Full Length Article

EVALUATION OF OXIDATIVE STRESS IN DOGS WITH HEPATIC DYSFUNCTION

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ABSTRACT

The study was conducted to evaluate the oxidative stress in the dogs affected with parenchymal, biliary and neoplastic hepatic disorders compared with apparently healthy dogs. Twenty apparently healthy dogs framed the control group. The experimental group consist of twenty dogs in which 9 dogs formed the parenchymal disorder, six dogs with biliary disorder and five dogs with neoplastic disorder based on the serum biochemical and ultrasonographic investigation. The biochemical parameters such as Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), total bilirubin and direct bilirubin were significantly elevated in experimental group whereas glucose concentration was decreased. In the present study Oxidative biomarkers such as Catalase (CAT), Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) were significantly reduced and Thiobarbituric Acid Reacting Substance (TBARS) was significantly increased in parenchymal, biliary and neoplastic disorders of experimental group when compared with control group. Results of this study showed that the significant increase in oxidative stress in all three disorders of hepatic dysfunction in dogs could be the cause for the progress of hepatic pathology.

Keywords: Antioxidant, Canines, hepatic dysfunction, Oxidant

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INTRODUCTION

Liver is the most important vital metabolic organ in the body with vast reserves of function (about 70 to 80 %) and capacity to regenerate and perform adequately despite extensive pathological damage to its integrity. Oxidants are formed as a product

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of aerobic metabolism, but the oxidants gets elevated during the pathophysiological conditions resulting in imbalance between the oxidants and antioxidants leading to related oxidative stress damage of cells causing oxidative stress (Sies, 1997). The three major enzymatic antioxidants present in any cell are Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT) (Sies, 1997). In liver, under physiologic conditions, due to aerobic metabolism free radicals are produced, but are eventually balanced by the antioxidants present *in situ*. Regardless of etiology, the increased reactive oxygen species formed from the mitochondria, cytochrome P450, Kupffer cells and neutrophils forms the basis for all types of hepatic injury (Bellanti *et al.,* 2014). In the present study, an attempt has been made to study the oxidative stress in the dogs affected with parenchymal, biliary and neoplastic hepatic disorders compared with apparently healthy dogs.

MATERIALS AND METHODS

The study was conducted in dogs that were brought to Small Animal Clinical Medicine Outpatient Unit, Madras Veterinary College Teaching Hospital and at Department of Veterinary Physiology, Madras Veterinary College, Chennai. The study consisted of apparently healthy dogs as Control group (n=20) and dogs with clinical signs suggestive of hepatic dysfunction on clinical examination and serum biochemistry as experimental group (n=20). Based on these parameters and ultrasonographic examination, experimental group animals were further classified into three groups viz., parenchymal disorder (n=9), neoplastic disorder (n=6) and biliary disorders (n = 5) based on ultrasonographic studies.

About two millilitres of blood was collected in an aseptic manner by venipuncture from the dogs in clot activator vacutainer and serum was separated by centrifugation at 1500 rpm for 20 min was stored at -20˚C until further biochemical, antioxidant and oxidant analysis. Another 2ml of blood was collected in NaF vacutainer for plasma glucose estimation. The biochemical parameters viz., Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total protein (TP), total bilirubin and direct bilirubin were estimated using diagnostic kits as per standard protocol. Catalase (CAT), Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) were estimated as per the procedure described in the ELISA kits supplied by Sincere Biotech, Beijing, China. The experimental data were statistically analysed as per Snedecor and Cochran (1994). The data were subjected to one-way analysis of variance (ANOVA) and post hoc analysis was carried out using Duncan's test for multiple comparisons by using SPSS software.

RESULTS AND DISCUSSION

Biochemical Profile

The results of biochemicals parameters of dogs with parenchymal, neoplastic and biliary disorders compared with control group is presented in Table 1. The Alanine aminotransferase (ALT) level was highly elevated in parenchymal, neoplastic and biliary disorder of dogs of experimental group when compared with the control group of dogs. The highest ALP activity was recorded in dogs with biliary disorder (Table 1). The present result was similar with the findings of Neer (1992); Sevelius (1995) and Harrison *et al*. (2018). On contrary to the present study, a significant decrease in the total proteins was reported in dogs with hepatobiliary dysfunction by Sevelius (1995) and Tantary *et al*. (2014). A significant decrease ($p< 0.01$) in plasma glucose was observed in the dogs with parenchymal, biliary disorders and neoplastic disorders when compared to the control group of dogs. The diminished glycogen store and impaired gluconeogenesis and lipolysis in liver due to injury had resulted in hypoglycemia (Younus *et al*., 1977).

