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Kavita Jaidiya
MVSc. Department of Veterinary Medicine, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Durga Bai Sodha
MVSc. Department of Veterinary Parasitology, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Chitra Jaidiya
MVSc. Department of Veterinary Public Health and Epidemiology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Manoj Kumar Dhaka
Ph.D. Department of Veterinary Medicine, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Corresponding Author:
Kavita Jaidiya
MVSc. Department of Veterinary Medicine, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Determination of oxidative stress in cattle with subclinical mastitis

Kavita Jaidiya, Durga Bai Sodha, Chitra Jaidiya and Manoj Kumar Dhaka

Abstract
Fifty cows' blood samples were taken and placed in vials coated with EDTA in order to estimate the levels of oxidative stress indicators, glutathione peroxide and malondialdehyde. In comparison to healthy cows (47.98±4.04 ng/ml), the mean blood level of reduced glutathione was lower in subclinical mastitis (30.44±1.87 ng/ml). The concentration of malondialdehyde (10.026±0.21 mmol/L) in cows with subclinical mastitis was notably greater than that of the healthy group (2.19±0.23 mmol/L).

Keywords: Sub clinical mastitis, malonaldehyde (MDA), reduced glutathione

Introduction
Mastitis is one of the most serious diseases that cows may have, and it still has a significant financial impact on the dairy industry globally. (Bachaya *et al*., 2012) [2]. Mastitis is divided into clinical and subclinical types according to the degree of inflammation and the severity of the condition. (Awale *et al.*, 2012) [1]. The milk seems normal since the subclinical mastitis is not causing any symptoms. This kind of mastitis is more than 30-40 times more common than clinical mastitis and produces large losses in the majority of dairy herds. Subclinical mastitis is a severe condition in the dairy industry across the world, and it is especially prevalent and concerning in India. The antioxidant glutathione (GSH) contains the tripeptides cysteine, glycine, and glutamic acid. These tripeptides are crucial for protecting cells from oxidative damage to proteins, lipids, and nucleic acids. (1996, Gérard and Chaudiere). Lipid peroxidation produces malondialdehyde, which is one of the most accurate and extensively used indicators of oxidative stress. (Esterbauer *et al.*, 1991) [4].

Materials and Methods
Collection of Blood
Blood samples were taken from each of the 50 cows and placed in vials coated with EDTA. from College of Veterinary and Animal Science, Bikaner for estimation of Oxidatives stress markers *viz.* Malondialdehyde (MDA) and Glutathione peroxide(GSH). After their plasma was separated, it was promptly frozen at -20˚C for examination.

Procedure
Oxidative Stress related parameters
Estimation of oxidative stress related biochemical parameters *viz*. malondialdehyde and glutathione peroxidase in plasma of cows was carried out as under.

Estimation of Malondialdehyde (MDA)
The MDA was estimated by using ELISA kits, supplied by Sino Gene Clon Biotechco. Ltd. (Table no.1)
Following materials were used for the estimation of MDA.
Microplate photometer (Wavelength: 450nm)
1. Incubator
2. Microplate washer

3. Digital single and multichannel pipette
4. Micro centrifuge tubes
5. Deionized or distilled water

Principle of the Assay

Sandwitch-ELISA detection was the basis for this kit. A microplate already coated with MDA antibody is included in this kit. which produces solid phase antibody. Add MDA to the sample or standard so that it competes with a predetermined quantity of MDA antibody on the solid phase supporter, and then incubate at 37˚C for 30 minutes. After removing any excess conjugate and unbound sample or standard from the plate, each microplate well was filled with MDA antibody and labeled HRA to create an antibody-antigen-enzyme-antibody complex, which was then incubated. Each well then receives an addition of TMB substrate solution. A stop solution is added to the enzyme-substrate reaction to end it, and the color change is detected spectrophotometric ally at 450 nm. Procedure followed was as per the instruction provided. The O.D. of the samples is then compared to the standard curve to determine the concentration of MDA in the samples. (Fig.-1).

