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Shaista Firdose
M.V.Sc Scholar, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, KVAFSU, Karnataka, India

Ravindranath Bislere Mallappa
Associate Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, KVAFSU, Karnataka, India

Sahadev Annayappa
Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, KVAFSU, Karnataka, India

Renukaradhya Gudepalya Jayanna
Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, KVAFSU, Karnataka, India

Gurubasayya P Kalmath
Associate Professor, Department of Veterinary Physiology, Veterinary College, KVAFSU, Karnataka, India

Rashmi Rajashekaraiah
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College, KVAFSU, Karnataka, India

Suchitra Bidare Raju
Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, KVAFSU, Karnataka, India

Corresponding Author:
Ravindranath Bislere Mallappa
Associate Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, KVAFSU, Karnataka, India

Studies on serum oxidative status and hematobiochemical profile in dogs affected with transmissible venereal tumor (TVT)

Shaista Firdose, Ravindranath Bislere Mallappa, Sahadev Annayappa, Renukaradhya Gudepalya Jayanna, Gurubasayya P Kalmath, Rashmi Rajashekaraiah and Suchitra Bidare Raju

Abstract
The present study was carried out to compare the haemato-biochemical profile and serum antioxidant enzyme status of TVT affected and healthy dogs. Group I comprised of healthy dogs (n=12) and Group II (n=12) involved TVT affected dogs (diagnosed based on clinical signs, nature of lesions, impression smear cytology and histopathology). Blood samples were collected from both the group of dogs for haemato-biochemical parameters and antioxidants evaluation. A significant leukocytosis with neutrophilia, significantly higher ALT, superoxide dismutase, glutathione peroxidase and significantly lower catalase activity were the prominent findings in TVT affected dogs. In conclusion, estimation of haemato-biochemical profile and antioxidant enzymes can be useful in the evaluation of clinical status of TVT affected dogs before initiation of suitable treatment.

Keywords: Antioxidants enzymes, dog, haemato-biochemical, TVT

Introduction
Transmissible venereal tumor (TVT) is a contagious, naturally occurring, horizontally transmitted venereal round cell tumor of dogs [1]. It is also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or Sticker tumor [2]. In India, TVT is the most commonly reported tumor of dogs, accounting for 23-43 per cent of all tumors in the canine population [3]. The TVT lesions are friable, hyperemic; multi nodular, cauliflower-like masses with an offensive odour from the hemorrhagic discharge [4]. The reduced production of several antioxidant defence enzymes can increase ROS levels, producing oxidative damage and increasing the risk of cancer in animals [5]. Changes in the haemato-biochemical and oxidant- antioxidant balance have been reported in earlier studies of TVT such as of Amruth *et al*. (2017) [6] and Ercan *et al*. (2020) [7] respectively. As the previous studies carried out on antioxidants evaluation in TVT had meager data, present study was carried out to ascertain antioxidant enzyme activity and haemato-biochemical status in dogs affected with TVT and to compare it with the haemato-biochemical profile of healthy dogs.

Materials and Methods
Dogs presented to the Department of Veterinary Gynaecology and Obstetrics were selected for the present study. The study was conducted on 24 dogs aged above 2 years. Group I comprised of healthy dogs (n=12) and Group II (n=12) involved dogs affected with TVT. TVT cases were initially diagnosed based on history, clinical symptoms and nature of the lesion. For further confirmatory diagnosis of TVT, impression smear cytology and histopathology of the tumor mass was performed.
Blood samples were collected from all the dogs to ascertain haemato-biochemical parameters such as total erythrocyte count (TEC), total leucocyte count (TLC), differential leukocyte count (DLC), haemoglobin (Hb), packed cell volume (PCV), platelet count, blood urea nitrogen (BUN), creatinine, total protein, alanine amino transferase (ALT) and aspartate amino transferase (AST) using semi auto analyzer. Antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase were estimated using methods described by Caliborne (1985) [8], Marklund and Marklund (1974) [9] and Rotruck *et al*. (1973) [10] respectively. Tumor samples collected from TVT affected dogs were subjected to histopathological evaluation.

The data obtained in the present study was tabulated and subjected to t- test to analyze the significant differences among means, where $p \leq 0.05$ was considered as statistically significant. The present statistical analysis was done with the help of SPSS (version 16.0) statistical software.

Result and Discussion

The results of the present study have been represented in Table 1 and 2. The mean TEC and Hb concentrations of TVT affected dogs and healthy dogs did not differ significantly which was in congruence with the findings of Das *et al*. (1991) [11] who recorded normal hemogram in TVT affected dogs. Similarly, mean PCV and platelet count showed no significant variations between Group I and Group II, as in line with the findings of Amruth *et al*., 2017 [6] and Cizmeci *et al*., 2012 [12] respectively.

Significant increase in the TLC in TVT affected dogs as compared to healthy dogs in the present study was in corroboration with the findings of Cizmeci *et al*. (2012) [12], Girmabirhan and Chanie (2015) [13] and Amruth *et al*. (2017) [6]. Similarly, significant neutrophilia ($p<0.05$) was observed in TVT affected dogs which was in line with the findings of Kabuusu *et al*. (2010) [14] and Amruth *et al*. (2017) [6]. Leukocytosis along with neutrophilia in TVT affected dogs might be due to inflammation or as a response to increased endogenous corticosteroids released in response to pain and stress as opined by Duncan *et al*. (1995) [15].

Further, the mean lymphocyte, monocytes and eosinophil count in TVT affected dogs and healthy dogs showed no significant variation in the present study as in agreement with the reports of Kabuusu *et al*. (2010) [14] and Amruth *et al*. (2017) [6].

