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ORIGINAL ARTICLE

Evaluation of oxidative stress in brucella infected cows

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Oxidative stress can influence the metabolism of cells in vital organs of the body. Oxidative stress is extremely dangerous as it does not exhibit any symptom and is recognisable with great difficulty by means of laboratory methods. It can be monitored with several biomarkers like antioxidants and pro-oxidants which can be assessed in serum. The inexorableness of exposure of cows to brucella infection makes oxidative stress associated with this infection an appropriate field of investigation. There is paucity of work to detect stress, which is essential to take timely corrective measures and to save the animal population. Therefore the investigation was carried out to evaluate oxidative stress in the cows suffering from brucellosis. For this serum biomarkers of oxidative stress *viz*. vitamin C, vitamin E, catalase, monoamine oxidase, glutathione reductase, superoxide dismutase, glutathione, xanthine oxidase, oxidase and peroxidase were determined. Results indicated that vitamin C, vitamin E and glutathione activity decreased significantly in affected cows as compared to healthy cows. Serum catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities increased significantly in affected cows as compared to healthy cows. Decreased activity of vitamin C, vitamin E and glutathione indicated towards their depletion which generally occurs in the oxidative stress to scavenge the free radicals. It was concluded that oxidative stress was there in the animals. This study recommends the use of antioxidants in affected cows

key words: Biomarkers/ brucellosis/ cattle/ oxidative stress

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Oxidative stress can influence the metabolism of cells in vital organs of the body like heart, nervous tissue and liver. Disturbances in the normal redox state can cause toxic effects through the production of

peroxides and free radicals that damage vital components of the cell. Oxidative stress is a large rise in the cellular reduction potential (Schafer and Buettner, 2001). Oxidative stress can be monitored with several biomarkers (antioxidants and pro-oxidants) which can be assessed in plasma and/or erythrocytes (Passi *et al*., 2001). Superoxide dismutase (SOD), catalase, xanthine oxidase, peroxidase, monoamine oxidase, oxidase, glutathione reductase, vitamin E, vitamin C, and glutathione are considered as good serum markers of oxidative stress. Heat, cold, drought, dehydration, infection, trauma, transportation, regrouping, crowding etc. are common stressors to the animals in arid and semi-arid tracts. To combat the stress, physiological changes occur according to the priorities of the body (Kataria and Kataria, 2005).

Oxidative stress is extremely dangerous as it does not exhibit any symptom and is recognisable with great difficulty by means of laboratory methods (Nazifi *et al*., 2009). Measures of oxidative stress allow the assessment of real status of physiological defenses and prevention of the appearance of correlated pathologies (Piccione *et al*., 2007). Brucella organisms are able to cause chronic infection in a wide range of mammals including humans. Brucella pathogen can stimulate cerebral lipid peroxidation in the infection without causing significant inflammation (Melek *et al*., 2006). Oxidative stress against brucella infection have not yet been well elucidated. The inexorableness of exposure of cattle to brucella infection makes oxidative stress associated with this infection an appropriate field of investigation as present investigation tried to determine oxidative / antioxidative status in serum of affected animals.

MATERIALS AND METHODS

During a brucellosis surveillance study in an organised private dairy farm sera were collected from 250 animals. From the lot, 50 sera each from healthy and brucella infected cattle were used in the study to evaluate oxidative stress.

Serum biomarkers of oxidative stress included vitamin C, vitamin E, catalase, monoamine oxidase, glutathione reductase, superoxide dismutase, glutathione, xanthine oxidase, oxidase and peroxidase. They were determined by the methods of Varley (1988); Nair and Magar (1955); Goldblith and Proctor (1950); Green and Haughton (1961); King (1965); Winterbourn *et al.* (1975); Owens and Belcher (1965); Litwack *et al.* (1953); Snell and Snell (1954) and Snell and Snell (1954), respectively.

RESULTS AND DISCUSSION

The mean values of serum vitamin C, vitamin E, catalase, superoxide dismutase, monoamine oxidase, glutathione, glutathione reductase, xanthine oxidase, oxidase and peroxidase in healthy and brucella infected cows are presented in table1.

Results indicated that vitamin C, vitamin E and glutathione activity decreased significantly in affected cows as compared to healthy cows. Serum catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities increased significantly in affected cows as compared to healthy cows.

In the present study MAO activity in the serum increased in affected cows as compared to healthy cows which indicated towards stress to the animals.Oxidative deamination of monoamines is catalysed by monoamine oxidase. Inactivation of neurotransmitters by MAOs is an important feature and their dysfunction results in stress or depression (Meyer *et al*., 2006). Monoamine oxidase type B catalyses the breakdown of dopamine in brain and during stress activity of it increases.

