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SHORT COMMUNICATION

Evaluation of antioxidant status in dogs with cataract

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The aim of the study was to compare oxidative status in clinically presented cataractous dogs with that of apparently healthy dogs. The enzymes Superoxide-Dismutase (SOD), Xanthine Oxidase (XOD), Total Antioxidant Capacity (TAC) and Lipid Peroxidation Product (LPO) (malonaldialdehyde) in 15 cataractous dogs were analysed in plasma and compared with apparently healthy dogs' plasma. The enzyme analysis was done by in vitro *enzyme immunoassay ELISA kits and the estimation of LPO was done by the formation of thiobarbituric acid reactive substances (TBARS) which was then assayed spectrophotometrically at 548 nm wavelength. The overall enzyme values of SOD, XOD, TAC and LPO in cataractous dogs were 0.7793±0.048 ng/mL, 0.2593±0.006 pg/mL, 1.3787±0.13 U/mL and 160.46±16.54 nmoL/g Hb, respectively and that in apparently healthy dogs were 0.9353±0.027 ng/mL, 0.2540±0.005 pg/mL, 1.9307±0.15 U/mL and 147.62±12.97 nmoL/g Hb, respectively. It was concluded that total antioxidant capacity was lower in cataractous dogs compared with normal dogs (control group).*

Keywords: Antioxidant status, Cataract, Dog

Cataract is defined as opacity of lens, and cataract formation is the major cause of vision loss accounting for 33.4% of all causes of blindness in dogs (Babizhayev and Yegorov, 2014). Various risk factors like ageing, diabetes mellitus, UV radiations, free oxygen radicals and oxidative stress have been regarded as important factors leading to age related cataract (Brennan and Kantoro, 2009). As majority of surveys were accomplished on human cells and data about oxidative stress parameters in cataractous dogs are scarce, the present study was done to investigate the levels of some oxidative stress parameters in the blood of cataractous dogs and to compare it with apparently normal healthy dogs.

The levels of superoxide dismutase (SOD), xanthine oxidase (XOD), total antioxidant status (TAS) and lipid peroxidation products (LPO) (malondialdehyde) were analysed in 15 dogs from both sexes with mean age of 7.87±0.77 yr affected by cataract in one or both eyes and compared with 15 apparently healthy dogs with mean age 5.80±0.77 yr.

A total of 5 mL blood was drawn from cephalic / saphenous vein out of which 3 mL was then transferred into pre-heparinised centrifuge tube and was centrifuged at 3000 rpm for 15 min for separation of supernatant plasma which was separated and transferred to plasma tubes and stored at -20ºC and kept for later use for enzyme estimation. To the remaining sediment of RBC an equal amount of NSS solution was mixed and again centrifuged. Two repeat washings of NSS were done and later distilled water was added to the RBCs to form hemolysate. This hemolysate was also stored at -20ºC and it was later used for the estimation of Malondialdehyde.

The mean LPO values in the group I consisting of cataractous dogs was 160.46±16.54 nmoL/g Hb (range 124.98-195.93 nmoL/g Hb) which was higher when compared with group II apparently normal dogs in which the mean value was 147.62±12.97 nmoL/g Hb (range 119.80-175.44 nmoL/g Hb). However, the difference was not statistically significant (P>0.05). Age wise comparison within group I revealed that level of LPO (164.90±51.16 nmoL/g Hb) was higher in cataractous dogs in the age group of 5-10 years (n=8) compared with cataractous dogs in the age group of 0-5 years (n=4) (164.90±51.16 nmoL/g Hb). The results suggest that such senile conditions may have an effect on the development of age-related cataract and is consistent with the theory of free radical age-related cataract development (Madany, 2016).

The mean value of Superoxide Dismutase (SOD) in group I dogs was 0.7793±0.048 ng/mL (range 0.6758 to 0.8829 ng/mL) which was lower when compared with healthy dogs in which the mean value was 0.9353±0.027 ng/mL (range 0.8767 to 0.9940 pg/mL) but statistically insignificant between the two groups (P>0.05). It was stated that a diminution of ROS scavenger enzymes and decreased DNA repair capability makes lens susceptible to oxidative damage and cataract (Donma *et al.*, 2002). In the present study the value of SOD enzyme in group I cataract dogs with diabetes (n=2) was 0.70±0.27 ng/mL, which was significantly lower when compared with group II apparently healthy dog with diabetes (n=1) in which the level of SOD was 1.03 ng/mL. The comparison of SOD value in diabetic cataract was significantly lower when compared with group II dogs without diabetes. This could be due to down regulation of SOD enzyme in diabetes(Moffat *et al.*, 1999).