Estimation of glutathione peroxidase (GSH-px)

Reduced Glutathion was estimated by using ELISA kits, Supplied by Sino Gene Clon Biotech co., Ltd (Table no.2)

Principle

Glutathione (GSH) is the major intracellular low-molecular-weight thiol that plays a critical role in the cellular defense against oxidative stress in mammalian cells. The kit was based on sandwitch-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with GSH-Px antibody which makes solid phase antibody Then GSH-Px in the sample or standard was added which competes with a fixed amount of GSH-Px antibody on the solid phase supporter. It was incubated at 37^0C for 30 minutes. The excess conjugate, unbound sample, and standard were removed from the plate by washing. GSH-Px antibody with labeled HRA was added in each microplate well. This formed antibody-antigen-enzyme-antibody complex and then incubated. Each well received an addition of TMB substrate solution. A stop solution was added to the enzyme-substrate reaction to end it, and the color change was detected spectrophotometric ally at 450 nm in wavelength. The steps taken were in accordance with the directions given. The samples' O.D. was then compared to the standard curve to determine the concentration of GSH-Px in the samples. (Fig.-2).

Results and Discussion

Oxidative stress related parameters (MDA and GSH)

Glutathione Peroxidase (GSH-Px)

The glutathione peroxidase (ng/ml) mean ± SE value of subclinical mastitis was 30.44 ± 1.87 (ng/ml), lower than that of healthy cows (47.98 ± 4.04 ng/ml). Table 3. According to Yang *et al*. (2011) [15], Sharma *et al*. (2016) [14], and Mir *et al*. (2017) [12], cows with mastitis had significantly lower glutathione peroxidase (GSH-Px) concentrations than healthy cows. These results were consistent with their findings.

Glutathione peroxidase (GSH-Px) plays a key role iin scavanging-butyl hydro peroxidase, an agent which includes lipid peroxidation. GSH is a significant endogenous antioxidant that cells produce and plays a direct role in scavenging reactive oxygen species and free radicals. (Scholz and others, 1989) [13]. Glutathione peroxidase actitivity has been employed by a number of previous researchers as a biomarker to measure oxidative stress in animals. Refer to Kataria *et al*. (2010) [8].

In current study, GSH concentration was found significantly lower in SCM affected cows which might be attributed that reduced glutathione is present in living cells at high concentrations. When it reacts with reactive oxygen species, it becomes oxidized and transforms into glutathione radical, which glutathione reductase may then restore into its reduced form (Kohen and Nyska, 2002) [10]. The conversion of reduced form to oxidized form of glutathione in sub clinical mastitis by excessive production of reactive oxygen species from inflammed gland.

In contrast to our study, an enhanced GSH concentration in plasma has been demonstrated in cows with mastitis by Kizil *et al*. (2007) [9] and Ibrahim *et al*. (2012) [3] with the explanation that there might be enhanced activities of GSH dependent enzymes leading to intense regeneration of reduced glutathione (GSH) from oxidized form (GSSH). This variation could be attributed to different GSH-Px activity associated with different bacterial pathogens.

The results of this study showed that the concentration of malondialdehyde in subclinical mastitis increased to 10.02 ± 0.21 (mmol/L), which was considerably higher than the concentration in healthy cows (2.19 ±0.23 (mmol/L)) (Table no 4). The increased formation of reactive oxygen species, such as hydroxyl radicals, by activated neutrophils from the clinically inflamed mammary gland may be the cause of the elevated plasma MDA levels and membrane damage

Present study findings were similar with Yang (2011) [15], Jhambh *et al.* (2013) [6] and Kachhawa (2018) [7] who found that the mean level of MDA was significantly higher in Sub clinical mastitis milk than normal animals.

Similarly the findings of Kushwaha (2016) [11] found that the mean level of MDA was significantly higher (61.14 ± 2.94 nano mol/gm) in SCM milk than in normal milk (44.06±2.55 nano mol/gm).

