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Pakistan Veterinary Journal
ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

Reference Values of Oxidative Stress Parameters in Adult Iranian Fat-Tailed Sheep

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ARTICLE HISTORY
Received: August 08, 2009
Revised: September 10, 2009
Accepted: October 24, 2009

Key words:
Malondialdehyde
Superoxide dismutase
Catalase
Glutathione peroxidase
Reference values
Iranian fat-tailed sheep

ABSTRACT

The present study was performed on 111 adult clinically healthy Iranian fat-tailed sheep from both sexes (28 male and 83 female). Blood concentrations of oxidative stress parameters viz. malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were determined. The reference values for oxidative stress parameters of adult clinically healthy Iranian fat-tailed sheep were as followed: MDA 0.53-0.60 µmol/l, SOD 948.65-1011.50 U/gHb, CAT 1834.29-1915.63 U/gHb and GPX 191.67-196.52 U/gHb. There were no significant differences in oxidative stress parameters among animal of the two sexes.

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To cite this article: Nazifi S, N Ghafari, F Farshneshani, M Rahsepar and SM Razavi, 2010. Reference values of oxidative stress parameters in adult Iranian fat-tailed sheep. Pakistan Vet J, 30(1): 13-16.

INTRODUCTION

A stressful condition leads to the excessive production of the radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system (Khadija *et al*., 2009). Generation of free radicals is an integral feature of normal cellular function. In contrast, excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell (Lopaczyski and Zeisel, 2001). Reactive oxygen species (ROS) including superoxide radical, hydrogen peroxide and hydroxyl radical have a great impact on the normal function of biomolecules like nucleic acids, proteins and cell membrane phospholipids. Free radicals are generated during stepwise reduction of molecular oxygen (Singh *et al*., 1999). Hallwell and Gutteridge (1999) described several lines of defense against reactive oxygen species in animals.

Enzymes with important antioxidant functions include: i) superoxide dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water, ii) catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water, and iii) glutathione peroxidase (GPX), which facilitates the destruction of both hydrogen peroxide and organic peroxides. Reduced glutathione (GSH), a tri-peptide thiol, is an important antioxidant, as well as a co-factor for various antioxidant enzymes (Kidd, 1997). SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical (Gonzales *et al.,* 1984). The hydrogen peroxide generated in this reaction is restored to water in the presence of CAT and GPX. Polyunsaturated fatty acids present in membrane phospholipids are the main target substrates for oxygen radical activity which results in disorganization of cell framework and function (Patterson and Leacke, 1998). Lipid peroxidation is an indicator of oxidative stress in cells and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and are decomposed to form a series of compounds, including malondialdehyde (MDA). The quantization of MDA is widely used as an indicator of lipid peroxidation (Simsek *et al.,* 2006).

Increased levels of lipid peroxidation products such as MDA have been reported in a variety of diseases like *Dicrocoelium dendriticum* infection in sheep (Simsek *et al.,* 2006) and kidney diseases in dogs (Kargin and Fidanci, 2001). The brain injury is reported to be caused by superoxide radical and hydrogen peroxide (Kotos and Wel, 1986). Distomatosis (*Fasciola hepatica, Fasciola gigantica* and *Dicroceolium dendriticum* infections) in sheep causes production of reactive oxygen species and lipid peroxidation by significant increase in liver MDA (Deger *et al.,* 2008).

There is little information concerning the oxidative stress enzymes in sheep. Comparative aspects of plasma antioxidant status in sheep and goats, and the influence of experimental abomasal nematode infection were investigated by Lightbody *et al.* (2001). Also, Kizil *et al.* (2007) reported oxidative stress and antioxidant status in goats naturally infected with *Mycoplasma agalactiae*. The aim of this study was to present the reference values

of oxidative stress parameters in adult clinically healthy Iranian fat-tailed sheep. Such information would allow us to make comparisons between normal and abnormal states and provide a better understanding in diseases accompanied by oxidative stress.

MATERIALS AND METHODS

Experimental animals

The study was performed on 111 adult, clinically healthy Iranian fat-tailed sheep from both sexes (28 male and 83 female; 3-4 years old) reared mainly in South Iran (Fars province). Animals were fed with hay (mainly alfalfa and grass). All animals were treated against internal and external parasites. They were treated with Fenbendazole (Damloran Company, Borujerd, Iran) 10 mg/kg, 30 days prior to the study. A healthy condition was established by clinical and laboratory examination (haematology, clinical biochemistry and other relevant tests).

Sample analysis

Blood samples for determination of oxidative stress parameters were obtained between 8 and 9 am, to avoid diurnal influences, by jugular venepuncture into vacutainers containing EDTA (in a 10:1 ratio) as an anticoagulant. After centrifugation at 750 *g* for 15 min, plasma was separated and erythrocyte lysate was prepared (Ivanov, 1999). After triple washing of erythrocyte mass with physiological solution, 0.5 mL of cell suspension was dissolved in 2 mL cold water for lysis of erythrocytes. Haemoglobin was then precipitated by adding 1.8 mL water and 0.2 mL ethanol/chloroform (3:5/v:v) to 0.2 ml lysate. The tubes were shaken for 5 min and centrifuged at 750 *g* for 20 min. The supernatant was used for the determination of enzyme activities.

SOD activity was measured by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride (RANSOD Kit, Randox, UK). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl-tetrazolium chloride (INT) to form a red formazan dye. SOD activity was then measured by the degree of inhibition of this reaction. One unit of SOD was that which caused a 50% inhibition of the reduction rate of INT under the assay condition.

