Biochemical and Circulating Oxidative Stress Biomarkers in Egyptian Buffaloes (Bubalus bubalis) Infested by Sarcoptic Mange

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Abstract: Oxidative stress is an imbalance between radical-generating and radical scavenging activity, resulting in oxidation products and tissue damage. This study was aimed to evaluate the biochemical and circulating oxidative stress biomarkers in blood of Egyptian buffaloes naturally infested by Sarcoptes scabiei var. bubalis. Thirty male buffaloes (Bubalus bubalis) were divided according to the extent of the infested area with S. scabiei into four groups, group (A) represents healthy control (n=15), group B represents low infestation (n=10), Group C represents moderate infestation (n=5), group D represents sever infestation (n=5). The parasitological examination of skin scraping of infested buffaloes reveals the presence of S. scabiei var. bubalis. The mean values of the hematological parameters in comparison with control were significant increase in WBCs and significant decrease in RBCs, Hb and PCV in groups C and D, but there were no significant change in these values in group B. In groups B, C and D, there were significant decrease in values of total protein, albumin, urea and creatinin, while there were significant increase in values of AST, ALT, GGT and LDH. In regard to oxidative stress, there was a significant increase in malonyldialdehyde and significant decrease in reduced glutathione, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, catalase and serum ascorbate in groups B, C and D with the most significant decrease was recorded in group D.

Key words: Sarcoptes scabiei • Antioxidant enzymes • Hematology • Biochemistry

INTRODUCTION

Sarcoptic mange is an important parasitic disease in buffaloes, caused by Sarcoptes scabiei var. bubalis. A high prevalence of the disease among water buffaloes has been reported from various parts of Egyptian subcontinent [1]. It causes heavy economic losses to dairy farmers because of high morbidity and mortality rates, especially in buffalo calves below one year of age [2]. The disease is manifested as severe skin lesions distributed all over the body in form of alopecia, thickened skin, dry, exudative crusts and hemorrhagic and non hemorrhagic fissures on the upper neck, skin of the face, upper eyelids, poll and ear [3]. Animals in poor condition appear to be most susceptible for the disease. Several other factors like stress, overcrowding, poor nutrition, cold weather and immunosupression predisposes the animal for the disease [4, 1]. Sarcoptes scabiei infestation is often accompanied by hypersensitivity reactions. Proliferation of mast cells and resultant increase in chymase and tryptase activity is supposed to play an important role in development of skin lesions [5]. Affected animals show severe pruritis and reduction in feed intake. Besides other pathological changes, decrease in feed digestibility, nutrient absorption and alteration in hepatic structure and function [6, 7]. Excess production of free radicals beyond the endogenous counteracting mechanism has been reported in various infections and inflammatory conditions [8]. Proinflammatory cytokine production appears to be the effector key in the pathogenesis of scabies [9]. Extract of S. scabiei have been shown to stimulate interleukin-1α (IL-1α) and IL-1β, tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ) secretion from keratinocytes, spleen, lymph node and
peripheral blood mononuclear cells [10]. These cytokines can also be generated from the inflammation of the skin itself caused by physical stimulation of the burrowing mites [11]. Triggering of this proinflammatory cascade can lead to excessive generation of the reactive oxidants, free radicals which include reactive oxygen species (ROS) such as hydroperoxide radical (OH), superoxide anion radical (O2) and reactive nitrogen species (RNS) such as nitric oxide radical (NO) in the biological system [12].

The free radicals play a key role in host defense against the invading parasite [13], but when generated at high levels they can result in metabolic dysfunction and biomolecular oxidative damage, which contribute to pathological changes in the tissues [14, 15]. Lipids and proteins are targets of various ROS and RNS and oxidized to give a diverse array of toxic products [16]. Lipids especially polyunsaturated fatty acids are sensitive to oxidation; leading to the term lipid peroxidation (LPO), of which malondialdehyde (MDA) is the most abundant [17]. The side chains of amino acid residues of proteins are also susceptible to oxidation by the action of free radicals, forming protein carbonyls [15]. Carbonylated proteins tend to form high-molecular-weight aggregates that are resistant to degradation and accumulate as damaged proteins [18]. In skin diseases, the body possesses an array of a potent antioxidant protection such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), GSH-peroxidase and the antioxidant vitamins A, E and C [12]. Synergistic and co-operative interactions of these antioxidants rely on the sequential degradation of peroxides and free radicals [11]. This study was aimed to evaluate hematological, biochemical and circulating oxidative stress biomarkers in Egyptian buffaloes naturally infested by S. scabiei var bubalis.