Table 1: Kit Contents

Content	Specifications (96T)
Micro ELISA Plate	8×12
Standard (9mmol/L)	0.5 ml
Standard diluent	1.5 ml
HRP- Conjugate reagent	6 ml
Sample diluent	6 ml
Chromogen solution A	6 ml
Chromogen solution B	6 ml
Stop solution	6 ml
Wash buffer (30X)	20ml
Adhesive strip	2 pieces
Product Description	1 Copy

Table 2: Kit content

Components	Specifications (96T)
Micro ELISA Plate	8×12
Standard (135 ng/ml)	0.5 ml
Standard diluents	1.5 ml
HRP- Conjugate reagent	6 ml
Sample diluents	6 ml
Chromogen solution A	6 ml
Chromogen solution B	6 ml
Stop solution	6 ml
Wash buffer (30X)	20ml
Adhesive strip	2 pieces
Product description	1 Copy

Table 3: Mean values GSH-Px

Cows	Glutathione peroxidase (ng/ml per) Mean ± SE
Normal	47.98± 4.04
Subclinical mastitis	30.44 ±1.87

Table 4: Mean value of Malondialdehyde (MDA)

Cows	Malondialdehyde (mmol/L) Mean ± SE
Normal	2.19± 0.23
Subclinical mastitis	10.02 ±0.21

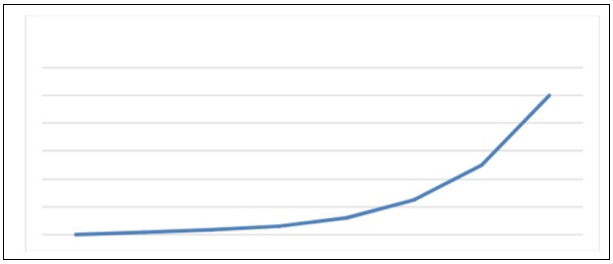

Fig 1: Malondialdehyde standard curve

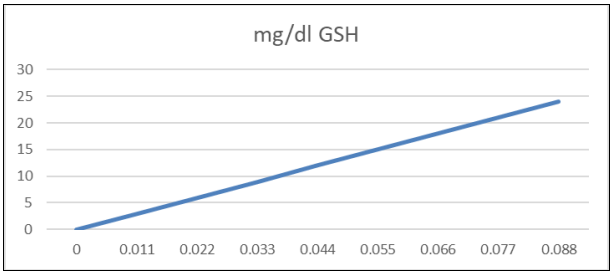

Fig 2: Reduced glutathione standard curve

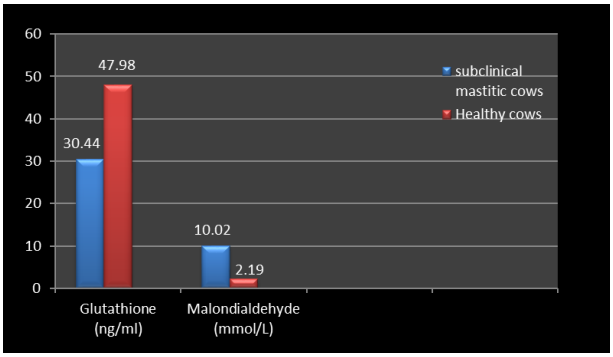

Fig 3: Mean value of MDA and GSH in Subclinical mastitis affected cows and control

Conclusion

The current study, "Studies on some aspects of sub clinical mastitis in cattle," was conducted to identify oxidative stress in animals with subclinical mastitis. The mean ± SE value of malondialdehyde in subclinical mastitis was (10.26 ± 0.21 mmol/L), which was greater than in healthy cows (2.19 ± 0.23 mmol/L). Subclinical mastitis had a lower mean ± SE value of glutathione peroxidase (GSH-Px) (30.44 ± 1.87 ng/ml) compared to healthy cows (47.98 ± 4.04 ng/ml).

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