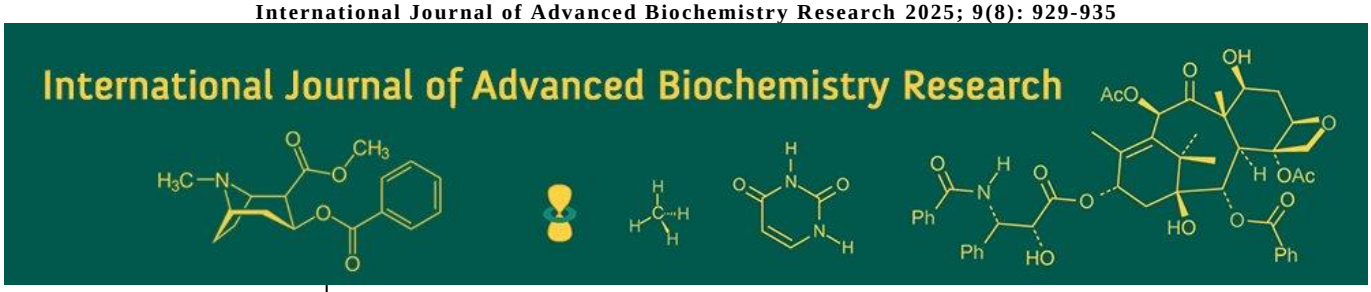

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Analysis of hematological and oxidative stress parameters in the rat experimental mastitis model

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Abstract
The present study, entitled 'Analysis of Hematological and Oxidative Stress Parameters in the Rat Experimental Mastitis Model', was conducted to evaluate hematological changes and oxidative stress-related alterations in the mastitis model. *Staphylococcus aureus*-induced experimental rat mastitis model and to assess the efficacy of *Allium cepa* ethanolic extract and Cefuroxime sodium in mastitis. The mastitis model was developed by intra-mammary inoculation of *Staphylococcus aureus* bacteria 10 µL (1.5×10^4 CFU/mL) in the left fourth, left fifth (L4, L5) and right fourth, right fifth (R4, R5) teat of lactating female Wistar rats (*Rattus norvegicus*). The cup borer method is used for the confirmation of the dose rate herbal drug against bacteria. A total of 40 rats were divided into 5 groups. Group 1 served as a normal/healthy control. Group II served as vehicle control in which sterile deionized water @ 10 µL per mammary gland was infused via the intra-mammary route. *S. aureus* culture of 10 µL (1.5×10^4 CFU/mL) per mammary gland was given via the intra-mammary route to induce mastitis in groups III, IV and V. Development of mastitis was confirmed by marked swelling, hardness and reddening at the teat area. After the development of mastitis, group III was kept as the mastitis control (disease control) group and no treatment was given. After the development of mastitis, group IV rats were treated with *Allium cepa* ethanolic extract @ 10 µL (50 mg/mL concentration) per mammary gland via the intra-mammary route, while group V rats were treated with Cefuroxime sodium infusion @ 10 µL (10 µg/mL concentration) per mammary gland via intra-mammary route for 5 days continuously. All the intra-mammary inoculations/infusions were made in the left fourth, left fifth (L4, L5) and right fourth, right fifth (R4, R5) teat of rats. The mastitis control group showed decreased values of TEC, Hb, MCV, MCH, MCHC and lymphocytes and increased values of PCV, TLC, neutrophils, eosinophils, and platelets as compared to their normal counterparts (*i.e.*, Group I). The *Allium cepa* ethanolic extract-treated group showed non-significantly decreased values of TEC, PCV, MCV, TLC, neutrophils and platelets and increased values of Hb, MCH, and lymphocyte as compared to the mastitis disease control group. The haematological effects of Cefuroxime sodium were comparable to those of *Allium cepa* extract. The present research was designed to evaluate the haematological changes and oxidative stress-related alterations associated with *Staphylococcus aureus-induced* experimental rat mastitis model and to evaluate the efficacy of *Allium cepa* extract and Cefuroxime sodium to counteract the same.

Keywords: Mastitis, *Staphylococcus aureus*, *Allium cepa*, cefuroxime sodium, hematological and oxidative stress parameters

Introduction
The Greek words "MAST", which refers to the 'mammary gland' and "ITIS" meaning 'inflammation' are the source of the word "MASTITIS". Mastitis is the inflammation of the mammary glands (Ruegg, 2003) [23]. Abnormalities in glandular tissue that are pathological, bacteriological, chemical and physical accompany the mammary gland diseases (Tripathy *et al.*, 2018). It causes swelling, redness, localized pain, reduced milk synthesis and other symptoms like fever, flu-like aches, chills and fatigue (Demon *et al.*, 2013; Amir *et al.*, 2014) [5, 2].

