EFFECT OF MASTITIS ON MAMMARY GLAND BIOCHEMICAL AND OXIDATIVE STRESS PARAMETERS IN EXPERIMENTALLY INDUCED LACTATING MICE

Jayaraj F. Chinchali and Basappa B. Kaliwal*
Department of Studies and Research in Biotechnology and Microbiology, Karnatak University, Dharwad, INDIA.

ARTICLE INFO

Article history
Received 15/03/2014
Available online
05/04/2014

Keywords
Mastitis,
Reactive Oxygen Species (ROS),
Biochemical Contents,
Oxidative Stress Parameters and Polymorphonuclear (PMN) Neutrophil Leukocytes.

ABSTRACT

The aim of the present study is to investigate the effect of experimentally induced bacterial mastitis on mammary gland biochemical and oxidative stress parameters in lactating mice. 25 Lactating mice were divided into five groups, PBS vehicle served as control and four induced groups of 5 mice each i.e., Lipopolysaccharide (LPS) an endotoxin, Staphylococcus aureus, Escherichia coli and Bacillus subtilis were used to induce mastitis by intramammary inoculation into the mammary gland of mice. Clinical observations, rectal temperature (ºC) of mice and body weight (g) were recorded for every 12 h of post infection during the study period. After 48 h of infection blood was collected through cardiac puncture for analysis of oxidative stress parameters, mice were euthanized and mammary glands were dissected out for the evaluation of biochemical contents such as DNA, RNA, protein, glycogen, cholesterol, lactate dehydrogenase (LDH), succinic dehydrogenase (SDH), acid phosphatase (ACP), alkaline phosphatase (AKP) activities and oxidative stress parameters such as, levels of glutathione (GSH), thiobarbaturic acid reactive substances (TBARS); superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities. Histopathological responses of the tissue damage were studied. The results exhibited contrasting indications depicting the effects of experimentally induced bacterial mastitis on mammary gland biochemical and oxidative stress parameters in lactating mice. There was a significant decrease in biochemical contents such as DNA, RNA, protein, glycogen levels with increased cholesterol level in the induced groups compared to control group. LDH and AKP activities were significantly increased with decreased SDH and ACP activities compared to control group. Equally oxidative stress parameters such as level of GSH and SOD, CAT and GST activities were decreased with increase in the level of TBARS in induced groups compared to control group of both mammary gland tissue and blood serum. Histopathological evidences revealed massive infiltration of polymorphonuclear neutrophil leukocytes (PMN), damage of alveoli and secretory products in the induced groups compared to control group of the mammary gland tissue.

Copyright © 2014 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

www.iajpr.com
INTRODUCTION

Mastitis in dairy cattle is the most economically important disease affecting the dairy industry [1]. One of the most striking features of acute mastitis is the massive transmigration of leukocytes from the circulating pool into the mammary gland [2, 3]. In veterinary medicine, mastitis is referred to as an intramammary inflammatory reaction caused by an infectious agent. Understandably, biochemical and structural differentiation of the cells of the mammary gland tissue of mice undergo alterations from pre-gestation, parturition, lactation and weaning. The secretory tissue of mammary gland contains lobes and each lobe consists of several lobules, each lobule contains clusters of alveoli, sac-like structures where milk is synthesized and secreted to the ductular system [4]. The endocrine system plays a major role in the mammary gland development (mammogenesis), in the onset of lactation (lactogenesis) and maintenance of milk secretion (galactopoiesis) [5].

