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BASELINE VALUES OF OXIDATIVE STRESS BIOMARKERS IN CATTLE

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ABSTRACT

The goal of this study is to summarize the baseline values of both enzymatic and non enzymatic oxidative stress biomarkers in cattle. Blood samples were collected from ten (10) crossbred cattle from Instructional Bovine Farm, Ranchi Veterinary College, BAU, Ranchi that were screened on the basis that the animals were clinically healthy. Different antioxidants biomarkers were estimated using standard methods. The superoxidase dismutase, glutathione peroxidase and glucose-6-phosphate dehydrogenase activities in hemolysate of healthy animals were 391.023±25.930 U/L, 123.556±5.208 U/ mg and 11.907±1.287 U/gm Hb respectively. The malonyl dialdehyde produced was 1.904±0.132 nmol/g Hb. The serum albumin and uric acid concentration were 4.325±0.222 gm/dl and 2.927 ± 0.159 mg/dl respectively. The concentration of total serum bilirubin, direct bilirubin (conjugated) and indirect bilirubin (non-conjugated) in the healthy animals in the study were 0.467 ± 0.055, 0.329 ± 0.015 and 0.138 ± 0.023 mg/dl respectively. This study will provide a database that will be useful in clinical Biochemistry.

Keywords: Baseline values, Oxidative stress biomarkers, Cattle.

In biological entities, free radicals or oxidants are formed from the body's natural metabolic activity and as a by product of the immune system's response to foreign organisms. The body has its own defence system to protect from its ill effects by the antioxidant system. Oxidative stress (OS) occurs when the rate of production of oxidants exceeds the capacity of the individual to convert them to less reactive molecules. Oxidative stress is a secondary aggravating factor in most diseases. A biomarker for OS is a biological molecule that changes when Reactive Oxygen Species (ROS) and/or free radicals (FRs) change and can be objectively measured and evaluated . OS is an active field of research in Veterinary medicine and has been implicated in numerous disease processes[16]. OS may impair health in cows both directly and indirectly. Direct effects include peroxidative damage to important lipids and macromolecule. Indirectly, changes induced by ROS in cellular membranes and components can modify metabolic

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pathways, resulting in altered physiology and has a negative effect on animal health and productivity as well as it has been implicated as a major initiator of tissue damage[1,3]. OS is extremely dangerous as it does not exhibit any symptoms and is recognizable with great difficulty by means of laboratory methods[17]. . A limited number of conditions in ruminant medicine have been investigated in regard to the effects of oxidative stress[5] . The goal of this study is to summarize the baseline values of both enzymatic and non enzymatic oxidative stress biomarkers in cattle.

MATERIALS AND METHODS

Blood samples were collected from ten (10) adult crossbred cattle of either sex from Instructional Bovine Farm, Ranchi Veterinary College, BAU, Ranchi that were screened on the basis that the animals were clinically healthy and free of disease since last six months and were non pregnant. Blood samples were collected, taking all aseptic precautions, from the animal through jugular vein puncture. Blood samples were collected between 10 am and 12 noon in order to reduce the variation associated with diurnal rhythms. Blood was collected with anticoagulant and plasma was separated by centrifugation at 3000 rpm for 15 minutes. The samples were kept in aliquots and were stored at -20^{0}C till analysis.

Preparation of hemolysate

For preparation of hemolysate, blood with anticoagulant (ethylene diamine tetra acetic acid) were collected in graduated centrifuged tube up to the marked level. Blood samples were centrifuged at 3000 rpm for 15 minutes to remove plasma. Plasma was separated and erythrocytes were washed with chilled normal saline solution and centrifuged thrice at 3000 rpm for 5 minutes and the supernatant were discarded including the buffy layer of WBC each time. Then distilled water was added to erythrocyte pellet slowly and with constant stirring up to the marked level to prepare hemolysate.