India is referred to as "The Botanical Garden of the World" because of the variety of plant species found here. It has also been a pioneer in the creation and application of traditional medical systems, including Ayurveda, Siddha and Unani. Numerous innovative compounds for preventive and curative medicine have been brought to modern science by plants used in traditional medicine. A significant portion of the global population uses plants as a source of medicine and as a cure for the majority of illnesses.

Even though synthetic antibiotics play an important role in mastitis treatment, there are many reports of adverse effects of antibiotic residue in milk (Vishnuraj *et al.*, 2016; Kumaraswamy *et al.*, 2018) [33, 15]. There is an increasing trend of ethnological treatment of bovine mastitis with an intention to decrease antibiotic residues in animal by-products. There are so many herbal therapeutic agents including *Allium cepa* (onion), *Hibiscus rosa-sinensis* (Jasud), *Syzygium aromaticum* (clove), *Lagenaria siceraria* (bottle gourd), *Citrus lemon* (lemon), *Aloe vera*, *Curcuma longa* (turmeric) etc. The novel indigenous herbal agents for mastitis treatment like onion need to be evaluated scientifically by studying their efficacy and safety in the rat mastitis model.

The rat model of mastitis

Rat models of mastitis involve the administration of a mastitis-inducing agent (e.g., Culture of *Staphylococcus aureus*) in the mammary gland. The rat model is a suitable tool for research focusing on the pathogenesis and control of bovine intramammary infections. Rat has 6 pairs of mammary glands 1. Cervical, 2. Cranial thoracic, 3. Caudal thoracic, 4. Abdominal, 5. Cranial inguinal and 6. Caudal inguinal.

Bacteria: *Staphylococcus aureus* (*S. aureus*)

There are so many pathogens causing mastitis. Many studies have determined that *Staphylococcus*, *Streptococcus* and *Coliforms* are the most frequently found infectious agents that cause mastitis (Shoaib *et al.*, 2020) [29]. *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* are the main bacteria associated with the occurrence of mastitis in dairy cows (Lundberg *et al.*, 2016) [18]. As per the studies conducted in India, the major bacterial pathogens involved in mastitis are *Staphylococcus spp.* Mastitis is typically treated with synthetic antibiotics, but many times the therapeutic effect is not satisfactory (Swinkels *et al.*, 2015) [30].

***Allium cepa* (Onion)**

There is a trend of treatment of mastitis by herbal agents like *Allium cepa* (onion), *Lagenaria siceraria* (bottle gourd), *Hibiscus rosa-sinensis*, *Citrus lemon* (lemon), etc. Among them, *Allium cepa* is the promising herbal candidate that needs to be evaluated for pathological changes associated with its therapeutic usage in rat mastitis models. Herbs have a few advantages over pharmaceuticals, being widely available, affordable and free of side effects. They are also safe to use over time. Scientific information on the efficacy of onions for the treatment of mastitis is lacking and requires further studies. The study of the efficacy of onion for mastitis treatment is also needed to prove the quality, safety and importance of *Allium cepa* extracts worldwide. The present research was intended to study the effects of *Allium cepa* extracts in the rat mastitis model. The data generated from this study will be highly relevant and essential for the effective use of onion in mastitis treatment. The aim of study was the experimental mastitis in the rats and study the *Staphylococcus aureus* induced mastitis associated hematological changes and oxidative stress in *Staphylococcus aureus*-induced mastitis in rats.

Materials and Methods

The current interventional laboratory animal study was undertaken to appraise the haematological changes and oxidative stress-related changes in the *Staphylococcus aureus*-induced rat mastitis model and to study the efficacy of *Allium cepa* extract and cefuroxime sodium to counteract the same. The experimental study was conducted at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari-396450, Gujarat, India.

A total of 40 adult lactating females (Wistar rats) of 200-240 grams body weight were used for the experiment. The rats were procured from the Aarsh Research and Development Center, Daman-396210, Gujarat, India (CCSEA registration no.2239/PO/RcBiBt/S/23/CCSEA). Ethical clearance for performing the experiments on the rats was obtained from the Institutional Animal Ethical Committee (IAEC) vide Project No. 133-VCN-VPP-2022.

The rats were maintained under standard laboratory conditions (temperature 24±2 °C and relative humidity of 40 to 70%). The photoperiod was 12 hours of light and 12 hours of dark. Ten days were given for the acclimatization. The animals were housed (one lactating female in one cage) in a polypropylene cage with a solid bottom having autoclaved rice husk as bedding material. The bedding material was changed every day. The experimental rats were fed standard rodent pellet feed. The rats were provided with ad libitum feed and clean and filtered drinking water in polypropylene bottles throughout the experiment.