Inflammation caused by intramammary infection of bacterial pathogens leading to mammary gland tissue damage is due to the response of the immune system. During inflammatory conditions neutrophils and macrophages are the cell types found in mammary gland tissue, which migrate from the blood to mammary gland as defense mechanism for the invading bacteria. Bacterial toxins released at the site of infection destroy the host cells leading to oxidative stress causing uncontrolled inflammatory responses and tissue injury. Phagocytosis by polymorphonuclear (PMN) leukocytes produces reactive oxygen species (ROS) that are needed for killing bacteria [6]. Polymorphonuclear (PMN) leukocytes contain highly cytotoxic granules and via enzymatic degradation and the production of reactive oxygen species (ROS) have been shown to induce tissue damage [7]. Directly, ROS can oxidize macromolecules such as lipids, proteins and DNA and cause oxidative cell injury. Indirectly, ROS can damage cellular components and membranes and thus modify metabolic pathways [8]. Excessive production of free radicals and ROS, and/or a decrease in body antioxidant defense, lead to damage of biological macromolecules and disruption of normal metabolism and physiology [9]. Oxidants are known to be cytotoxic through many different mechanisms such as protein and amino acid oxidation, lipid peroxidation, and DNA damage. Oxidative stress is a condition that occurs when the production of ROS exceeds the capacity of antioxidant defenses to neutralize these pro-oxidants, resulting in oxidative damage to lipids, DNA, proteins, and other macromolecules [10].

The purpose of the present investigation was to know the experimental bacterial mastitis induced pathogenesis by Lipopolysaccharide (LPS), *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* on mammary gland of mice. The evaluation of biochemical contents such as DNA, RNA, Protein, Cholesterol, Glycogen levels, activities of dehydrogenases such as LDH and SDH, phosphatases such as AKP and ACP and oxidative stress parameters such as GSH and TBARS levels with SOD, CAT and GST activities were performed to comprehend the effect of mastitis on mammary gland. The inferences drawn from the present investigation may help in augmenting the progress of new approaches for improving mammary gland health in lactating animals.

MATERIALS AND METHODS

**Inoculum preparation**

The intramammary inocula preparation was based on the method described by Brouillette *et al.*, with minor modifications [11]. *S. aureus*, *E. coli* and *B. subtilis* were isolated from a clinical case of bovine mastitis and grown overnight at 37°C in the Tryptic soy broth medium to reach mid-exponential growth. The actual number of colony forming unit (CFU) injected was confirmed by spreading the inoculum on to an agar plate and counting the colonies after overnight incubation. Bacterial concentration of the culture was determined using a standard curve plotting CFU as a function of the absorbance at 600 nm, the cultures were further serially diluted and suspended in phosphate buffer saline (PBS) to the desired number of CFU/ml.

**Animals**

Laboratory bred adult female Swiss albino mice were used in the experiments. Mice aged 90-120 days old weighing between 30-32 g was used. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. The mice were provided with standard mice pellet diet “Gold Mohar” (Krish Scientist’s Shopee, Bangalore) and water *ad libitum*. The mice were maintained under normal day/night schedule (12 L: 12 D) at room temperature 25 ± 2°C.

**Mouse mastitis model of infection**

The intramammary inoculation technique was based on the method described by Brouillette and Malouin [12]. The lactating mice used for inducing intramammary infection were of 12-14th day of the parturition weighing 36-38 g. The pups were removed 1-2 h before bacterial inoculation of mammary glands and a mixture of ketamine/xylazine at 87 and 13mg/kg of weight, respectively, was used for anesthesia of the lactating mice. A 1ml syringe with 28 gauge blunt needle was used to inoculate both L4 (on the left) and R4 (on the right) abdominal mammary glands. The study was approved by the Institutional Animal Ethical Committee, Department of Biotechnology and Microbiology, Karnataka University, Dharwad, India. CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) (Animal House Registration No. 639/02/a/ CPCSEA) guidelines were followed for maintenance and use of the experimental animals.

**Infection profile**

Lactating mice were divided into five groups of 5 mice each. Group I were injected with PBS (PBS vehicle serves as control), Group II were inoculated with 100µl/gland of LPS (20µg/ml), group III mice were inoculated with *S. aureus* inoculum doses of 2.8×10⁷ CFU, group IV mice were inoculated with *E. coli* inoculum doses of 2.8×10⁷ CFU and group V mice were inoculated with *B. subtilis* inoculum doses of 2.8×10⁷ CFU suspended in PBS (100µl/gland) were inoculated through R4 and L4 teat canals of mice for the induced groups II, III, IV and V respectively.
Clinical observations, body weight and temperature

Clinical observations, rectal temperature (°C) of mice and body weight (g) were recorded for every 12 h of post infection during the study period 48 h after the intramammary infection, the mice were weighed; blood was drawn by cardiac puncture to collect serum and sacrificed under sodium pentobarbital anesthesia. The mammary gland tissues were dissected out and collected in PBS for estimation of biochemical contents, dehydrogenases and phosphatases activities and oxidative stress parameters. Blood serum collected was estimated for oxidative stress parameters.

