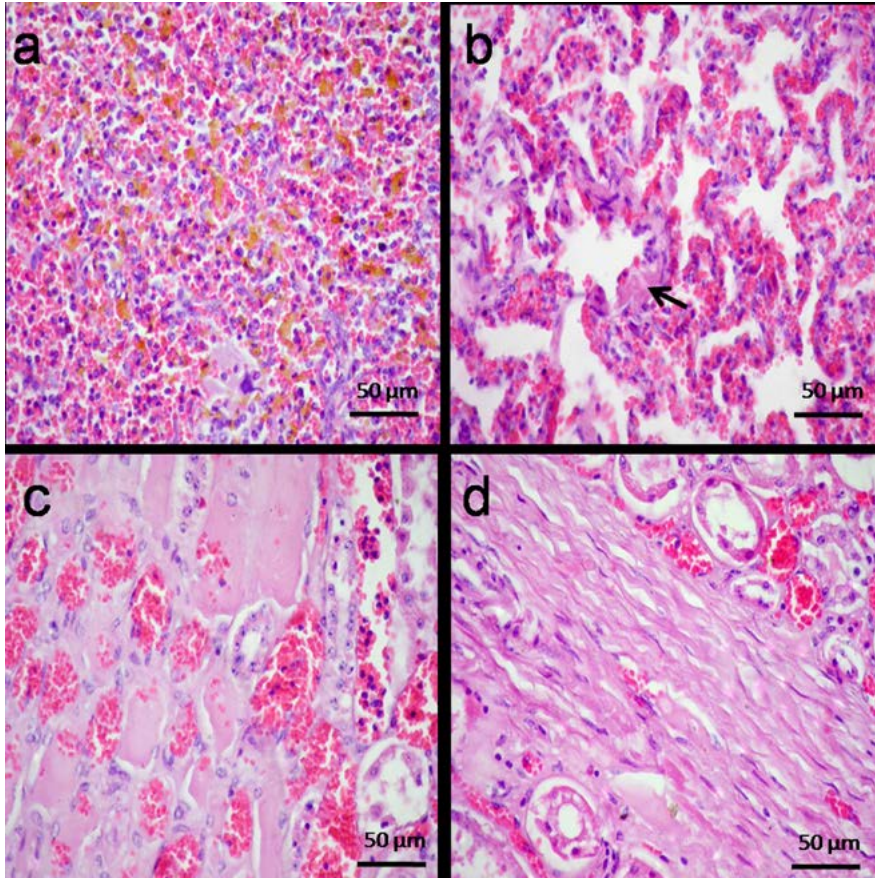


Figure 3. (a) Hemosiderosis of spleen. (b) Hemorrhage and interstitial fibrosis (arrow), lung. (c) Degeneration of renal tubules, hyalinization, and congestion. (d) connective tissue proliferation in the interstitium of kidney. H&E.

(AST, GGT) and total bilirubin were noticed. These findings were compatible with an inflammatory process that was attributable to advanced cardiac disease, impaired liver function, chronic infectious disease or other inflammatory complications (Tennant and Center, 2008). In the present study, impaired liver and renal function in TP group, indicating development of congestive heart failure (Hussein and Staufenbiel, 2014). Estimation of liver enzymes, total bilirubin, total proteins and albumin are helpful diagnostic techniques for evaluation of liver function (Tennant and Center, 2008). In diseased cattle, the significantly increased activities of AST and GGT could be attributed to leakage of these enzymes from hepatocytes as a result of liver congestion. In the current study, biochemical findings indicated that advanced stage of heart failure in cattle may be associated with hepatic and renal failure.

Post-mortem examination revealed severe thickening of the pericardium (fibrinous pericarditis) and accumulation of pericardial effusions either mild or severe in the pericardial sac. The hearts were covered with yellow fibrin shreds with fibrinous adhesions. The same gross lesions were observed by (Elhanafy and French, 2012). They found that the pericardial sac was totally lined by a large mat of thick yellow fibrinous material. Fibrous adhesions also found at necropsy in all cases in animals with fatal traumatic pericarditis (Roth and King, 1991). Microscopically, organized fibrinous pericarditis (constrictive pericarditis) was the most prominent pathological feature in the examined animals. Braun et al. (2007) found constrictive pericarditis in five cases of traumatic

pericarditis, ten cases had fibrinous pericarditis and three suppurative pericarditis with abscessation in all three cases. Chronic venous congestion of liver and interstitial fibrosis of lung and hemosiderosis in spleen were observed. These lesions indicated also the final stage of heart failure and the compression of the cardiac chambers associated with pericarditis (Braun, 2009)

CONCLUSIONS

Oxidative stress is involved in the complications of traumatic pericarditis. An association between oxidative stress indices and pathological changes has also been demonstrated, suggesting that oxidative stress and lipid peroxidation are involved in the complications and aggravations of traumatic pericarditis in cattle.

COVERING LETTER

This manuscript was prepared for publication to evaluate the level of oxidative stress indices and the possible pathological changes associated traumatic pericarditis in cattle. The content of this manuscript has not been published or submitted for publication elsewhere. All authors are in agreement with the content of the manuscript. This research work was carried out according to the ethical regulations in Egypt, where this study was done.

CONFLICT OF INTEREST STATEMENT

None of the authors of the paper entitled "Oxidative stress indices and pathological changes in cattle with traumatic pericarditis" has any financial or personal relationships that could inappropriately influence or bias the content of the paper. ■

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