Pure culture of *Staphylococcus aureus sub. spp. aureus* (MTCC-737), Chandigarh-160036, India and stored at-20 °C until use. To verify the culture's purity, the preserved culture was brought back to life and put through standard microbiological testing. Pure culture of *S. aureus* was subjected to serial fold dilutions in PBS solution till they reached the concentration of 1.5×10^4 CFU/mL. The solution was infused 10 µl/teat (4^{th} and 5^{th} pair of mammary glands) of female rats (*i.e.*, Intra-mammary route). The intra-mammary infusion was done as per Zhong's protocol and Cai's method. (Zhong *et al.*, 2005; Cai *et al.*, 2020) [34, 3].

The onion (*Allium cepa*) plant along with onion peels was collected from the surrounding regions of Navsari-396450, Gujarat, India. Only the red-coloured onion peels were used for the extraction of polyphenol compounds. The onion peels were collected by removal of the leaves, stems etc.

The onion peels were separated from red onions and washed with distilled water. The peels were crushed and made paste-like consistency by using an electric mixer. Consequently, the paste was stored at 4 °C until used. For making 70% ethanolic extraction, The mixture was heated for 3 hours at 60 °C in a water bath. The slurry was filtered through filter paper (Whatman No. 2; Whatman, Kent, UK). The solvent was evaporated using a rotary evaporator. The sticky extract was transferred into a sterile petri dish and subjected to evaporation using ethyl alcohol in the oven at 40 °C. The dried extract was weighed and stored at-20 °C till further use (Hassas-Roudsari *et al.*, 2009) [9].

Cup borer method

This technique is applied for the confirmation of an effective dose of herbal concentration against the mastitic bacteria. The sterile plates, cryovial, L-spreader, sterile water, sterile Muller Hinton (MH) agar, sterile Muller Hinton (MH) broth, and sterile centrifuge tube are required in the cup borer method. The sterile petri dish plate was filled with Muller-Hinton (MH) agar to maintain the 3 to 4 mm depth in the petri dish plate. The even thickness in

medium layers was achieved by setting the dishes or plates on a levelled surface. The sterile media was flooded with 2 mL standardised inoculum 1.5×10^4 of CFU/mL *Staphylococcus aureus* (equivalent to 0.5 McFarland 1.5×10^8 CFU/mL). Equally spaced cups were drilled into the agar plates using a sterile cork borer (4 mm). To prevent the extract from seeping beneath the agar, the bottom of the borehole was sealed with a single drop of sterile molten agar. Different concentrations of the extracts (i.e. 400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL) were prepared by serial dilution using sterile distilled water. The bored holes were filled with 100 microliters of each of the extract concentrations. The plates were incubated for 18 hours at 37 °C. After incubation, the zones of inhibition were measured in millimetres by using of zone scale.

Experimental Designs

A total of 40 adult lactating female rats were procured and housed as per CCSEA (Committee for the Purpose of Control and Supervision Experiments on Animals) guidelines. The rats were divided into five groups. Group 1 served as a healthy/normal control in which no treatment was given. The rats from groups II, III, IV and V were anaesthetized using ketamine @ 22-25 mg/kg body weight through the intraperitoneal route and placed on the dorsal surface for intra-mammary inoculation of the test/vehicle items. Before bacterial inoculation, the mammary gland area was cleaned and made aseptic with 70% ethyl alcohol. Group II served as the vehicle control in which the sterile deionized water @ 10 µL per teat (i.e., by intra-mammary route) was infused. In groups III, IV and V, *S. aureus* culture of 10 µL (1.5×10^4 CFU/mL) per teat (*i.e.* by intra-mammary route) was given in left fourth, left fifth (L4, L5) and right fourth, right fifth (R4, R5) mammary glands.

The clinical signs of mastitis were observed after 6, 12, 24 and 48 hrs of intra-mammary inoculation. The development of mastitis was confirmed by the appearance of clinical signs like reddening and swelling of the mammary gland area along with hot area and pain sensation. After confirmation of mastitis, group IV rats were treated with *Allium cepa* ethanolic extract @ 10 µL (50 mg/mL) per teat (i.e. by intra-mammary route) while group V rats were treated with Cefuroxime sodium infusion 10 µL (10 µg/mL) by intra-mammary route up to 5 days.

Collection of Samples

At the end of the experiment, blood samples were collected from the rat's retro-orbital plexus using micro capillaries at the end of the experiment. Blood was collected from all the rats in K_3EDTA vials and clot activator vials for haematology and oxidative stress analysis respectively. The clot activator vials were initially kept at room temperature for 30 minutes and later centrifuged at 1000 rpm for 10 minutes at 4 °C to obtain serum. Later, the sera were aliquoted and stored at-20 °C till further analyses. Serum samples were used to measure the oxidative stress. At the time of blood collection, blood smears were prepared for differential leukocyte count.