Biochemical Studies

The biochemical contents such as DNA and RNA carried out as per the method described by Schneider [13], protein by Lowry et al.,[14], glycogen by Carrol et al.,[15], cholesterol by Abell et al.,[16], activities of enzymes such as LDH by King [17], SDH by Nachlas et al., [18], ACP and AKP by method of Bergmeyer and Bernt [19].

Oxidative stress parameters

The oxidative stress parameters such as GSH level was measured following the method of Ellman [20], the product of the reaction between malondialdehyde (MDA) and thiobarbaturic acid reactive substances (TBARS) by Okhawa et al., [21] were measured by a modified method of Esterbauer and Cheesman, [22], SOD activity by Kakkar et al.,[23], CAT activity by Aebi [24] and GST activity by Habig and Jakoby [25].

Histological Observations

For histopathological examination the mammary gland tissues were fixed in aqueous bouin's fluid for 24h and dehydrated in graded series of alcohol, cleared in benzene and embedded in paraffin wax. The paraffin blocks were sectioned at 5 μm thicknesses by LEICA RM 2255 microtome and the tissue sections were subjected to rehydration by exposing them to decreasing concentrations of alcohol 10-10% and then stained with haematoxylin. The sections were dehydrated by using increasing concentrations of alcohol 100-10% and then stained with eosin. The stained slides were photographed under Axio Imager M2 for histological studies.

Statistical analysis

Statistical significance between the control and experimental data were subjected to one way analysis of variance (ANOVA) together with post-hoc Dunnett's test (P≤0.05).

RESULTS

Clinical observations, body weight and temperature

In the present study the inoculated mice were examined for clinical observations, body weight (g) and rectal temperature (°C) of mice were recorded for every 12 h of post infection during the study period. Clinical observations ranged from reddening of the skin at the base of the teat to obvious swelling of the infected mammary gland. Symptoms included ruffled coat, hunched appearance, suppressed intake of food and weakness. Significant decrease in the body weight of mice in the induced groups, respectively, compared to that of control (Table 1). There was a significant increase in rectal temperature after 12 h of post infection and successively decreased after 24, 36 and 48h of post infection in the induced groups, respectively, compared to that of control (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum (CFU)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>36.8±0.6</td>
</tr>
<tr>
<td>II</td>
<td>LPS</td>
<td>36.6±0.3</td>
</tr>
<tr>
<td>III</td>
<td>S. aureus</td>
<td>37.3±0.9</td>
</tr>
<tr>
<td>IV</td>
<td>E. coli</td>
<td>37.2±0.4</td>
</tr>
<tr>
<td>V</td>
<td>B. subtilis</td>
<td>37.4±0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals *Significant P ≤ 0.05 vs Control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum (CFU)</th>
<th>Rectal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>37.1±0.6</td>
</tr>
<tr>
<td>II</td>
<td>LPS</td>
<td>37.7±0.4</td>
</tr>
<tr>
<td>III</td>
<td>S. aureus</td>
<td>37.2±0.2</td>
</tr>
<tr>
<td>IV</td>
<td>E. coli</td>
<td>36.8±0.5</td>
</tr>
<tr>
<td>V</td>
<td>B. subtilis</td>
<td>36.8±0.8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals *Significant P ≤ 0.05 vs Control
Biochemical Studies

Biochemical contents and the enzyme activities revealed that there was a significant decrease in the levels of DNA, RNA, protein and glycogen of mice mammary gland tissue in the induced groups. But there was a significant increase in the level of cholesterol in the induced groups respectively, compared to that of control (Table 3). There was a significant decrease in SDH and AKP enzyme activities in mice mammary gland tissue of the induced groups, respectively, compared to that of control. But there was a significant increase in the level of the LDH and AKP activities in the induced groups, respectively, compared to that of control (Table 4).

