Oxidant and Antioxidant Status in Pneumonic Goats with Special Reference to Bacterial Etiology

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Abstract

The current investigation was carried out to evaluate oxidative stress serum biomarkers, antioxidant concentrations and isolation of the most common etiology of bacterial pneumonia. Thirty goats of different age and sex belonging to private farms in Giza governorate were used in this study. All goats were exposed to complete and comprehensive clinical examination. The animals were divided into two equal groups apparently healthy (15), diseased (15). Blood samples were collected from both groups to determine enzymatic activates of superoxide dismutase (SOD), glutathione peroxidase (GPX) in erythrocyte haemolysate, catalase (CAT), vitamin C, vitamin E and Albumin, aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), urea and creatinine, and nasal swabs were collected for isolation of possible bacteria. The levels of CAT, SOD and GPX were significantly higher (p≤0.05), vitamins C, E had significant decrease (p≤0.05), Significant decrease (p≤0.05) in Albumin in pneumonic goats. Of 30 goats’ nasal swabs, 73.3% (22/30) yielded different bacterial isolates. Mannheimia (Pasteurella) haemolytica was the predominant isolates (81.8%; 18/22) and other isolates. It can be concluded that pneumonia served to produce oxidative stress in the goat with increased activities of antioxidant enzymes to facing excessive production of free radicals. For healthier status in small ruminants antioxidant supplementation is very important as supportive treatment in diseased cases and preventive aid in healthy cases.

Key words: Oxidative Stress, Antioxidant, Vit. C, Bacteria, Pneumonia, Goat


Introduction

Sheep and goat industry in Egypt is considered one of the main important sources for meat and milk production and consequently important for human life maintenance (Donia et al., 2014). The goat
populations in Egypt are estimated at 4.20 million head and sheep population 5.48 million head (FAO, 2014). Respiratory diseases in sheep and goat continues to be a major problem and generally resulting from exposure of animals to environmental and managerial stressors such as long period of starvation, long time of transportation, overcrowdings, bad ventilation and bad weather (Rahal et al., 2014; Assad and El Sherif, 2002) that predispose to bacterial and viral infectious agents (Emikpe et al., 2010, Emikpe et al., 2013a). Exposure to stressful condition leads to excessive production of free radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant resulted from excessive production of free radicals without removal leads to irreversible cell damage (Rahal et al., 2014 and Nazief et al., 2009). Oxidative stress can influence the metabolism of cells in vital organs of the body (Kataria et al., 2010 b). Research in the area of oxidative stress in veterinary field is still little and requires more and more scientific attention and research studies especially in small ruminant sector. For evaluation of oxidative stress in animals require estimation of certain blood biomarkers that reflect the oxidative profile of affected cases (Pilania et al., 2013). Antioxidant is the first line of defense against free radicals and have a great role in protecting against the dangerous effects of oxidants in organs as lung, kidney etc (Aytekin et al., 2015). Antioxidant system includes antioxidant enzymes as superoxide dismutase (SOD), Glutathione peroxidase (GPX), Glutathione reductase (GR) and catalase (CAT) (Singh et al., 2009) trace elements as zinc, iron, copper and selenium (Mcdowell et al., 2007) and vitamins as vitamin E and vitamin C (Donia et al., 2014).

As the oxidative stress have a great negative impact on health and production of the animals and with the fact those efforts to detect oxidative stress in small ruminant are still in the infancy. So, this study was carried out to evaluate selected serum oxidative and antioxidants biomarkers and selected biochemical parameters in pneumonic goat in Egypt.

Materials and Methods

Animals

A thirty native breed (Balady) goats of 2.3 ± 0.6 years old, 38.2±0.2 9kg and different sex belonging to different private farms in Giza governorate and the hospital of department of medicine and infectious diseases, Faculty of Veterinary Medicine, Cairo University were used in this study. This study was start in October 2015 till March 2016. The animals were divided into two equal group (15 in each), apparently healthy (control group) and diseased (pneumonic group). All animals were exposed to complete and comprehensive clinical examination including estimation of respiratory rate, pulse rate and rectal temperature. Pneumonic goats showed symptoms of respiratory distress which including rhinitis and congested mucous membranes, serous or mucoid nasal discharge, dry or moist cough, increase respiratory
and pulse rates in addition to increase rectal temperature. Auscultation on lung area revealed dry rals in early stage and moist rals in late stage.