A significant increase ($p< 0.01$) in the total bilirubin and direct bilirubin was observed in dogs with hepatic dysfunction especially in dogs with biliary disorder, when compared to the control group of dogs. The current finding was similar to that of Neer (1992). Hyperbilirubinemia in biliary disorders may be due to diminished excretion of bilirubin by damaged hepatocytes and also due to biliary obstruction in elevated levels of bilirubin (Rothuizen and Brom, 1987).

Antioxidant and Oxidant profile

The Table 2 represents the antioxidant and oxidant profile of dogs with hepatic dysfunction compared with control. Physiologically, a balance exists between oxidants and antioxidants activity in the body to main homeostasis. In the present study, antioxidants Superoxide dismutase, Glutathione peroxidase and catalase were found to be reduced in the liver affected dogs when compared to control dogs.

In the present study, the catalase concentration was significantly decreased in the experimental groups when compared with the control group. Parenchymal dysfunctions which occurs secondary to infectious organisms cause increased free radical formation by heme, released due to haemolytic anaemia. The increased heme through Fenton's reaction increases H_2O_2. In order to reduce the free radical, catalase activity gets increased and hence, there is decreased concentration of catalase in the circulation. Hepatic injury causes release of cytokines and chemokines from the hepatic stellate cells and kupfer cells. The TNF alpha produced from hepatic cells causes increased production of Reactive Oxygen Species (ROS) resulting in increased consumption of antioxidant enzymes. The respiratory burst mediate killing of infected cells by neutrophils increases the reactive oxygen species during severe infection. To attenuate the levels of H_2O_2 produced by the action of superoxide dismutase, catalase activity gets increased leading to decreased concentration (Center, 1999). Biliary disorder of dogs showed a significant reduction in the catalase concentration which may be due to increased consumption of CAT to scavange the free radical produced during oxidative stress. GPx concentration in the experimental group was reduced significantly and the findings were similar to Sato *et al*. (2003). The reduction may be due to increased utilization of GPx to neutralize the ROS.

In the present study, the concentration of SOD was significantly reduced in parenchymal, neoplastic and biliary disorders when compared to the control group. The findings were similar to that of Sato *et al*. (2003) and Sadiem *et al*. (2014). In parenchymal disorders, due to inflammatory response, neutrophil undergoes phagocytosis

Table 1. Biochemical profile of control and experimental groups

Parameters	Control group (n=20) Mean ± SE	Experimental groups (n=20)			F- value
		Parenchymal disorders (n=9) Mean ± SE	Neoplastic disorders (n=6) Mean ± SE	Biliary disorders (n=5) Mean ± SE	
ALT (IU/L)	63.70^{a} ± 5.90	267.11^{b} ± 56.26	197.00^{b}± 51.89	178.00^{b}± 8.27^{b}	9.376**
ALP (IU/L)	108.00^{a} ± 5.66	517.11^{b} ±102.56	710.16^{b} ± 174.64	1263.80^{c} ±92.10	39.350**
Total protein (g/dL)	6.35^{a} ± 0.17	6.48^{a} ± 0.26	5.60^{a} ± 0.63	5.86^{a} ± 0.45	1.410^{NS}
Glucose (mg/dL)	96.75^{c} ± 3.55	68.77^{ab} ± 9.52	78.33^{bc} ±12.27	52.20^{a} ± 7.29	7.415**
Total bilirubin (mg/dL)	0.63^{a} ± 0.49	3.16^{b} ±1.07	2.58^{ab} ± 1.14	4.13^{b} ± 1.38	5.494**
Direct bilirubin (mg/dL)	0.50^{a} ± 0.04	2.65^{b} ± 1.00	2.03^{ab} ± 1.00	3.23^{b} ± 1.06	4.510**