**Table 3. Effect of mastitis induced infection on biochemical contents of mammary gland of mice.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum (CFU)</th>
<th>DNA (µg/mg wt wet tissue)</th>
<th>RNA (µg/mg wt wet tissue)</th>
<th>Protein (µg/mg wt wet tissue)</th>
<th>Glycogen (µg/mg wet tissue)</th>
<th>Cholesterol (µg/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2.12±0.16</td>
<td>3.62±0.12</td>
<td>185.34±6.32</td>
<td>2.98±0.24</td>
<td>18.23±0.11</td>
</tr>
<tr>
<td>II</td>
<td>LPS</td>
<td>1.84±0.28</td>
<td>2.63±0.27</td>
<td>166.82±8.58</td>
<td>2.64±0.45</td>
<td>22.58±0.35</td>
</tr>
<tr>
<td>III</td>
<td>S. aureus</td>
<td>1.46±0.25</td>
<td>2.12±0.15</td>
<td>135.52±7.26</td>
<td>2.04±0.37</td>
<td>26.38±0.26</td>
</tr>
<tr>
<td>IV</td>
<td>E. coli</td>
<td>1.68±0.33</td>
<td>2.54±0.42</td>
<td>159.73±9.63</td>
<td>2.43±0.29</td>
<td>24.47±0.18</td>
</tr>
<tr>
<td>V</td>
<td>B. subtilis</td>
<td>1.74±0.18</td>
<td>2.78±0.28</td>
<td>168.46±6.28</td>
<td>2.58±0.16</td>
<td>21.16±0.29</td>
</tr>
</tbody>
</table>

Values are mean± SEM of 5 animals  * Significant P ≤ 0.05 vs Control

**Table 4. Effect of mastitis induced infection on dehydrogenases and phosphatases enzyme activities of mammary gland of mice.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum (CFU)</th>
<th>LDH* (µmole/min/g tissue)</th>
<th>SDH* (µmole of pyruvate formed/min/g tissue)</th>
<th>ACP* (µmole/min/g tissue)</th>
<th>AKP* (µmole of formazan byproduct/min/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>10.46±0.37</td>
<td>11.78±0.28</td>
<td>18.16±0.68</td>
<td>19.22±0.34</td>
</tr>
<tr>
<td>II</td>
<td>LPS</td>
<td>14.82±0.23</td>
<td>10.13±0.35</td>
<td>16.78±0.52</td>
<td>20.98±0.39</td>
</tr>
<tr>
<td>III</td>
<td>S. aureus</td>
<td>18.25±0.15</td>
<td>7.19±0.22</td>
<td>12.58±0.34</td>
<td>24.73±0.27</td>
</tr>
<tr>
<td>IV</td>
<td>E. coli</td>
<td>16.73±0.44</td>
<td>9.13±0.37</td>
<td>14.83±0.48</td>
<td>22.68±0.55</td>
</tr>
<tr>
<td>V</td>
<td>B. subtilis</td>
<td>14.26±0.31</td>
<td>10.76±0.56</td>
<td>16.48±0.74</td>
<td>21.24±0.43</td>
</tr>
</tbody>
</table>

Values are mean± SEM of 5 animals  a µmoles of pyruvate formed/min/g tissue  b µmoles of formazan formed/min/g tissue  c µmoles of inorganic phosphorus formed/min/g tissue  d µmoles of p-nitrophenyl formed/min/g tissue  * Significant P ≤ 0.05 vs Control

**Oxidative stress parameters**

In the present study the oxidative stress parameters results revealed that there was a significant decrease in the level of GSH, CAT, SOD and GST activity in mice mammary gland tissue and serum of the induced groups, respectively, compared to that of control. However, there was a significant increase in the level of TBARS in mice mammary gland tissue and serum of the induced groups, respectively, compared to that of control (Table 5 and 6).