Results

This technique was used to confirm the concentration of *Allium cepa* ethanolic extract to control or kill the bacteria. Initially, Muller Hinton agar was flooded in a sterile plate and the equally spaced cups were drilled into the agar plates using a cork borer. The bottom holes were sealed with molten agar. Different concentrations of the *Allium cepa* ethanolic extract (400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL) were assessed to assess their antimicrobial activity. *Allium cepa* ethanolic extract showed 32 mm and 20 mm zones of inhibition at 400 mg/mL concentration and 200 mg/mL concentration, respectively. Also, 100 mg/mL and 50 mg/mL show very mild activity against *Staphylococcus aureus* bacteria. Based on the above findings, it was noted that a 50 mg/mL concentration of *Allium cepa* ethanolic extract has effective anti-*Staphylococcus aureus* bacterial activity.

Haematological parameters

In the present study, blood samples were collected from all rats at the end of the experiment. The data of mean value of Total Erythrocyte Count (TEC), Haematocrit/ Packed Cell Volume (PCV), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Total Leucocyte Count (TLC), Differential Leukocyte Count (DLC) and Platelets of all the groups are presented in table 1. In the current experiment, on the 11th day, TEC (10^6/µL) values were measured in groups. Results showed (Table 1) a non-significantly decreased TEC value of group III (mastitis disease control animals) as compared to group I (normal healthy control group). There was a significant decrease in TEC values of group IV (*Allium cepa* treated group) and group V (cefuroxime sodium treated group) as compared to group I (normal healthy control group). However, there was a non-significant difference in TEC values between groups IV and V. Group IV was noted to have a highly significant (p<0.001) decreased TEC value as compared to the mastitis disease control group (group III). Yet, there was a non-significant difference between groups III and V.

Here, there was a non-significant difference between all the groups. In groups I, II, III and IV the Hb concentration values were found to be comparable among each other, but in group III, non significantly decreased Hb concentration was noted as compared to group I. However, a significant increase in Hb concentration in group V as compared to group III. Interestingly, group IV showed an increase in the value of Hb concentration as compared to group III, but this difference was non-significant. In the present study, the mean of PCV (%) values showed there was a non-significant difference between Groups I, III and Group IV. There was also a non-significant difference between group III (Mastitis disease control) and IV (*Allium cepa* treated group). There was a non-significant decrease in the PCV value of group IV as compared to group III. However, group III and group V (Cefuroxime sodium-treated group) showed highly significant (p<0.001) differences in PCV (%) values. Group IV was found to have significantly increased PCV (%) values as compared to group V (Cefuroxime sodium-treated group). In the current experiment, MCV (fL) values were recorded for different groups. There was a non-significant difference between groups II, III and V. Here, group III and group IV showed significantly decreased (p<0.001) MCV values as compared to group I. There was a non-significant difference between group III and group V. However, there was a non-significantly decreased MCV value in group IV as compared to group III.

At the end of the experiment, MCH values (Pg) were recorded. The obtained data showed that there were non-significant differences between groups I, II, IV and V. Group III (mastitis control group) showed significantly decreased MCH value as compared to group I (normal control group). Both the *Allium cepa-treated* group (group IV) and the cefuroxime sodium-treated group (group V) showed increased MCH values compared to group III (mastitis disease control group). But these differences were non-significant. In this experiment, MCHC (g/dL) values of groups showed there was a non-significantly decreased value of MCHC in the *Allium cepa* treated group (group IV) and cefuroxime sodium treated group (group V) as compared to the mastitis control group (group III). Yet, there was a non-significant difference in the MCHC value of group IV and group V. In the current study, the mean TLC (thousand per µL) values of different groups were obtained. There were non-significant differences between groups I, II, IV and V. However, significantly ($p \leq 0.001$) decreased TLC values were noted in the *Allium cepa-treated* group and cefuroxime sodium.

Table 1: Details of haematological values of different treatment groups of rats.