Samples

i. Two separate blood samples of about (8-10 ml) were collected from apparently healthy and pneumonic goats. One sample was taken in a plan test tube to separate non-hemolyzed sera that stored in eppendorf at -20 c until analysis of selected biochemical parameters.

ii. Commercial kits were used for spectrophotometric determination serum concentration of albumin level (Spinreact company, Spain), creatinine (Bio-Diagnostic, Giza, Egypt), BUN (Biosystems company, Spain), AST, ALT (Specterum company, Egypt), vitamin C (Bio-Diagnostic, Giza, Egypt) vitamin E using a specific spectrophotometer (Apple 302, USA).

iii. The other sample was taken into a clean dry labeled heparinized test tube. Heparinized blood was centrifuged at 3000rpm/5 min and pipette off plasma without disturbing the white buffy layer. Plasma was kept and stored at -20c till analysis.

iv. Thirty nasal swabs were collected aseptically from both apparently healthy as well as diseased pneumonic goats for bacteriological examination.

Determination of Catalase (CAT)
Catalase was determined by colorimetric method by commercial kits provided by (Bio-Diagnostic, Giza, Egypt) according to Aebi (1984) and the instruction of the enclosed pamphlet.

Determination of Superoxide Dismutase (SOD)
Superoxide dismutase was determined by colorimetric method by commercial kits provided by (Bio-Diagnostic, Giza, Egypt) according to Nishikimi et al., (1972) and the instruction of the enclosed pamphlet.

Determination of Glutathione Peroxidase (GPX)
Glutathione peroxidase was determined by colorimetric method by commercial kits provided by (Bio-Diagnostic, Giza, Egypt) according to Paglia and Valentine (1976) and the instruction of the enclosed pamphlet.

Bacteriological Methods
Isolation of Bacteria
Nasal swab samples were inoculated separately onto the nutrient agar and blood agar, eosin methylene blue (EMB) agar and MacConkey agar media and incubated at 37 °C for 24 hours. The colonies on primary cultures were repeatedly sub-cultured by steak plate method until the pure cultures with
homogenous colonies were obtained (Cheesbrough, 1985). Characterization of bacteria: In order to identify and differentiate bacterial pathogens isolated from pneumonic goats different characteristics of bacteria such as- cultural characteristics, staining reaction, shape, size and arrangement and biochemical characteristics were studied.

**Statistical Analysis**

The statistical significance between means was compared using Student’s t-test; p≤0.05 was considered significant. All data are expressed as means±standard error of means (SEM). All tests were performed using computer package of statistical analysis system (SPSS, version 16) (SAS, 2002).

**Results and Discussion**

Oxidative stress is a growing field of research in small ruminant medicine and has a great role in various diseases processes. The understanding of the role of oxidant and antioxidants in physiological and pathological conations (Donia et al., 2014) is very important. Respiratory rate, pulse rate and rectal temperature are significantly higher (p≤.005) in pneumonic goats in compared with healthy one the same findings were recorded by (Radostitis et al., 2007) in pneumonic goats. The activity of CAT, SOD and GPx enzymes were significantly higher (p≤0.05) in pneumonic goats in compared with healthy one, the same findings were recorded by (Kataria et al., 2012 a) in goats infected with PPR.

**Table 1: Physical Parameters in Healthy and Pneumonic Goats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Goats (N=15)</th>
<th>Pneumonic Goats (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory rate (/min)</td>
<td>27.5 ± .58</td>
<td>28.3 ± 1.54*</td>
</tr>
<tr>
<td>Pulse rate (/min)</td>
<td>81 ±1.26</td>
<td>84 ±1.5*</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>39.3 ± 0.03</td>
<td>39.4 ± 0.15*</td>
</tr>
</tbody>
</table>

*p≤0.05, **p≤0.01, ***p≤ 0.001. The results were expressed as mean ± standard error of mean (M ±SEM).