MATERIALS AND METHODS

Animals: Thirty male buffaloes (Bubalus bubalis) aged 18-30 months were included in this study. These animals were selected from buffaloes reared in different areas of Menofia governorate, Egypt. All the selected animals have been reared similarly under unorganized farming with unsatisfactory standards of animal management and feeding.

All animals were subjected to careful clinical and laboratory investigations. Accordingly, the selected animals were divided into four groups. The first group showed 3–5 skin lesions, each with an approximate area of 5–10 cm² (n=10, mild infested group, group B). The second contained more scabies lesions, which approximately cover less than 25% of the skin (n= 5, moderate infested group, group C). The third group in which the scabies lesions cover more than 25% of the skin were considered severely infested group (n=5, severe infested group D). The remainder animals (n=10) were clinically healthy and served (as a control group, group A).

The signs in positive cases of S. scabiei var bubalis infestation were observed and recorded. No abnormal clinical signs or mites were observed in the control group and they had good body condition. All the selected animals were free from other ectoparasites as well as blood and gastrointestinal parasites. Scabby lesions were free from pathogenic bacteria or fungi infections. Infested animals were free from other systemic diseases.

Samples: From each buffalo two blood samples were collected by the jugular vein puncture, one in a tube containing EDTA and the second in a tube without anticoagulant for subsequent serum collection. Blood samples collected in EDTA were used for parasitological examination, hematological investigations and preparation of erythrocyte hemolysate. Blood without anticoagulant was centrifuged at 1,000×g for 10 min. Serum was collected and stored at -20°C until processing.

Parasitological Examination: On the edge of active lesions, the skin was painted with glycerin and deeply scraped with a sterile scalpel until blood oozes. Scraps were collected on 10% potassium hydroxide solution and examined under a microscope after gentle heating. The parasite was identified according to its morphological characteristics [19]. Animals with scabby skin and presence of S. scabiei var bubalis on microscopic examination were accepted as clinically affected with sarcoptic mange.

Hematological Parameters: The evaluated hematological parameters included estimation of red blood cell count (RBCs), white blood cells (WBC), hemoglobin concentration (Hb) and packed cell volume (PCV). These parameters were done according to the routine hematological procedures.

Biochemical and Oxidative Stress Biomarkers: Serum samples were evaluated for the concentrations of total protein (TP), albumin (Alb), Total globulin (determined by subtracting albumin from serum total protein), blood urea nitrogen (BUN), creatinine and serum enzymatic activities of alanin aminotransferase (ALT) aspartate
aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and lactic dehydrogenas (LDH). All these parameters were determined by spectrophotometric method using commercially available test kits supplied by Biomed diagnostics (Germany) and following the manufacturer's instructions.

Oxidative and antioxidative status was evaluated by measuring malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), superoxide dismutase (SOD), Serum ascorbate and catalase (CAT). All these parameters were determined by spectrophotometric method using commercial kits of biodiagnostics (Egypt).

**Statistical Analysis:** The data were presented as mean± standard error (SE) and were subjected to statistical analysis using one-way analysis of variance (ANOVA) [20] followed by a multiple comparison Duncan test. Differences at $P < 0.05$ were considered significant.

**RESULTS**

**Clinical Findings:** Scabby lesions appeared as erythematous skin with irregular alopecic areas and scab formation. In group B which represented by low infested buffaloes, skin lesions were confined to patchy areas in the wither, neck, flank, thighs, abdomen and inguinal region. In group C which represented by moderate infestation was characterized by wide alopecic areas covering less than 25% of the body especially in the wither, abdomen and extremities. The skin was roughened, corrugated, covered with grayish chalky scabs and cracked into deep fissures oozing sero-hemorrhagic exudates. In group D which represented by severely affected group, lesions covered more than 25% of the skin and less than 50% of the skin surface was affected. Moderate and severely affected cases showed varying degrees of anemia (pale mucus membranes), poor body condition (weight loss, small wither and projection of the ribs), leg edema, loss of appetite and debilitation.

**Parasitological Findings:** Mites were present in the skin scrapings of infested buffaloes, all of which were identified morphologically as *S. scabiei var bubalis*.

**Hematological Findings:** The mean values of the hematological parameters in control and infested buffaloes are presented in Table 1. In comparison with control group (group A) there were significant increase in WBCs and significant decrease in RBCs, Hb and PCV in groups C and D, but there were no significant change in these values in group B. These results indicate that anemia was developed in groups C and D, which was more severe in the latter group.