Parameters studied

Estimation of lipid peroxidation and enzyme activities were estimated on the day of collection. Plasma was used for estimation of vitamins. The estimation of superoxide dismutase (SOD)[18], glutathione peroxidase (GSH-Px)[9], glucose -6-phosphate dehydrogenase[7], β carotene and ascorbic acid[2] ,uric acid[18], albumin[20] and bilirubin[15] in plasma and malonyl dialdehyde (MDA) [19] in whole blood was done by standard methods. All the parameters were estimated in spectrophotometer.

Statistical analysis

Statistical evaluation of the data obtained from the experiments were analysed by using standard methods [21].

RESULTS AND DISCUSSION

Enzymatic antioxidants provide the first line of defence against the indiscriminate damage that FRs and ROS cause cellular components. The first group comprises enzymatic antioxidants including superoxide dismutase (SOD) and glutathione-peroxidase (GSH-Px), and represents the main form of intracellular antioxidant defence. The superoxidase dismutase (SOD), glutathione peroxidase (GSH-Px) and glucose-6-phosphate dehydrogenase (G6PD) activities in hemolysate of healthy animals in our study were 391.023±25.930 U/L, 123.556±5.208 U/ mg and 11.907±1.287 U/gm Hb respectively (Table 1). Similar results were reported by earlier workers [1,4,11,13] regarding the different enzymatic biomarkers.

Considering a biomarker for OS, GPx belongs to a family of selenoproteins that function to oxidative defence of animal tissues by

catalysing the reduction of hydrogen and lipid peroxides and is also considered an indicator of oxidative stress[4] . GSH-Px functions in cellular oxidation-reduction reactions to protect the cell membrane from oxidative damage caused by free radicals. It catalyses the oxidation of reduced glutathione (GSH) with the help of hydrogen peroxidase to oxidised glutathione (GSSG) and water. The enzyme glucose-6-phosphate dehydrogenase (G-6-PD) is one of the enzymes of the pentose phosphate pathway. This pathway is involved in keeping an adequate amount of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) in cells. NADPH in turn maintains the levels of glutathione which protects the red cell from oxidative damage. Superoxide dismutase (SOD) catalyses the dismutation of O_2^- to H_2O_2.

The principal antioxidant vitamins for tissue defense against free-radical damage includes vitamins E, C and â-carotene. Antioxidant properties of carotenoids include scavenging singlet oxygen and peroxyl radicals, sulfur, thiyl, sulfonyl and NO_2 radicals and provide protection of lipids from superoxide and hydroxyl radical attack. Therefore, carotenoids can actively quench singlet oxygen (O_2) and prevent lipid peroxidation caused by O^-_2 and they can intercept the propagation step of lipid peroxidation. Vitamin C efficiently scavenges superoxide, hydrogen peroxide, hypochlorite, the hydroxyl and peroxyl radicals, and singlet oxygen. Vitamin C protects cells from lipid peroxidation and increases the fluidity of cell membranes, thus enhancing the immune response. In our study the concentration of â carotene and ascorbic acid in plasma were 1.075±0.134 and 11.229±0.814 mg/dl respectively . Our results were in accordance with earlier workers[1,12,17, 24].

Lipids, in particular those that are polyunsaturated, are prone to oxidation. Increased malonyl dialdehyde (MDA) concentration in plasma is a marker of lipid peroxidation. Since membrane phospholipids are the major targets of oxidative damage, lipid peroxidation is often the first parameter analysed for proving the involvement of free radical damage. Lipid peroxidation level was assessed in hemolysate by estimating the amount of malonyl dialdehyde (MDA) produced in different groups. The increase in the amount of MDA may result in the destruction of membrane integrity. The amount of MDA produced in our study was 1.904±0.132 nmol/g Hb in the healthy animals. Earlier workers[1,8,24] also reported similar results.