Catalase enzyme its function to catalyze the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al., 2004). In this study higher serum activity of CAT could be due to higher rate of formation of hydrogen peroxide (Kataria et al., 2010 b) that indicating higher oxidative stress. On the other hand (Donia et al., 2014) were recorded lower serum activity of CAT in pneumonic sheep. Super oxide dismutase enzyme activity was higher in pneumonic goats in this study and the same result was recorded by (Pilania et al., 2013), while (Donia et al., 2014) were recorded lower serum activity of SOD in pneumonic sheep. SOD is responsible for scavenge of super oxide radicals which are results during various metabolic pathways. It catalyzes the destruction of super oxide into oxygen and hydrogen peroxide (Kataria et al., 2012 c). Also researches (Rana et al., 2013) have used SOD as one of oxidative stress biomarkers. Glutathione peroxidase serum activity in this study was higher in pneumonic goats,
while (Donia et al., 2014) were recorded lower serum activity of GPx in pneumonic sheep. Increased activities of serum enzymes of oxidative stress (CAT, SOD and GPx) signified the presence of oxidative stress in order to combat excessive production of free radicals (Pilania et al., 2013; Kataria et al., 2012 c; Nisbet et al., 2007). It can be said that pneumonia in goats results in excessive production of free radicals and so in balance between oxidant and antioxidant system in body (Pilania et al., 2013; Maan and Kataria et al., 2012).

Table 2: Activities of Serum Enzymes of Oxidative Stress in Healthy and Pneumonic Goats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Goats(N=15)</th>
<th>Pneumonic Goats (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (CAT) (U/L)</td>
<td>711.63± 0.73</td>
<td>713.61 ± 0.73</td>
</tr>
<tr>
<td>Superoxidedismutase (SOD) (U/L)</td>
<td>227.54± 1.09</td>
<td>230.32± 0.95</td>
</tr>
<tr>
<td>Glutathione peroxidase (GPx)(U/L)</td>
<td>149.85 ± 0.77</td>
<td>152.44 ± 0.94</td>
</tr>
</tbody>
</table>

* p≤0.05, ** p≤0.01, ***p≤ 0.001. The results were expressed as mean ± standard error of mean (M ±SE).

Table 3: Serum Antioxidants Concentrations in Healthy and Pneumonic Goats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Goats(n=15)</th>
<th>Pneumonic Goats (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>0.36 ± 0.004</td>
<td>0.34±0.004</td>
</tr>
<tr>
<td>Vitamin E (µg/dl)</td>
<td>0.15 ± 0.003</td>
<td>0.14 ± 0.004</td>
</tr>
</tbody>
</table>

* p≤0.05, ** p≤0.01, ***p≤ 0.001. The results were expressed as mean ± standard error of mean (M ±SE).

The results of this study showed significant decrease (p≤0.05) in the concentrations of vitamin C and vitamin E in pneumonic goats in compared with healthy one and the same findings were recorded by (Kataria et al., 2012a) in goats and (Kataria et al., 2012 b) in sheep. Vitamins C and E are considered non enzymatic antioxidants (Bose et al., 2012). Low levels of serum vitamins C and E indicate their depletion in the trial to combat free radicals (Kataria et al., 2010 a). Vitamin C works as an antioxidant because ascorbate free radical reacts poorly with oxygen and hence super oxide is not created. Instead two semi-dehydro ascorbate radicals will react and form one ascorbate and one dehydroxy ascorbate with help of glutathione, dehydroxyascorbate is converted back to ascorbate (Kataria et al.,2010 c). Vitamin E stops the production of reactive oxygen species during oxidation of fat and protects cell membrane from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reactions. The oxidized alpha tocopheroxyl radical are recycled back to the active reduced form through reduction by ascorbate and retinol. Regarding to the results obtained in this study the serum concentrations of albumin; ALT, AST, creatinine and BUN were significantly decreased (p≤0.05) in pneumonic goats in compared with healthy one. The same findings were recorded by (Donia et al., 2014) in sheep suffering from respiratory affections. Mean value of albumin in pneumonic goat was decreased the same finding was reported by (Krupakaran et al., 2013 and Alfartosi et al., 2010) in cattle and sheep. This decrease could be due to
albumin is considered the major extracellular source of thiols which are scavengers of free radicals allowing albumin to function as an antioxidant (Halliwell, 1998).