Superoxide dismutase is the key antioxidant enzyme because superoxide is one of the main reactive oxygen species in the cell. Superoxide

dismutase is responsible for the quenching of superoxide radicals which are released during the chemical reactions of the various metabolic pathways. There are several ways by which free radicals are formed. Inside the body, oxygen as well as hydroxyl radicals are generated during energy productions. Once formed, these free radicals initiate their own reactions thereby exerting potentially harmful effects on the various systems of the body. In the normal course, the free radicals are converted to a less reactive form by the free radical quenching enzymes which are naturally found in the cell. This enzyme catalyzes the removal of the O_2 free radical, thus protecting the O_2 metabolizing cells against the harmful effects of the superoxide free radicals. However, the enzymatic levels of SOD are altered to a considerable extent in various diseased states exhibiting either elevation or depletion in activity (Bauer and Bauer, 1999). In the present study elevation was noticed in affected cows.

Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. Xanthine oxidase is a form of xanthine oxidoreductase that generates reactive oxygen species (Ardan *et al*., 2004). Therefore it can be used as a marker of oxidative stress. In stressed animas higher serum xanthine oxidase may indicate oxidative stress.

Glutathione is an endogenous antioxidant which protect cells from free radicals (Pompella *et al*., 2003). It also helps in maintaining exogenous antioxidants such as vitamins C and E in their reduced or active forms. Glutathione is found almost exclusively in its reduced form, since the enzyme that reverts it from its oxidized form, glutathione reductase, is constitutively active and inducible upon oxidative stress. The ratio of reduced glutathione to oxidized glutathione within cells is used as a measure of cellular toxicity (Pastore *et al*., 2003). Decreased glutathione concentration in affected animals indicated its use in an attempt to reduce oxidative stress.

Glutathione reductase reduces glutathione disulfide to the sulfhydryl form GSH, which is an important cellular antioxidant (Meister, 1988). The activity of glutathione reductase is used as an indicator for oxidative stress. Its increased activity in affecetd cows indicated oxidative stress.

Serum peroxidase activity is considered as the main indicator of the antioxidant activity (Podil'chalk *et al.*, 1996). Its activity increased in affected animals. Peroxidase catalyses the oxidation by hydrogen peroxide of a number of substrates such as ascorbate, ferrocyanide, cytochrome C etc.

The enzyme oxidase, subclass of oxidoreductases, catalyzes an oxidation/reduction reaction involving molecular oxygen as the electron acceptor. Then oxygen is reduced to water or hydrogen peroxide. Higher oxidase activity in affected cowss showed the higher rate of oxidative reactions which could lead to oxidative stress.

Catalase functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al.*, 2004). Hydrogen peroxide is a harmful by-product of many normal metabolic processes. To prevent damage, it must be quickly converted into other, less dangerous substances. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani *et al*., 1996). Higher catalase activity in affected animals indicated the higher rate of formation of hydrogen peroxide. This indicated the body's response to combat the oxidative stress.

In present study serum vitamin C level was lower in affected animals which indicated its depletion in the process to prevent oxidative stress. Vitamin C is an anti-oxidant which protects the

Table 1. Mean ± SEM values of serum biomarkers of oxidative stress in healthy cows and cows with brucellosis

S.No.	Markers of oxidative stress	Healthy cows	Cows with Brucellosis
1	Vitamin C, µmol L^{-1}	21.0± 3.0	12.0[b]± 1.8
2	Vitamin E , µmol L^{-1}	5.7 ±0.8	2.0 [b] ±0.01
3	Catalase, kU L^{-1} (MU/L)	70.9 ±10.0	109.4 [b] ±10.1
4	Superoxide dismutase, kU L^{-1}	127.0 ±12.9	218.9 [b] ±12.0
5	Monoamine oxidase, U L^{-1}	297 ± 10.0	449 [b] ± 12.7
6	Glutathione, µmol L^{-1}	3.3±0.1	2.1 [b] ±0.08
7	Glutathione reductase, kU L^{-1}	3.0± 0.1	5.1 [b] ± 0.07
8	Xanthine oxidase, mU L^{-1}	51 ±2.0	79 [b] ±6.0
9	Oxidase , U L^{-1}	59.0 ±4.1	90 [b] ±4.7
10	Peroxidase, mU L^{-1}	72 ±5.0	109 [b] ±7.9

Superscript ‘b’ on the means showed the significant ($p \leq 0.05$) difference(z- test) from the respective mean value of healthy cows.

body against oxidative stress (Padayatty *et al*., 2003). L-ascorbate is a strong reducing agent. It is converted to its oxidized form, L-dehydroascorbate which can then be reduced back to the active L-ascorbate form in the body by enzymes and glutathione. During this process semidehydroascorbic acid radical is formed. Ascorbate free radical reacts poorly with oxygen, and thus, will not create a superoxide and two semidehydroascorbate radicals will react and form one ascorbate and one dehydroascorbate. With the help of glutathione, dehydroxyascorbate is converted back to ascorbate. The presence of glutathione is crucial since it spares ascorbate and improves antioxidant capacity of blood (Gropper *et al.*, 2004).

In present study serum vitamin E level was lower in affected animals which showed its depletion in an attempt to reduce the production of reactive oxygen species. Vitamin E is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction.

Results showed that the status of markers of oxidative stress was changed in affected cows. Decrease in the levels of antioxidants indicated towards oxidative stress which can deplete the body’s antioxidant resources. It was concluded that oxidative stress was there in the animals. This study recommends the use of antioxidants in affected cows

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