GPX activity was measured by the method of Paglia and Valentine (1967), using RANSEL Kit, (Randox, UK). GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of GR and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance was measured at 340 nm. The thiobarbituric acid method was used to quantitate MDA-reactive products (Plaser and Cushman, 1966). Thiobarbituric acid (TBA) and MDA react to form a schiff base adduct under high temperature/acidic conditions to produce a chromogenic/fluorescent product that can be easily measured employing various analytical techniques such as spectrophotometric or fluorometric methods. CAT activity was estimated in erythrocyte lysate by the method of Beers and Sizer (1952), using the ferrous oxidation in xylenol orange (FOX) assay. Samples containing CAT were incubated with H_2O_2 for varying time intervals prior to rapid mixing of aliquots of the incubation mixtures with FOX reagent, which measures residual H_2O_2. Absorbance was then read at 560 nm after 30-min incubation at room temperature. Decay of H_2O_2 is proportional to CAT activity in the original sample. The haemoglobin concentration of the lysate was determined by the cyanmethaemoglobin method (Mahoney *et al.,* 1993).

Data analysis

Results are presented as mean, standard error of mean (SEM), median, mode, standard deviation (SD), range and minimal and maximal values. Raw data were tested for normal distribution using SPSS software and the Kolmogorov-Smirnov method. All reference ranges had a normal distribution by confidence interval 90%. The reference range was determined according to mean ± 2.397 × SD. Statistical analysis was performed using t-test for comparison of the differences between animals of two sexes.

RESULTS

Reference values of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and malondialdehyde (MDA) in 111 adult male and female clinically healthy Iranian fat-tailed sheep are shown in Table 1. The values were as followed: SOD 948.65-1011.50 U/gHb, GPX 191.67-196.52 U/gHb, CAT 1834.29-1915.63 U/gHb and MDA 0.53-0.60 µmol/l. There were no significant differences in oxidative stress parameters (SOD, GPX, CAT and MDA) between both sexes (P>0.05).

DISCUSSION

There is little information about the oxidative stress parameters (SOD, GPX, CAT and MDA) of sheep. The activity of antioxidant enzymes in adult clinically healthy Iranian fat-tailed sheep were comparable to the values reported by Lightbody *et al.* (2001) and Kizil *et al.* (2007). A comparison of our results to those reported by Todorova *et al.* (2005) showed that reference values of oxidative stress indices such as MDA, CAT and SOD in carnivores (dogs and cats) were higher than the values obtained in Iranian fat-tailed sheep. This outcome was consistent in both sexes. This status may be due to their different diets, in other word, generation of free radicals in carnivores is more than that in herbivores. The oxidative status is variable and can be changed by different factors. In the present study, all Iranian fat-tailed sheep were adult. In normal conditions, age influences greatly free radical generation and consequently, the level of antioxidant defense enzymes. In similar investigations on rats at different ages, decreased plasma levels of antioxidant vitamins C and E, decreased SOD activity, and increased CAT activity was observed (De and Durad, 1991).

Table 1: Reference values of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and malondialdehyde (MDA) in adult male and female clinically healthy Iranian fat-tailed sheep

Sex	Parameter	No	Mean	SEM	SD	Reference range	Median	Mode	Mini-mum	Maxi-mum
Male	SOD(U/gHb)	28	987.3	15.9	84.26	954.68-1020.03	968	920.00	860	1160
	GPX(U/gHb)	28	193.5	1.34	7.13	190.77-196.30	194.00	186.00	180	205
	CAT(U/gHb)	28	1880.0	24.5	129.8	1829.68-1930.38	1910.50	1826.0	1587	2067
	MDA(μmol/l)	28	0.56	0.01	0.08	0.53-0.60	0.54	0.53	0.46	0.77
Female	SOD(U/gHb)	83	973.24	8.33	75.95	956.65-989.82	961.00	954.00	861	1136
	GPX(U/gHb)	83	193.68	0.81	7.41	192.06-195.30	194.00	198.00	180	205
	CAT(U/gHb)	83	1867.5	16.33	148.8	1835.05-1900.05	1913.00	1836.0	1518	2062
	MDA(μmol/l)	83	0.57	0.01	0.09	0.55-0.59	0.57	0.48	0.41	0.76
Total	SOD(U/gHb)	111	984.58	7.48	80.91	948.65-1011.50	964.50	947.00	861	1145
	GPX(U/gHb)	111	193.54	1.00	7.54	191.67-196.52	194.00	194.00	180	205
	CAT(U/gHb)	111	1874.9	12.33	141.7	1834.29-1915.63	1912.00	1833.0	1561	2064
	MDA(μmol/l)	111	0.57	0.008	0.009	0.53-0.60	0.5600	0.4900	0.44	0.76

In the present study, there were no significant differences in oxidative stress parameters between both sexes (P>0.05). There is a good evidence to show that sex differences in oxidative status exist in different species. In many species, females live longer than males and it is probably associated with free radicals which are in lower amount in the mitochondria of females than males (Sastre *et al.,* 2002). The longer lifespan in females may be due to the higher gene expression of antioxidants and the lower oxidative damage of mitochondria (Borras *et al.,* 2003). Moreover, there is evidence for the strong antioxidant properties of estrogen (Tudus, 2000) but not for progesterone and testosterone (Barp *et al.,* 2002). In animal species, further investigations are needed to interpret these changes.

In conclusion, the results of the present investigation provide a baseline data for comparisons between normal and abnormal states; therefore, can provide a better understanding in diseases accompanied by oxidative stress.

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