Mean values of ALT and ALT concentrations in pneumonic goats were significantly higher in compared to healthy goats. The same findings were reported by (Deger et al., 2008) in sheep. This increase could be due to changes in the antioxidants abilities of the liver and in the phospholipids structure of the cell membrane were accompanied by increase in ALT and AST as markers of liver damage. Mean values of creatinine and BUN concentrations in pneumonic goats were significantly higher in compared to healthy goats. This could be due to kidney damage as a result of free radicals produced from inflammatory process and inability of antioxidants to combat it.

**Table 4:** Selected Serum Biochemical Parameters in Healthy and Pneumonic Goats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Goats (N=15)</th>
<th>Pneumonic Goats (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (gm/dl)</td>
<td>3.67 ± 0.08</td>
<td>3.43 ± 0.10 *</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>15.33 ±0.35</td>
<td>15.96 ± 0.23 *</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>46.57 ± 0.64</td>
<td>47.92 ± 0.63 *</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.74 ± 0.01</td>
<td>0.76 ± 0.01 *</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>24.84 ± 0.19</td>
<td>24.90 ± 0.19 *</td>
</tr>
</tbody>
</table>

* p≤0.05, ** p≤0.01, ***p≤ 0.001. The results were expressed as mean ± standard error of mean (M ±SE).

Regarding the bacteriological examination of the nasal swabs, our results revealed that the percentage of nasal swabs that obtained bacterial isolates i.e., bacteriologically positive samples were 73.3% (22/30). *M. haemolytica* was represented the vast majority of the isolates (81.8%), followed by *P. multocida* (18.1%), *K. pneumoniae* (9%) and *P. aeruginosa & E. coli* were represented (4.5%). The prevalence rate of bacterial isolates from apparently healthy animals were 46.6% that means only 7 cases were bacteriologically positive out of all apparently healthy goats (7/15) as well as the prevalence rate of bacterial isolates from diseased animals were 100% which also means that all diseased cases were bacteriologically positive (15/15). Also, the prevalence of apparently healthy animals that were bacteriologically positive was 31.8% (7/22), while the prevalence of diseased cases that were bacteriologically positive was 68.2% (15/22). Single and mixed bacterial infection were recorded as only 4 cases were had mixed bacterial infection with two bacteria and those were from the diseased cases, while all the positive samples from the apparently healthy cases (7 samples) and the rest diseased cases (11 diseases cases) were have had single bacterial infection. From our results, Mannheimiosis is the most common bacterial respiratory disease in goats and this agreed with (Hussein and Elsawi, 1984). This finding was most likely due to abrupt environmental changes and other stressful conditions like transportation, over crowdness and different infections. Thus the interaction of stress factors, defense mechanism and *M. haemolytica* which naturally habitats the respiratory tract of goats would predispose
the disease (Brogden et al., 1998). So, improvement of environmental conditions and management systems will help a reduction of respiratory diseases especially mannheimiosis.

Table 5: Prevalence Rate of Bacterial Isolates from Apparently Healthy and Pneumonic Goats

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Nasal Swabs</th>
<th></th>
<th></th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannheimia haemolytica</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsella pneumoae</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeroginosa</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

From this study oxidative stress is a phenomenon occurs due to increase in reactive oxygen species or deficiency in antioxidant defense mechanisms or system either enzymatic or non-enzymatic. This imbalance leads to damage in lipid, protein and DNA containing macromolecules of the cells of the body. Based upon the decreased antioxidant levels and increase enzyme activities, it is concluded that pneumonia in goat considered as strong oxidative stress.

Recommendations

Based upon the obtained results periodical assessment of oxidative stress in small ruminant is important, with supplement of proper antioxidants as supportive treatment in pneumonia and in healthy contact animals, mannheimiosis is the most common bacterial cause of pneumonia in goat.

References


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