Parameters	Group I (N = 8)	Group II (N = 8)	Group III (N = 8)	Group IV (N = 8)	Group V (N = 8)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
TEC (x 10^6/µL)	8.58^{d}±0.12	7.91^{a}±0.08	8.45^{cd}±0.14	8.02^{ab}±0.06	8.20^{bc}±0.12
Hb (g/dL)	15.00^{ab}±0.13	14.58^{ab}±0.22	13.91^{a}±0.18	15.11^{ab}±0.59	15.42^{b}±0.73
PCV (%)	43.82^{b}±0.56	47.35^{c}±0.22	44.62^{b}±0.25	43.97^{b}±0.06	42.74^{a}±0.13
MCV (fL)	57.54^{c}±0.39	57.01^{bc}±0.46	56.45^{b}±0.17	55.03^{a}±0.11	56.32^{b}±0.50
MCH (Pg)	20.24^{b}±0.24	19.60^{ab}±0.18	19.20^{a}±0.18	19.82^{ab}±0.34	19.88^{ab}±0.29
MCHC (g/dL)	34.32^{b}±0.26	35.28^{c}±0.30	33.06^{a}±0.25	32.67^{a}±0.30	32.20^{a}±0.32
TLC (x 10^3/µL)	9.44^{a}±0.41	9.55^{a}±0.64	12.22^{b}±0.46	9.93^{a}±0.33	9.63^{a}±0.36
Neutrophils (%)	32.42^{a}±1.88	32.47^{a}±1.34	34.93^{a}±0.52	32.60^{a}±0.60	32.26^{a}±0.71
Eosinophils (%)	1.50^{a}±0.22	1.16^{a}±0.31	1.57^{a}±0.20	1.33^{a}±0.21	1.25^{a}±0.11
Basophils (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Lymphocyte (%)	64.52^{a}±1.89	63.82^{a}±1.41	61.67^{a}±0.54	64.31^{a}±0.63	64.88^{a}±0.71
Monocyte (%)	3.06^{a}±0.7	3.72^{b}±0.19	3.40^{ab}±0.36	3.09^{a}±0.13	2.86^{a}±0.9
PLT (x 10^3/µL)	1013.00^{a}±5.40	1130.83^{b}±14.79	1223.17^{c}±14.14	1154.14^{b}±9.53	1161.20^{b}±15.34

Note: Mean bearing different superscripts in a row differ significantly ($p \leq 0.05$).
N = Number of experimental animals

The neutrophil results showed a non-significant increase in the neutrophil value of group III (mastitis control) as compared to group I (normal control). The treatment groups i.e. group IV (*Allium cepa* treated) and *i.e.* group V (Cefuroxime sodium treated) group showed a non-significant decrease in the neutrophil count as compared to the mastitis control (group III) group. The mean of lymphocyte values (%) showed a non-significant increase in the value of lymphocyte (%) in group IV and group V as compared to group III.

In the present study, the mean monocyte (%) count showed a non-significant difference between groups I, III, IV and V. Yet, group IV and group V showed a non-significant decrease in the monocyte value (%) as compared to group III. In the current study, platelet count (10^3/µL) was measured in the groups. The result showed a non-significant difference between group IV and group V. Interestingly, the *Allium cepa-treated* (group IV) group showed a highly significant ($p<0.001$) decrease in the platelet count as compared to the mastitis control (group III) group.

Oxidative Stress Parameters

In the present investigation, mastitis-induced oxidative stress-induced damage and its counteracting effects were estimated by measuring lipid peroxidation (LPO) and superoxide dismutase (SOD) levels. The superoxide dismutase (SOD) level was expressed in terms of nanograms per millilitre (ng/mL) and lipid peroxidation (LPO) was expressed in terms of malondialdehyde (MDA) concentration (nM/mL). The data is presented in Table 2.

Lipid peroxidation (LPO)

In the current study, lipid peroxidation values (nM/mL) in groups I, II, III, IV and V were found to be 13.13^{a}±0.65, 11.71^{a}±1.40, 19.33^{c}±0.17, 15.92^{b}±0.35 and 16.36^{b}±0.5 respectively Table 2. There was a significant increase in the LPO value of the mastitis disease control group (group III) in comparison to the normal control group (group I) and vehicle control group (group II). There was a highly significant ($p<0.001$) decrease in the lipid peroxidation (LPO) values in *Allium cepa* treated (group IV) and the Cefuroxime sodium treated (group V) groups as compared to the mastitis disease control (group III). However, there was a non-significant difference in the LPO values between the *Allium cepa* treated (group IV) group and the Cefuroxime sodium treated group (group V). Hence, it could be interpreted that both the therapeutic agents (*i.e. Allium cepa* ethanolic extract and Cefuroxime sodium) have similar effects on lipid peroxidation values.