Similarly there was no significant variation in the biochemical parameters of mean creatinine, BUN and total protein concentration of TVT affected dogs and healthy dogs in accordance with the findings of Albanese *et al*. (2006) [16] and Naveen *et al*. (2019) [17].

However there was significant increase ($p<0.05$) in the ALT activity of TVT affected dogs as compared to healthy dogs in congruence with the findings of Girmabirhan and Chanie (2015) [13] and Amruth *et al*. (2017) [6]. Edoardo *et al*. (2005) [18] reported that the aminotransferases typically elevate with hepatocellular damage, hepatitis, hepatic tumor, anorexia, toxemia, and biliary stasis. But increased aminotransferase levels were not significant until they reached double than the normal values. Hence the results of the present study indicate that the liver is not affected.

No significant disparity was observed in the mean AST levels between TVT affected dogs and healthy dogs. The present finding was in agreement with the findings of Cizmeci *et al*. (2012) [12] who reported that AST levels are not affected in TVT affected dogs.

With respect to the serum antioxidant evaluation in the present study, mean CAT activity was significantly lower ($p<0.05$) in TVT affected dogs as compared to healthy dogs. Contrary to the present findings, Ercan *et al*. (2020) [7] reported a non-significant decrease in the serum catalase level in TVT affected dogs. The significant reduction in CAT activity in TVT affected dogs as observed in the present study might be ascribed to enhanced lipid peroxide scavenging and tumor cell sequestration [19]. Further, Cullen *et al*. (2003) [20] and Cobanoglu *et al*. (2010) [21] reported that in certain cancers, catalase expression was down regulated.

Further significantly higher ($p<0.05$) mean SOD activity was recorded in the TVT affected dogs as compared to healthy dogs. The present findings are in consonance with the findings of SzczubiaŁ *et al*. (2004) [22] who also reported high SOD activity in dogs with mammary tumors. On the contrary, Ercan *et al*. (2020) [7] reported low levels of SOD in TVT affected dogs. Bauer and Bauer (1999) [23] reported that SOD enzyme levels are altered in a variety of pathological conditions. The increase in antioxidant enzyme activity in the present study could be attributed to the adaptive mechanism of the body in response to oxidative stress [24].

As in line with SOD, the mean GPx activity was significantly higher ($p<0.05$) in TVT affected dogs as compared to control dogs. Ercan *et al*. (2020) [7] reported significantly decreased serum GPx activity in TVT affected dogs as compared to the healthy dogs which is in contrast to the present findings.

The significantly higher levels of serum GPx activity in TVT affected dogs as recorded in the present study might be attributed to the overexpression of the GPx enzyme to protect cells against oxidative stress as reported by Sies *et al*. (1995) [25] or to compensate the lack of catalase [26]. Further, Ghalia and Fouad (2000) [27] and Iscan *et al*. (2002) [28] reported significantly higher levels of GPx in tumorous condition, as it is the first step in enzyme defense against H_2O_2 and other hydroperoxides.

It is reported that at low hydrogen peroxide concentrations, catalase acts as a peroxidase [26]. Further, Li *et al*. (1998) [29] reported that increased ROS levels may also trigger signaling pathways that cause SOD and GPx to be expressed at the mRNA level. As a result, SOD and GPx overexpression may have a role in the detoxification of hydrogen peroxides, electrophilic poisons, and carcinogens in the TVT affected dogs.

Table 1: Mean±SE haemato-biochemical profile of control (n=12) and TVT affected dogs

Parameters	Control (n=12)	TVT affected dogs (n=12)
TEC ($x10^6$/cmm)	5.93±0.33	5.76±0.47
Hb (g/dL)	13.45±0.72	11.83±0.99
PCV (%)	37.14±1.18	38.99±3.36
Platelet ($x10^3$/cmm)	260.33±21.41	221.75±46.84
TLC ($x10^3$/cmm)	13.71±0.72^a	18.63±1.74^b
Neutrophil (%)	69.82±2.11^a	78.85±2.31^b
Lymphocyte (%)	25.82±2.28	19.67±2.19
Monocyte (%)	2.68±0.31	3.54±0.31
Eosinophil (%)	2.98±0.31	3.01±0.77
Creatinine (mg/dL)	1.09±0.90	1.51±0.30
BUN (mg/dL)	15.46±1.67	12.84±2.09
Total Protein (g/dL)	7.24±0.19	6.98±0.30
ALT (U/L)	39.49±2.80^a	48.64±7.24^b
AST(U/L)	35.71±3.59	37.91±4.64

Note: Means bearing different superscripts within a row differ significantly ($p<0.05$)

Table 2: Mean serum antioxidant enzyme activity in control and TVT affected dogs

Parameter	Control Group	TVT Affected Group
CAT (µmoles/min/mg of protein)	1.27±0.15^a	0.58±0.14^b
SOD (U/min/mg of protein)	10.61±1.90^a	23.48±3.22^b
GPx (µmole/mg of protein)	147.30±11.26^a	283.53±16.93^b

Note: Means bearing different superscripts within a row differ significantly ($p<0.05$)

Conclusion

Leukocytosis with neutrophilia and significantly higher ALT activity were the prominent haemato-biochemical changes whereas, significantly increased serum SOD and GPx activities with decreased serum CAT levels were the salient findings in the enzymatic antioxidant evaluation in TVT affected dogs. It could be concluded that the increase in SOD and GPx in TVT affected dogs may suggest the activation of antioxidative defense mechanism to protect the cells against oxidative damage and the decrease in CAT may be due to its down regulation in its expression or its exhaustion due to increased ROS in the tumor condition. Further studies involving large sample size may throw better light to draw any inference by evaluating other oxidative stress markers and antioxidant parameters in dogs with TVT, which could not be undertaken due to time and financial constraints.

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Conflict of interest

Authors have no conflict of interest

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