Mean values having the same superscript within a row do not differ significantly (P>0.05)

**- Significant (p<0.01)

^{NS} – Non significant (p>0.05)

Table 2. Antioxidant and oxidant profile of control and experimental groups

Parameters	Control group (n=20) Mean ± SE	Experimental groups (n=20)			F- value
		Parenchymal disorders (n=9) Mean ± SE	Neoplastic disorders (n=6) Mean ± SE	Biliary disorders (n=5) Mean ± SE	
Catalase (ng/mL)	10.72^{b} ± 0.23	5.15^{a} ± 0.75	4.71^{a} ± 0.25	4.69^{a} ± 0.26	39.56**
Glutathione peroxidase (ng/mL)	10.28^{b} ± 0.34	4.72 a± 0.73	4.18^{a} ± 0.24	4.24^{a} ± 0.24	33.459**
Superoxide Dismutase (ng/mL)	10.16^{b} ± 0.45	5.97^{a}± 1.04	5.26^{a} ± 0.81	4.01^{a} ± 0.58	13.392**
TBARS (ng/mL)	10.36^{b} ± 0.25^{b}	13.14^{a} ± 0.42	14.57^{a} ± 0.82	14.90^{a} ± 1.29	12.140**

Mean values having the same superscript within a row do not differ significantly (P>0.05)

**- Significant (p<0.01)

which results in oxidative burst with increased O_2 release leading to increased production of superoxide anions. In order to render the superoxide anion less hazardous by dismutation of two superoxide anion into H_2O_2 and water (Root and Metcoff, 1977). SOD activity gets increased thereby reducing its concentration in plasma. The chemokines and cytokines such as TGF- β1, TNF-α, PDGF, IL-1 that are derived from Kupffer cells contribute to chronic liver diseases and hepatic neoplasms (Chatterjee and Mitra, 2015). The ROS during immune response resulted in increased consumption of SOD, thereby reducing its concentration in the circulation.

Biliary disorder of dogs showed a significant decrease in the SOD concentration when compared to the control dogs. The findings are similar to that of Sadiem *et al*. (2014). Persistent hepatocellular injury induces abnormal bile synthesis which results in cholelith formation. Choleliths can induce inflammation in the gallbladder wall and a change in the bile composition leading to altered bilirubin metabolism. This results in reduction in its antioxidant property. This change in bile composition induces further increase in the biliary free radical formation. Moreover, inflammatory response to injury of gall bladder mucosa is associated with granulocyte infiltration. The activated phagocytes further produces ROS resulting in oxidative stress (Sipos *et al*., 2001). Free radicals and other peroxides produced physiologically initiate the activity of antioxidant enzymes. SOD are consumed more to scavenge the free radical.

A high significant increase ($p < 0.01$) in the mean ± SE values of TBARS concentration was observed in the experimental group of dogs when compared to control group. The increased ROS causes increased consumption of antioxidant enzyme which led to inactive antioxidant enzymes involved in reducing the ROS. Thus, it initiate the peroxidation of lipids. TBARS concentration in the liver parenchymal diseases secondary to the infections such as erhlichiosis, babesiosis, leptospirosis showed a significant increase. The findings were similar to that of Crnogaj *et al*. (2017) and Da Silva *et al*. (2013). Antioxidants protect cells and tissues from harmful oxidative damage. The increase in TBARS concentration indicated the persistence of oxidative stress (Rubio *et al*., 2017). In hepatic tumours, most of the factors that initiate tumourigenesis causes increased free radical synthesis in the cell. The release of free radicals further induce tumour formation and development by affecting the initiation, development and progression stages of carcinogenesis. Hence, significant increases in the TBARS concentration in the neoplastic disorders in this study recorded got agreed with the findings of Unsal and Kurutas, 2017.

CONCLUSION

In the present revealed that the antioxidant status of dogs afffected with hepatic dysfunction of parenchymal, biliary and neoplastic cause is significantly decreased when compared with the normal dogs indicating oxidative stress and this could also be one of the major cause for the progression of hepatic pathology in companion animals.

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