Table 2: Details of oxidative changes in different treatment groups of rats

Parameter studied	Group I (N = 8)	Group II (N = 8)	Group III (N = 8)	Group IV (N = 8)	Group V (N = 8)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
LPO (nM/mL)	13.13^{a}±0.65	$11.71^{a\pm}$1.40	$19.33^{c\pm}$0.17	15.92^{b}±0.35	16.36^{b}±0.5
SOD (ng/mL)	$3.33^{a\pm}$0.83	$3.43^{a\pm}$0.32	$2.8^{a\pm}$0.25	$3.65^{a\pm}$0.22	$3.22^{a\pm}$0.41

Note: Mean bearing different superscripts in a row differ significantly ($p \leq 0.05$).
N = Number of experimental animals

Superoxide dismutase (SOD)

In the current study, superoxide dismutase values (ng/mL) in the groups I, II, III, IV and V were found to be $3.33^{a}\pm0.83$, $3.43^{a}\pm0.32$, $2.8^{a}\pm0.25$, $3.65^{a}\pm0.22$ and $3.22^{a}\pm0.41$ respectively Table 2. In the present study, superoxide dismutase (SOD) showed a non-significant difference among all the groups. However, it could be said that the mastitis control (group III) group showed a non-significantly decreased SOD level as compared to the normal control (group I) group. Yet, the *Allium cepa-treated* (group IV) group showed a non-significantly increased value of SOD as compared to the mastitis control (group III) group. Similarly, the Cefuroxime sodium-treated (group V) group showed a non-significantly increased SOD value as compared to the mastitis control group. Here, there was a non-significant difference between the *Allium cepa-treated* group and the Cefuroxime sodium-treated group. Overall, the results indicated similar antioxidant properties of both therapeutic agents against mastitis.

Discussion

In the present study, the mastitis disease control group showed decreased values of TEC, Hb, MCV, MCH, MCHC and lymphocytes and increased values of PCV, TLC, neutrophils, eosinophils, and platelets as compared to their normal counterparts (*i.e.*, group I). Our findings conformed with the findings of Abba *et al.* (2013) [1] and Sayhood *et al.* (2018) [27] who reported decreased mean values of RBC count, haemoglobin, PCV, MCV, MCH and MCHC compared to the normal control group and increased mean values of neutrophils and WBCs count. The present study showed similar out comes as those of Saleh *et al.* (2022) [25] and Sadat *et al.* (2023) [24] who mentioned significantly increased value of TLC, neutrophils and lymphocytes. Increased mean value of Hb, PCV and RBCs was also reported by Sadat *et al.* (2023) [24]. Saleh *et al.* (2022) [25] also mentioned non-significant changes in the basophils, eosinophilic and monocytic counts in subclinical mastitis.

Irrespective of statistical considerations, it can be inferred that the *Allium cepa* treated group showed decreased values of TEC, PCV, MCV, TLC, neutrophils and platelets and increased values of Hb, MCH and lymphocyte as compared to the mastitis disease control group. Similar findings were also reported by Samson *et al.* (2012) [12], Meraiyebu *et al.* (2013) [20], and Lee (2020) [16], who described significantly increased values of RBCs, Hb, haematocrit and lymphocytes in their study. Our study findings support the report of Dawud *et al.* (2016) [4] who observed a significantly decreased number of neutrophils. Earlier researchers have noticed significantly reduced total WBC and neutrophil counts and increased lymphocyte counts. (Dawud *et al.*, 2016) [4].

Previously, many scientists, including Lykkesfeldt and Svendsen (2007) [19], Sharma *et al.* (2010) [28], Jhambh *et al.* (2013) [12], Eslami *et al.* (2015) [7], Zigo *et al.* (2019) [35]; Taifa *et al.* (2022) [31] and Sadat *et al.* (2023) [24] researched oxidative stress wherein they all found decreased SOD values and increased LPO values in mastitis affected animals. Various scientists have reported the protective effect of *Allium cepa* against *Staphylococcus aureus*-induced infections and other systemic infections (Helen *et al.*, 2000) [10]; Nuutila *et al.* (2003) [21]; Gulsen *et al.* (2007) [8]; Prakash *et al.* (2007) [22]; Izawa *et al.* (2008) [11]; Kim *et al.* (2013) [13]; Kumar *et al.* (2013) [14]; Duan *et al.* (2015) [6] and Liguori *et al.* (2017) [17].

Conclusion

The present research was designed to evaluate the haematological changes and oxidative stress-related alterations changes associated with *Staphylococcus aureus-induced* experimental rat mastitis model and to evaluate the efficacy of *Allium cepa* extract and Cefuroxime sodium to counteract the same. Before the In-vivo study, the In-vitro study was conducted, whereby the zone of inhibition was found to be 32 mm zone at 400 mg/mL, 20 mm at 200 mg/mL and 100 mg/mL and 50 mg/mL showed moderate zone of inhibition in the cup borer method. In the present study, the mastitis disease control group showed decreased values of TEC, Hb, MCV, MCH, MCHC and lymphocytes and increased values of PCV, TLC, neutrophils, eosinophils, and platelets as compared to their normal counterparts (group I). Irrespective of statistical considerations it can be inferred that the *Allium cepa* treated group showed decreased values of TEC, PCV, MCV, TLC, neutrophils and platelets and increased values of Hb, MCH and lymphocyte as compared to mastitis disease control group. The haematological effects of Cefuroxime were comparable to those of *Allium cepa* extract. There was a significant increase in the LPO value of the mastitis disease control group in comparison to the normal control group and vehicle control group. There was a highly significant ($p<0.001$) decrease in the Lipid Peroxidation (LPO) values of *Allium cepa* treated and Cefuroxime sodium treated group as compared with mastitis control group. However, there was a non-significant difference in the LPO values between the *Allium cepa-treated* group and the Cefuroxime sodium-treated group. However, the superoxide dismutase (SOD) showed a non-significant difference between all the groups. During the necropsy, grossly prominent blood vessels around the mammary gland area in rats of group III were observed.