**Table 5. Effect of mastitis induced infection on oxidative stress parameters of mammary gland of mice.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum (CFU)</th>
<th>Antioxidant (µg/mg wet tissue)</th>
<th>Oxidative stress byproduct (µg/mg wet tissue)</th>
<th>CAT* (µg/mg wet tissue)</th>
<th>SOD* (µg/mg wet tissue)</th>
<th>GST* (µg/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>8.63±0.12</td>
<td>0.27±0.014</td>
<td>150.42±2.38</td>
<td>38.52±2.16</td>
<td>4.13±0.15</td>
</tr>
<tr>
<td>II</td>
<td>LPS</td>
<td>6.82±0.23</td>
<td>0.34±0.012</td>
<td>134.46±3.12</td>
<td>29.72±1.18</td>
<td>3.43±0.24</td>
</tr>
<tr>
<td>III</td>
<td>S. aureus</td>
<td>4.76±0.35</td>
<td>0.38±0.016</td>
<td>118.52±2.62</td>
<td>21.74±1.34</td>
<td>2.21±0.18</td>
</tr>
<tr>
<td>IV</td>
<td>E. coli</td>
<td>5.62±0.16</td>
<td>0.35±0.013</td>
<td>127.48±3.63</td>
<td>25.43±2.02</td>
<td>2.86±0.28</td>
</tr>
<tr>
<td>V</td>
<td>B. subtilis</td>
<td>6.62±0.28</td>
<td>0.33±0.015</td>
<td>132.37±2.76</td>
<td>30.56±1.98</td>
<td>3.36±0.35</td>
</tr>
</tbody>
</table>

Values are mean± SEM of 5 animals  a µmole of glutathione (GSH)/mg tissue  b µmole of H2O2  c µmole of H2O2  d superoxide dismutase (SOD) unit/mg protein  e Glutathione-s-transferase (GST) µmole/min/mg protein  * Significant P ≤ 0.05 vs Control

**Table 6. Effect of mastitis induced infection on oxidative stress parameters in serum of mice.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum (CFU)</th>
<th>Antioxidant (µg/mg wet tissue)</th>
<th>Oxidative stress byproduct (µg/mg wet tissue)</th>
<th>CAT* (µg/mg wet tissue)</th>
<th>SOD* (µg/mg wet tissue)</th>
<th>GST* (µg/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5.42±0.09</td>
<td>0.17±0.012</td>
<td>134.38±2.16</td>
<td>24.26±2.66</td>
<td>2.48±0.21</td>
</tr>
<tr>
<td>II</td>
<td>LPS</td>
<td>4.18±0.16</td>
<td>0.23±0.015</td>
<td>122.24±3.31</td>
<td>19.42±1.24</td>
<td>2.03±0.14</td>
</tr>
<tr>
<td>III</td>
<td>S. aureus</td>
<td>2.64±0.25</td>
<td>0.34±0.011</td>
<td>108.63±2.45</td>
<td>13.74±1.13</td>
<td>0.98±0.26</td>
</tr>
<tr>
<td>IV</td>
<td>E. coli</td>
<td>3.52±0.23</td>
<td>0.28±0.013</td>
<td>116.33±2.94</td>
<td>16.48±1.85</td>
<td>1.46±0.15</td>
</tr>
<tr>
<td>V</td>
<td>B. subtilis</td>
<td>4.05±0.13</td>
<td>0.25±0.016</td>
<td>123.45±2.38</td>
<td>20.62±1.76</td>
<td>1.98±0.17</td>
</tr>
</tbody>
</table>

Values are mean± SEM of 5 animals  a µmole of glutathione (GSH)/mg protein  b µmole of H2O2  c µmole of H2O2  d superoxide dismutase (SOD) unit/mg protein  e Glutathione-s-transferase (GST) µmole/min/mg protein  * Significant P ≤ 0.05 vs Control
Histopathology

Histopathological changes of lactating mammary gland tissue of mice inoculated with PBS served control group and infected groups with LPS, *S. aureus*, *E. coli* and *B. subtilis* are presented in Figure 1(a-e) at 20X. The present histological observations of control mammary gland showed no polymorphonuclear (PMN’s) neutrophils leukocyte infiltrations, compactly arranged epithelial and luminal cells of healthy alveoli filled with milk proteins, fat and lipid droplets as secretory products (Fig. 1a.). The mammary gland tissue of LPS, *S. aureus*, *E. coli* and *B. subtilis* inoculum induced groups caused massive PMN infiltrations, alveolar atrophy showing discontinuous epithelial and luminal cell lining and disturbed secretory products (Fig. 1b-e.). The aetiological symptoms were prominently observed in the *S. aureus* and *E. coli* induced groups, when compared with that of control, LPS and *B. subtilis*.