**Biochemical Parameters:** The mean values of the biochemical biomarkers in control and infested buffaloes are presented in Table 2. In comparison with control group there were significant decrease in values of total protein, albumin, urea and creatinin in groups B, C and D with the most significant decrease was recorded in group D. The mean values of AST, ALT, GGT and LDH were significantly increased in groups B, C and D with the most significant increase was recorded in group D. There were no changes in globulin and CK in different groups.

**Oxidative Stress Biomarkers:** In Table 3, there was a significant increase in malonyldialdehyde in groups B, C and D with the most significant decrease was recorded in group D. In the other hand there were significant decrease in reduced glutathione, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, catalase and serum ascorbate in groups B, C and D with the most significant decrease was recorded in group D.

**DISCUSSION**

Sarcoptic mange in buffaloes is responsible for high morbidity and mortality especially in calves below one year of age [1]. The condition is usually associated with inanition, anemia, decrease in plasma protein concentration and alteration in serum biochemical indices suggesting compromised hepatic functions [21, 7].

As shown above in Table 1, there were significant changes in different hematological parameters in buffaloes infested by Sarcoptic mange. There were significant leukocytosis and anemia manifested by significant decrease in PCV, Hb and RBCS in groups C and D but they did not change in group B when compared with control group. Leukocytosis may be attributed to the presence of infestation by mange. These results indicate that anemia was developed in group C and D, which was more severe in the latter group. Several authors reported that anemia is a prominent feature in mangy camels [22, 23]. Early reports suggested that anemia in mangy animals is a result of feeding behavior of mites on blood or suppression of erythropoiesis due to the effect of toxic substances secreted by mites [24].
Table 1: Hematological parameters in control and different groups of Egyptian buffaloes manifested by sarcoptic mange. Values are means ± SE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A Control</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs (x 10^6/µl)</td>
<td>11.45±0.15</td>
<td>11.95±0.15</td>
<td>12.45±0.15</td>
<td>13.65±0.26</td>
</tr>
<tr>
<td>RBCs (x 10^6/µl)</td>
<td>8.45±0.15</td>
<td>8.05±0.17</td>
<td>7.33±0.21</td>
<td>6.21±0.19</td>
</tr>
<tr>
<td>Hb (g %)</td>
<td>12.45±0.034</td>
<td>11.22±0.034</td>
<td>10.31±0.12</td>
<td>9.01±0.22</td>
</tr>
<tr>
<td>PCV(%)</td>
<td>35.23±0.15</td>
<td>33.0±0.24</td>
<td>31.4±0.16</td>
<td>28.5±0.034</td>
</tr>
</tbody>
</table>

WBCs: white blood cells RBCs: red blood cells Hb: hemoglobin PCV: packed cell volume

Means within the same row having the different letters are significantly different at (P<0.05).

Table 2: Biochemical biomarkers in control and different groups of Egyptian buffaloes manifested by sarcoptic mange. Values are means ± SE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A Control</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>8.38±0.47</td>
<td>8.12±0.21</td>
<td>7.38±0.33</td>
<td>6.98±0.41</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.31±0.31</td>
<td>4.11±0.2</td>
<td>3.31±0.01</td>
<td>3.01±0.03</td>
</tr>
<tr>
<td>globulin (g/dl)</td>
<td>4.07±0.31</td>
<td>4.01±0.11</td>
<td>3.97±0.31</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>96.20±0.48</td>
<td>110.20±0.15</td>
<td>144.20±0.50</td>
<td>155.30±0.15</td>
</tr>
<tr>
<td>ALT</td>
<td>19.80±0.53</td>
<td>39.60±0.81</td>
<td>45.70±0.68</td>
<td>67.80±0.53</td>
</tr>
<tr>
<td>GGT</td>
<td>46.80±0.40</td>
<td>55.90±0.63</td>
<td>72.50±0.03</td>
<td>85.90±0.63</td>
</tr>
<tr>
<td>AP</td>
<td>90.40±0.92</td>
<td>89.50±0.29</td>
<td>88.00±0.52</td>
<td>89.50±0.29</td>
</tr>
<tr>
<td>LDH</td>
<td>440.10±0.07</td>
<td>422.10±0.08</td>
<td>488.10±0.71</td>
<td>494.10±0.04</td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td>24.93±0.09</td>
<td>22.93±0.22</td>
<td>19.03±0.01</td>
<td>18.01±0.71</td>
</tr>
<tr>
<td>Creatinin (µmol/L)</td>
<td>84.02±0.28</td>
<td>80.19±0.11</td>
<td>72.20±0.71</td>
<td>70.19±0.11</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase ALT: alanine aminotransferase AP: alkaline phosphatase GGT: gamma glutamyl transferase LDH: lactic dehydrogenase

Means within the same row having the different letters are significantly different at (P<0.05).