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References

1. Abba Y, Igbokwe IO, Adamu L, Buba I. Alterations in hematological and serum biochemical parameters of Sahel goats with clinical mastitis. IOSR J Agric Vet Sci. 2013;4(4):74-77.
2. Amir LH, Academy of Breastfeeding Medicine Protocol Committee. ABM clinical protocol 4: Mastitis, revised March 2014. Breastfeed Med. 2014;9(5):239-243. doi:10.1089/bfm.2014.9984
3. Cai L, Tong J, Zhang Z, Zhang Y, Jiang L, Hou X, *et al.* Staphylococcus aureus-induced proteomic changes in the mammary tissue of rats: a TMT-based study. PLoS One. 2020;15(5):1-17. doi:10.1371/journal.pone.0231168
4. Dawud FA, Dubo AB, Yusuf NW, Umar IA. Effects of aqueous extract of Allium cepa (red onion) on ovalbumen-induced allergic asthma in Wistar rats. Bayero J Pure Appl Sci. 2016;9(2):95-101.

doi:10.4314/bajopas.v9i2.19
5. Demon D, Breyne K, Schiffer G, Meyer E. Antimicrobial efficacy of intramammary treatment with a novel biphenomycin compound against *Staphylococcus aureus*, *Streptococcus uberis*, and *Escherichia coli*-induced mouse mastitis. J Dairy Sci. 2013;96(11):7082-7087. doi:10.3168/jds.2013-7011
6. Duan Y, Jin DH, Kim HS, Seong JH, Lee YG, Kim DS, *et al.* Analysis of total phenol, flavonoid content and antioxidant activity of various extraction solvents extract from onion (*Allium cepa* L.) peels. J Korean Appl Sci Technol. 2015;32(3):418-426. doi:10.12925/jkocs.2015.32.3.418
7. Eslami H, Batavani RA, Asr S, Hobbenaghi R. Changes of stress oxidative enzymes in rat mammary tissue, blood and milk after experimental mastitis induced by *E. coli* lipopolysaccharide. Vet Res Forum. 2015;6(2):131-137.
8. Gulsen A, Makris DP, Kefalas P. Biomimetic oxidation of quercetin: isolation of a naturally occurring quercetin heterodimer and evaluation of its *in vitro* antioxidant properties. Food Res Int. 2007;40(1):7-14.
9. Hassas-Roudsari M, Chang PR, Pegg RB, Tyler RT. Antioxidant capacity of bioactives extracted from canola meal by subcritical water, ethanolic and hot water extraction. Food Chem. 2009;114(2):717-726.
10. Helen A, Krishnakumar K, Vijayammal PL, Augusti KT. Antioxidant effect of onion oil (*Allium cepa* Linn) on the damages induced by nicotine in rats as compared to alpha-tocopherol. Toxicol Lett. 2000;116(1-2):61-68.
11. Izawa H, Kohara M, Aizawa K, Suganuma H, Inakuma T, Watanabe G, *et al.* Alleviative effects of quercetin and onion on male reproductive toxicity induced by diesel exhaust particles. Biosci Biotechnol Biochem. 2008;72(5):1235-1241.
12. Jhambh R, Dimri U, Gupta VK, Rathore R. Blood antioxidant profile and lipid peroxides in dairy cows with clinical mastitis. Vet World. 2013;6(5):271-276.
13. Kim SH, Choi KC. Anti-cancer effect and underlying mechanisms of kaempferol, a phytoestrogen, on the regulation of apoptosis in diverse cancer cell models. Toxicol Res. 2013;29(4):229-234. doi:10.5487/TR.2013.29.4.229
14. Kumar KE, Harsha KN, Sudheer V. *in vitro* antioxidant activity and *in vivo* hepatoprotective activity of aqueous extract of *Allium cepa* bulb in ethanol-induced liver damage in Wistar rats. Food Sci Hum Wellness. 2013;2(3-4):132-138.
15. Kumaraswamy NP, Latha C, Vrinda KM, Sethukekshmi C, Mercy KA. Detection of antibiotic residues in raw cow milk in Thrissur, India. J Pharm Innov. 2018;7(8):452-454.
16. Lee JY. A study on the biological activity of *Allium cepa* extract *in vivo*. J Life Sci. 2020;30(3):267-277.
17. Liguori L, Califano R, Albanese D, Raimo F, Crescitelli A, Di Matteo M. Chemical composition and antioxidant properties of five white onion (*Allium cepa* L.) landraces. J Food Qual. 2017;2017(1):1-9.