Albumin is the protein found in the highest concentration in blood, making up over half of the protein mass. It is manufactured by the liver from the amino acids taken through the diet. Albumin is part of the antioxidant pool and it is a free radical scavenger .Range of albumin is 2.1 – 3.6 g/dl[10] in healthy cattle and in our study the concentration was 4.325±0.222 gm/dl. Uric acid is also an antioxidant molecule. It is a powerful scavenger of peroxyl and hydroxyl radicals [23]. Some workers [24] reported a concentration of uric acid as 2.11 ± 0.21 mg/dl in normal cattle and in our study we obtained a concentration of 2.927 ± 0.159 mg/dl. Bilirubin is the yellow breakdown product of normal heme catabolism. The concentration of total serum bilirubin, direct bilirubin (conjugated) and indirect bilirubin (non-conjugated) in the healthy animals in our study were 0.467 ± 0.055, 0.329 ± 0.015 and 0.138 ± 0.023 mg/dl respectively. Range of total serum bilirubin, conjugate and unconjugate bilirubin are 0.01-0.5 ,0.04- 0.44 and 0.03 mg/dl respectively [10] in healthy cattle.

Table 1. Mean ± S.E. of baseline values of Enzymatic Biomarkers of Oxidative stress in cattle

Enzymatic Biomarkers	Concentration
SOD (U/L)	391.023±25.930
GSH-Px (U/mg Hb)	123.556±5.208
G-6-PD (U/mg Hb)	11.907±1.287

Table 2. Mean ± S.E. of baseline values of Non-Enzymatic Biomarkers of Oxidative stress in cattle

Non-Enzymatic Biomarkers	Concentration
SOD (U/L)	391.023±25.930
GSH-Px (U/mg Hb)	123.556±5.208
G-6-PD (U/mg Hb)	11.907±1.287
β Carotene (mg/dl)	1.075±0.134
Ascorbic acid (mg/dl)	11.229±0.814
MDA (g /Hb)	1.904±0.132
Serum albumin (g/dl)	4.325±0.222
Serum uric acid(mg/dl)	2.927±0.159
Total serum bilirubin (mg/dl)	0.467±0.055
Direct serum bilirubin (mg/dl)	0.329±0.015
Indirect serum bilirubin (mg/dl)	0.138±0.023

Table 3 Cropping pattern followed by households

G. Total (480)	(B) Non-Tribal (240)	(A) Tribal (240)	Area	
480 (100)	240 (100)	240 (100)	Maize	Kharif Crops
350 (72.91)	190 (79.16)	160 (66.66)	Moong	
77 (16.04)	40 (16.66)	37 (15.41)	Urd	
97 (20.26)	57 (23.75)	40 (16.55)	Arhar	
112 (23.33)	75 (31.25)	37 (15.41)	Til	
249 (51.82)	189 (78.75)	60 (25.00)	Wheat	Rabi Crops
134 (27.91)	97 (40.41)	37 (15.41)	Barley	
87 (18.12)	67 (27.91)	20 (8.33)	Gram	
79 (16.45)	45 (18.75)	34 (14.16)	Masoor	
135 (28.12)	103 (42.91)	32 (13.33)	Mustard	
13 (2.70)	13 (5.41)	-	Moong	Zaid Crops
18 (3.75)	18 (7.50)	-	Urd	
7 (1.45)	7 (2.91)	-	Guar	
138 (28.75)	76 (31.66)	62 (25.83)	Moong	Fodder Crops
21 (4.37)	14 (5.83)	7 (2.91)	Urd	
236 (49.16)	140 (58.33)	96 (40.00)	Luxc cna	
75 (15.62)	75 (31.25)	-	Berseem	
62 (12.91)	48 (20.00)	14 (5.83)	Oat	
89 (18.54)	61 (25.41)	28 (11.66)	Sugarcant	
87 (18.12)	87 (36.25)	-	jawar	

CONCLUSION

The study was undertaken to find out the baseline values of different enzymatic and non enzymatic oxidative stress biomarkers in cattle. By estimating the biomarkers of OS, we can know the oxidative status of the animals and treat them by giving antioxidants like the antioxidant vitamins and micro nutrients. The study will provide a database for clinical Biochemistry.

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