DISCUSSION

Inflammation is a critical component of the innate defense system that involves complex biological responses of vascular tissues to harmful stimuli such as bacterial pathogens [26]. During infection of the mammary glands, the tissue damage is caused by bacteria that produce toxins which destroy cell membranes and damage milk-producing tissue. Immune response caused elevated neutrophil migration evoked to fight inflammation inadvertently rendered alveolar epithelial cells to non-secretory conditions [27]. Mastitis induced by intramammary infection of bacterial pathogens is characterized by strong release of PMN’s into the mammary gland. PMNs act by engulfing and destroying the invading bacteria via oxygen-dependent and oxygen-independent systems [28]. At the infection site, the accumulated PMNs activate the release of antibacterial peptides, proteases and reactive oxygen species (ROS) (i.e., superoxide anion, hydrogen peroxide, hydroxyl radicals and hypochlorous acid) which cause tissue injury if overproduced [29]. ROS play crucial roles in various physiological processes, including innate immunity, modulation of redox-dependent signaling cascades, and as cofactors in the production of hormones and pro-inflammatory cytokines [30].

Figure 1. Histopathological changes of lactating mammary gland tissue of mice inoculated with PBS control (a), LPS (b), *S. aureus* (c), *E. coli* (d) and *B. subtilis* (e) inoculum induced groups of infection. Arrow represents alveoli, arrow head denotes polymorphonuclear neutrophil infiltration and star indicates secretory products. Magnifications 20X.
In the present study, Lipopolysaccharide (LPS), *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* used to induce mastitis by intramammary inoculation of mice showed contrasting indications and significant differences when observed for body weight, rectal temperature, biochemical and oxidative stress parameters. Clearly indicating the effects were due to the mastitis induced in mice, when compared with that of control mice. There was significant decrease in the body weight of mice in the induced groups when compared with that of control group which might be due to the severe infection, progressive depression weakness and suppressed intake of the food. The bacterial load replicated rapidly and filled the alveolar space by PMN’s leading to decrease in milk volume, milk energy, or mammary gland weight and tissue damage [31]. Similar results were reported suggesting that the severity of mastitis may also progress to include systemic involvement such as fever, depression and loss of appetite which thereby results in decrease of body weight [32, 33].

The rectal temperature of mice at 12 h of post intramammary infection (PI) significantly increased in the induced groups when compared with that of control group and gradual decrease in the temperature after 24, 36 and 48 h of post intramammary infection in the induced groups of mice when compared with that of control group. Rectal temperature peaked at 12 h of PI in the induced groups, due to the inflammation causing fever and pyrexia. Rectal temperature thereby lowered after 24, 36 and 48 h of post intramammary infection in the induced groups as the pyrexia depressed due to the innate immunity and resistance. Similar results were reported in the mastitis induced cows, suggesting that the rise in temperature is due to the inflammation caused by intramammary infection [34, 35].

DNA and RNA levels of the mammary gland tissue were significantly decreased in the induced groups of mice when compared with that of control group which may be attributed towards the tissue damage occurred due to the bacterial toxins, apoptosis or necrosis of epithelial cells, secretory cells and breakdown of extracellular matrix. Similar results were reported suggesting polymorphonuclear neutrophils released to kill bacteria harm the mammary tissue by releasing reactive oxygen intermediates and hydrolytic enzymes [7]. Protein level of the mammary gland tissue is significantly decreased in the induced groups of mice when compared with that of control group owing to the decreased protein synthesis in mammary gland cells caused by oxidative cell injury. Comparable results were reported suggesting excess production of ROS, proteolytic enzymes, oxidants and proteases released by PMN to engulf bacteria have been associated with tissue damage [36]. Significant decrease in the glycogen level in the induced groups of mice when compared with that of control group is due to impaired cellular components and altered metabolic pathways via glucose oxidation as a result of bactericidal molecules released by PMN. Similar results were reported suggesting phagocytosis by PMN leads to the excess ROS production thereby damaging biological macromolecules and disruption of normal metabolism and physiology [9]. Significant increase in the cholesterol level in the induced groups of mice when compared with that of control group may be credited to the lipid peroxidation caused by bacterial toxins and free radicals. Similar results were reported suggesting respiratory burst initiate lipid peroxidation and partial breakdown of their vital proteins, RNA, and DNA [37].