18. Lundberg A, Nyman AK, Aspan A, Borjesson S, Unnerstad HE, Waller KP. Udder infections with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* at calving in dairy herds with suboptimal udder health. J Dairy Sci. 2016;99(3):2102-2117.
19. Lykkesfeldt J, Svendsen O. Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet J. 2007;173(3):502-511.
20. Meraiyebu AB, Olaniyan OT, Anjorin YD, Shekins O, Dare BJ, Shafe MO. Effects of aqueous extract of onion (*Allium cepa*) on blood parameters in adult Wistar rats (*Rattus novergicus*). IOSR J Pharm Biol Sci. 2013;5(4):71-74.
21. Nuutila AM, Puupponen-Pimia R, Aarni M, Oksman-Caldentey KM. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chem. 2003;81(4):485-493.
22. Prakash D, Singh BN, Upadhyay G. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). Food Chem. 2007;102(4):1389-1393.
23. Ruegg PL. Investigation of mastitis problems on farms. Vet Clin Food Anim Pract. 2003;19(1):47-73.
24. Sadat A, Farag AM, Elhanafi D, Awad A, Elmahallawy EK, Alsowayeh N, *et al.* Immunological and oxidative biomarkers in bovine serum from healthy, clinical, and sub-clinical mastitis caused by *Escherichia coli* and *Staphylococcus aureus* infection. Animals. 2023;13(5):892-906.
25. Saleh N, Allam TS, Omran A, Abdelfattah AM. Evaluation of changes in hemato-biochemical, inflammatory and oxidative stress indices as reliable diagnostic biomarkers for subclinical mastitis in cows. Alex J Vet Sci. 2022;72(2):23-34.
26. Samson ES, Olasunkanmi AK, Joel JS, Alfred EF. Haematological and hepatotoxic potential of onion (*Allium cepa*) and garlic (*Allium sativum*) extracts in rats. Eur J Med Plants. 2012;2(4):290-307.
27. Sayhood MH, Essa AH, Aldeewan A, Jawad NM. Study the effect of clinical mastitis caused by *Staphylococcus aureus* on blood parameter of buffalo in northern of Basra. Basrah J Vet Res. 2018;17(3):127-135.
28. Sharma N, Mukherjee R, Ingale SL, Jadhav RK. Therapeutic and antioxidant activity of vitamin E and selenium in bovine staphylococcal mastitis. Indian J Vet Res. 2010;19(1):25-31.
29. Shoaib M, Rahman SU, Aqib AI, Ashfaq K, Naveed A, Kulyar MFEA, *et al.* Diversified epidemiological pattern and antibiogram of mecA gene in *Staphylococcus aureus* isolates of pets, pet owners and environment. Pak Vet J. 2020;40(3):331-336. Available from: http://pvj.com.pk/in_press/19-580.pdf
30. Swinkels JM, Hilkens A, Zoche-Golob V, Kromker V, Buddiger M, Jansen J, *et al.* Social influences on the duration of antibiotic treatment of clinical mastitis in dairy cows. J Dairy Sci. 2015;98(4):2369-2380.
31. Taifa S, Muhee A, Bhat RA, Nabi SU, Roy A, Rather GA, *et al.* Evaluation of therapeutic efficacy of copper nanoparticles in *Staphylococcus aureus*-induced rat mastitis model. J Nanomater. 2022;2022(1):1-12.
32. Tripathy RK, Rath PK, Mishra BP, Panda SK, Jena B. Clinicopathological and compositional changes in milk of mastitis cows. Int J Curr Microbiol Appl Sci. 2018;7(6):1680-1687.
33. Vishnuraj MR, Kandeepan G, Rao KH, Chand S, Kumbhar V. Occurrence, public health hazards and detection methods of antibiotic residues in foods of

animal origin: a comprehensive review. Cogent Food Agric. 2016;2(1):1-15.
34. Zhong K, Wang YL, Zou SX, Chen WH. Establishment of experimental mastitis model by endotoxin via teat duct in rat. Chin J Vet Sci. 2005;13(5):654-658.
35. Zigo F, Elecko J, Vasil M, Ondrasovicova S, Farkasova Z, Malova J, *et al.* The occurrence of mastitis and its effect on the milk malondialdehyde concentrations and blood enzymatic antioxidants in dairy cows. Vet Med. 2019;64(10):423-432.