Table 3: Circulating oxidative stress biomarkers in control and different groups of Egyptian buffaloes manifested by sarcoptic mange. Values are means ± SE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A Control</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmolles/g Hb))</td>
<td>145.5±13.3</td>
<td>161.8±16.6</td>
<td>202.6±20.0</td>
<td>217.7±18.9</td>
</tr>
<tr>
<td>GSH (µmol/mg Hb)</td>
<td>36.46±2.62</td>
<td>30.16±2.62</td>
<td>26.46±2.62</td>
<td>20.46±0.12</td>
</tr>
<tr>
<td>GSH-Px (mU/mg Hb)</td>
<td>893.45±11.65</td>
<td>793.45±10.65</td>
<td>677.45±15.65</td>
<td>590.45±10.65</td>
</tr>
<tr>
<td>GST (mU/mg Hb)</td>
<td>1.22±0.04</td>
<td>0.9±0.01</td>
<td>0.7±0.04</td>
<td>0.4±0.03</td>
</tr>
<tr>
<td>SOD (U/mg Hb)</td>
<td>6.14±0.39</td>
<td>5.53±0.22</td>
<td>4.49±0.31</td>
<td>3.33±0.03</td>
</tr>
<tr>
<td>CAT (U/mg Hb)</td>
<td>22.4±0.73</td>
<td>20.0±0.55</td>
<td>16.3±0.61</td>
<td>12.2±0.81</td>
</tr>
<tr>
<td>Serum ascorbate (µmol/l)</td>
<td>23.6±0.09</td>
<td>21.4±0.32</td>
<td>17.6±1.73</td>
<td>13.3±1.04</td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde GSH: Reduced glutathione GSH-Px: Glutathione peroxidase GST: Glutathione-S-transferase SOD: Superoxide dismutase CAT: Catalase

Means within the same row having the different letters are significantly different at (P<0.05).

It was clear from table 2 that buffaloes infested by sarcoptic mange showed significant changes in biochemical biomarkers when compared with control group. There were significant decrease of total protein and albumin and this may be attributed to free radicals can cause protein oxidation, lipid peroxidation and DNA damage [25]. Also there were significant decrease of urea and creatinin in all buffaloes infested by sarcoptic mange. The decrease of urea should be linked with loss of appetite and with CK results that indicate reduced muscle activity. On the other hand Various pathological changes were observed in liver, spleen and kidney of wombats, sheep and buffaloes suffering from sarcoptic mange [26, 6, 7, 27] and this may be attributed to the increased toxic product of oxidative process in the circulation suggests that the oxidative stress in mangy camels may be a systematic syndrome and other tissues rather than the skin may be affected. Liver enzymes include AST, ALT, GGT and LDH were significantly increased in all buffaloes infested by mange and this may be due to sarcoptic mange in buffaloes is responsible for compromised very change in hepatic functions [21, 7].

In the present study, table 3 revealed that buffaloes infested by sarcoptic mange were in a state of significant oxidative stress and an altered antioxidant defense mechanism. Remarkably decreased levels of GSH and reduced activities of antioxidant enzymes for instance GSH-Px, GST, CAT, SOD and ascorbat [28]. This may be consequences of overproduction of free radicals by the inflammatory cells recruited to combat the parasites and consequently, exhaustion of infested buffaloe’s antioxidant system. Numerous studies demonstrated that a variety of inflammatory cells are activated which induce or activates various oxidant-generating enzymes to kill intra-cellular and extra-cellular parasites [25].
Elevated levels of MDA in Sarcoptes-infested buffaloes in present study signify the potential role of lipid peroxidation in pathogenesis of Sarcoptes-infestation in buffaloes. Increased levels of lipid peroxides may be implicated in the pathology of skin lesions induced by Sarcoptes mites. Lipid peroxidation is a well established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. Lipid hydroperoxides (LOOH) are by-products of lipid peroxidation and increased levels of lipid peroxidation products are associated with a variety of diseases including parasitic infestations [29, 30, 31]. Reactive oxygen species (ROS) are capable of initiating and promoting the over production of LPO as a result of oxidative damage [32, 28]. Lipid peroxidation (LPO) is known to cause cellular injury by inactivation of membrane enzymes and receptors, depolymerization of polysaccharide, as well as protein cross linking and fragmentation [33].

From the data of the present study it could be concluded that sarcoptosis in Egyptian buffaloes is accompanied by anemia, leucocytosis and a state of oxidative stress process, which increased by increasing the area of infestation and may contribute to the pathogenesis of the disease.

**REFERENCES**