The mean value of XOD in group I dogs was 0.259±0.006 pg/mL (range 0.245 to 0.273 pg/mL) and the mean value of XOD in group II healthy dogs was 0.254±0.005 pg/mL (range 0.242 to 0.265 pg/mL) with no statistically significant difference between the two groups (P>0.05). As such no literature has been found that compared the activity of Xanthine oxidase in cataractous dogs to that of healthy dogs.

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Table 1: Comparison of superoxide dismutase, xanthine oxidase, total antioxidant capacity and malondialdehyde between group 1 (cataractous) and group II (normal) dogs.

Enzymes/Groups	Superoxide Dismutase (ng/mL)	Xanthine Oxidase (pg/mL)	Total Antioxidant Capacity (U/mL)	LPO (Malondialdehyde) (nmol/g Hb)
Group I (n=15)	0.779±0.04	0.259±0.006	1.378±0.13	160.46±16.54
Group II (n=15)	0.935±0.02	0.254±0.005	1.930±0.15	147.62±12.97
Group I dogs with diabetes (n=2)	0.70±0.27	0.24±0.015	1.50±0.15	69.51±47.17
Group I dogs without diabetes (n=13)	0.79±0.04	0.26±0.007	1.35±0.07	174.45±14.74
Group I dogs in age group 0-5 yr (n=4)	0.66±0.12	0.25±0.006	1.46±0.13	164.90±51.16
Group I dogs in age group 5-10 yr (n=8)	0.81±0.06	0.26±0.009	1.35±0.17	169.36±16.68
Group II dogs in age group 0-5 yr (n=7)	0.96±0.04	0.26±0.002	1.99±0.22	137.34±14.38
Group II dogs in age group 5-10 yr (n=7)	0.89±0.03	0.25±0.012	1.79±0.23	167.76±20.88
Group I dogs with diabetes (n=2)	0.70±0.27	0.24±0.01	1.50±0.15	69.51±47.17
Group I dogs without diabetes (n=13)	0.79±0.04	0.26±0.00	1.35±0.07	174.45±14.74
Group II dogs with diabetes (n=1)	1.03	0.24	2.42	78.58
Group II dogs without diabetes (n=14)	0.92±0.02	0.25±0.00	1.89±0.00	152.55±12.88

The total antioxidant status (TAS) in group I dogs was 1.37±0.13 U/mL (range 1.09 to 1.63 U/mL), which was lower when compared with group II dogs in which the level was 1.93±0.15 U/mL (range 1.60 to 2.25 U/mL). However the difference was not statistically significant (P>0.05). The age-wise comparison of the TAS value in group I dogs showed a decrease in the level with increasing age as the values were 1.46±0.13 U/mL in the age group of 0-5 years (n=4), 1.35±0.17 U/mL in the age group 5-10 years (n=8) and 1.33±0.51 U/mL in the age group of > 10 years (n=3). A decrease in TAS activity in the serum of dogs with age-related cataract indicates weakening of the systemic defence mechanisms and depletion of the set of non-enzymatic antioxidants in the blood and the dominance of peroxidation processes over processes of free radical neutralization(Placer, 1966).

The level of LPO was higher in cataractous dogs compared to normal dogs. SOD was significantly lower in the cataractous patients compared with the normal controls. The level of SOD was also significantly lower in cataract diabetic patients (P<0.05). Total antioxidant capacity was lower in cataractous dogs compared with normal and it was also found to be lower in diabetic cataract animals compared than diabetic non-cataractous dogs. Xanthine oxidase revealed no variation among different age groups, gender or diabetic vs non-diabetic patients between cataractous and normal dogs. The enzyme estimation done *in-vivo* using ELISA kits was useful in determining the oxidative status in blood plasma of dogs.

From the results of this study, it was concluded that total antioxidant capacity was lower in cataractous dogs compared with normal dogs.

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