LDH and AKP enzyme activities were significantly increased with significantly decreased SDH and ACP enzyme activities of the mammary gland tissue in the induced groups of mice compared to control group may be due to the neutrophil promoting tissue injury and disturbed mammary function, via reactive oxygen metabolite generation (the respiratory burst) and granular enzyme release (degranulation) [38]. Similar results were reported suggesting high concentrations of ROS produced by PMN for phagocytosis of bacteria compromise cellular function by damaging nucleic acids and by altering proteins, carbohydrates and membrane phospholipids [26].

GSH level of the mammary gland tissue and serum was significantly decreased in the induced groups of mice when compared with that of control group is due to the oxidative stress induced by the inflammatory reaction. Similar results were reported suggesting free radicals released as a result of PMN infiltration reduce the antioxidant level [39]. Significantly increased TBARS level of the mammary gland tissue and serum in the induced groups of mice when compared with that of control group is because of the accumulation of ROS leading to lipid peroxidation. Similar results were reported suggesting neutrophils exert their bactericidal effect through a respiratory burst that produces hydroxyl and oxygen radicals, which are key components of the oxygen-dependent killing mechanism [40]. SOD, CAT and GST antioxidant enzyme activities significantly decreased in the induced groups of mice mammary gland tissue and serum when compared with that of control group may be ascribed PMN become activated and generate powerful reactive oxygen species, such as superoxide anions (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), halogen reactive species and hydroxyl radicals (·OH) by partial reduction of O$_2$ [41]. Comparable results were reported signifying that imbalance between increased production of free radicals and reduced availability of antioxidant defenses due to intramammary infection induced by bacterial pathogens causing inflammatory disease in rat [42].

Histopathological evidences revealed that massive infiltration of PMN leukocytes and migration to the mammary gland tissue was due to the inflammation induced by bacterial infection. Similar evidences were reported suggesting that intramammary infiltration of PMN’s in the mammary gland tissue during mastitis [31]. Mastitis leads to losses in mammary function are directly related to disruption of alveolar cell integrity, sloughing of cells, induced apoptosis, and increased appearance of poorly-differentiated cells [27]. In the present study, alveolar damage showed unstable and discontinuous linings of epithelial and luminal cell destructions. Alveolar destruction led to the decrease of secretory products such as milk proteins, lipid droplets and fat content due to the bacterial infection which act by rapidly dividing in the host by inhibiting the phagocytosis by PMN cells. Similar results were reported suggesting the presence of functional PMN is crucial to the host defense against bacterial pathogens but neutrophils may promote tissue injury and disturb mammary function, via reactive oxygen metabolite generation [43]. Thus, histopathological changes and atrophies revealed that the tissue damage was due to increase in PMN infiltration caused ROS production leading to oxidative stress.
CONCLUSION

In the present study experimentally induced mastitis by bacterial pathogens lead to deleterious effects on the mammary gland tissue and physiology of mice. The results of present investigation indicated that the effect of induced mastitis curtailed the mammary gland biochemical contents, dehydrogenases, phosphatases activities, oxidative stress parameter constraints in lactating mice. The pathogenesis of mice mammary gland tissue is due to bacterial toxins released by bacterial pathogens, PMN apoptosis or necrosis, overproduction of ROS released by PMN and peroxidation of lipids. An imbalance between increased production of reactive oxygen species and reduced availability of antioxidant defenses at the time of intramammary infection by bacterial pathogens resulted in increased oxidative stress.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, New Delhi, for funding the Interdisciplinary Program for Life Science Project (BT/PR/4555/INF/22/126/2010 dated 30-09-2010). Bioinformatics Infrastructure Facility Project (BT/BI/25/001/2006 VOL II dt 05-03-2012) and P. G Departments of Microbiology and Biotechnology Karnatak University, Dharwad for providing the facilities.

Authors’ Statements

Competing Interests

The authors declare no conflict of interest.

REFERENCES


www.iajpr.com


