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REFERENCE VALUES OF OXIDATIVE STRESS PARAMETERS IN ADULT NATIVE IRANIAN GOATS

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Summary

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The present study was performed on 132 clinically healthy Iranian native goats from both sexes (36 male and 96 female). The blood concentrations of 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 native goats were as followed: SOD 778.65−1190.50 U/g Hb, GPX 266.67−322.00 U/g Hb, CAT 1536.29−2215.63 U/g Hb and MDA 0.346−0.801 μmol/L. There were no significant differences in oxidative stress parameters between the two sexes.

Key words: catalase, glutathione peroxidase**,** Iranian native goats, malondialdehyde, reference values, superoxide dismutase

INTRODUCTION

A stressful condition leads to the excessive production of free radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system. Generation of free radicals is an integral feature of normal cellular functions. In contrast, excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell (Lopaczyski & Zeisel, 2001). Reactive oxygen species (ROS) – 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).

Halliwell & Gutteridge (1999) described several lines of defense against reactive oxygen species in animals. Enzymes with important antioxidant functions include superoxide dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water, catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water, and 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). Glutathione reductase (GR) catalyses the reduction of oxidased glutathione to GSH,

and thus maintains its antioxidant function (Halliwell & Gutteridge, 1999). SOD is the first line of defense against ROS and is active in catalyzing the 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 in membrane phospholipids are the main target substrates for oxygen radical activity which result in disorganization of cell framework and function (Patterson *et al.,* 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. One of them is malondialdehyde (MDA). The quantitation 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 *et al.,* 2001). In experimental animals brain injury is reported to be caused by superoxide radical and hydrogen peroxide (Kotos *et al.,* 1986). Distomatosis (*Fasciola hepatica, Fasciola gigantica* and *Dicroceolium dendriticum* infections) in sheep cause 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 goats. 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 is to present the reference values of oxidative stress in adult clinically healthy Iranian native goats. 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

The study was performed on 132 clinically healthy Iranian native goats from both sexes (36 male and 96 female; 2−3 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. Each animal had a separate file including all necessary records so that its characteristics, including age, sex, could be determined.

Blood samples for determination of oxidative stress parameters were obtained between 8 and 9 AM, to avoid diurnal influences, and were immediately examined. For the determination of oxidative stress parameters, blood samples were collected by jugular venepuncture into vacutainers containing EDTA as an anticoagulant (in a 10:1 ratio). After centrifugation at 750 *g* for 15 min the plasma was separated and erythrocyte lysate was prepared by the method of 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 plus 0.2 mL etha-

nol/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 Com, UK). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium 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 was measured by the method of Paglia & Valentine (1967) (RANSEL Kit, Randox Com, 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.

CAT activity was estimated in erythrocyte lysate by the method of Beers & Sizer (1952). CAT activity was measured using the ferrous oxidation in xylenol orange (FOX) assay. Samples containing CAT are 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 is then read at 560 nm after 30-min incubation at room temperature. Decomposition of H_2O_2 is proportional to CAT activity in the original sample.

The thiobarbituric acid method was used to quantitate MDA-reactive products (Plaser & 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.

The haemoglobin concentration of the lysate was determined by the cyanmethaemoglobin method (Mahoney *et al.,* 1993).

Results are presented as mean, standard error of mean (SEM), median, mode, standard deviation (SD), range, minimum and maximum values. Raw data were tested for normal distribution using the Kolmogorov-Smirnov method (SPSS software). All reference ranges had a normal distribution by confidence interval 90%. The reference range was determined as mean ± 2.397×SD. Statistical analysis was performed using t-test for comparison of the differences between two sexes. The value of $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

The values of SOD, GPX, CAT and MDA of clinically healthy male and female goats are shown in Table 1. The reference values for oxidative stress parameters (SOD, GPX, CAT and MDA) of adult clinically healthy Iranian native goats from both sexes were 778.65−1190.50 U/g Hb, 266.67−322.00 U/g Hb, 1536.29−2215.63 U/g Hb and 0.346− 0.801 μmol/L, respectively. There is little information about the oxidative stress parameters (SOD, GPX, CAT and MDA) of goats. The activity of antioxidant enzymes in adult clinically healthy Iranian native goats were comparable to the values reported by Lightbody *et al.*

Table 1. Reference values of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and malondialdehyde (MDA) in 132 adult male and female clinically healthy Iranian native goats

Parameter	Mean	SEM	SD	Reference range	Median	Mode	Min/max
Male (n=36)							
SOD (U/gHb)	989.31	16.36	98.14	754.06−1224.56	963.50	920.00	811/1180
GPX (U/gHb)	293.08	1.19	7.12	276.01−310.15	294.00	294.00	280/305
CAT (U/gHb)	1888.10	19.96	119.78	1601.04−2175.24	1912.50	1826.00	1587/2067
MDA (μmol/L)	0.57	0.004	0.006	0.366−0.778	0.5550	0.5300	0.43/0.77
Female (n=96)							
SOD (U/gHb)	982.80	8.30	81.35	787.81−1177.79	966.00	947.00	866/1162
GPX (U/gHb)	294.81	1.31	12.81	264.09−325.52	294.00	298.00	280/398
CAT (U/gHb)	1871.40	15.25	149.43	1513.23−2229.57	1915.50	1836.00	1511/2065
MDA (μmol/L)	0.57	0.007	0.009	0.337−0.810	0.5650	0.4900	0.42/0.79
Total (n=132)							
SOD (U/gHb)	984.58	7.48	85.91	778.65−1190.50	964.50	947.00	811/1180
GPX (U/gHb)	294.34	1.00	11.54	266.67−322.00	294.00	294.00	280/398
CAT (U/gHb)	1875.90	12.33	141.71	1536.29−2215.63	1914.00	1836.00	1511/2067
MDA (μmol/L)	0.573	0.008	0.009	0.346−0.801	0.5600	0.4900	0.42/0.79

(2001) and Kizil *et al.* (2007). Comparing our results to those reported by Todorova *et al.* (2005), it could be seen 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 native goats. This outcome was consistent in both sexes. This status may be due to their different diets, in other words, generation of free radicals in carnivores is more than that of in herbivores.

The oxidative status is variable and can be changed by the influence of different factors. Even during exercise, oxidative stress is provoked and antioxidant capacity was found to decrease (Marshall *et al.,* 2002). In the present study, all Iranian native goats were adult. Normally, age influences greatly free radical generation and consequently, the level of enzyme antioxidant defense. 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 & Durad, 1991). The role of free radicals in aging is one of the main hypotheses which explains this process (Sastre *et al.,* 2002). In the present study, there were no significant differences in oxidative stress parameters (SOD, GPX, CAT and MDA) between both sexes ($P>0.05$). There is good evidence to show that sex differences in oxidative status exist in different species. In many species, females live longer than males and this is probably associated with free radicals which are in lower amount in the mitochondria of females (Sastre *et al.,* 2002). The longer lifespan in females may be due to the higher gene expression of anti-

oxidants and the lower oxidative damage of mitochondria in females (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 baseline data for comparisons between normal and abnormal states and therefore, a better understanding in diseases accompanied by oxidative stress.

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