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Effect of *Thespesia populnea* mediated nanoparticles on haematological parameters in murine mastitis

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Abstract

Bovine mastitis is a prevalent inflammatory disease in dairy industry which causes reduced milk yield. It is mainly due to microbial infections involving various bacteria. Among them *Staphylococcus aureus* is the most common pathogenic agent whose resistance to antibiotics have led to difficulty in the treatment of mastitis in cattle. However, recent studies have demonstrated the antimicrobial effect of metal-based nanoparticles which can be applied for treating multidrug resistant bacterial infections. Therefore, the present study was undertaken to study the effect of metal-based nanoparticles viz Silver and zinc oxide nanoparticles which were biologically synthesised from the leaves of *Thespesia populnea* in the treatment of *Staphylococcus aureus* induced mastitis in mice by studying the variations in haematological indices. A total of 48 mice were equally divided into six groups where group I served as control group while remaining groups were induced with mastitis. Groups III, IV, V and VI were treated with *Thespesia populnea* leaf extract, *T. populnea* methanolic extract mediated nano silver (TPNS), *T. populnea* methanolic extract mediated nano ZnO (TPNZ) and Ceftriaxone respectively. The blood parameters like haemoglobin, RBC, PCV, MCV, MCHC, WBC, Neutrophils, Lymphocytes, Eosinophils and Monocytes were studied. The abnormal deviation of these parameters when compared with control group indicated induction of mastitis. However, in the treated groups restoration of the above blood parameters was seen in the increasing order of III, V, VI and IV. From the results, it can be inferred that silver and zinc oxide nanoparticles have more effect in the restoration of the haematological values when compared with *Thespesia populnea* leaf extract alone due to the cumulative effect.

Keywords: Murine mastitis, TPNS, TPNZ, haematological parameters

Introduction

Mastitis is an inflammation of the mammary gland of dairy cows along with morphological, pathological, and bacterial alterations in the glandular tissue and milk. It can be classified as subclinical, clinical, or chronic, and the degree of the condition depends on the type of cause, the animal's age, breed, immunological status, and stage of lactation (Viguier *et al.*, 2009) [1]. The primary cause of it are microorganisms most commonly bacteria, which enter the udder, grow, and release toxins that damage the mammary gland (Sharma *et al.*, 2006) [2]. It has been determined that *Staphylococcus aureus* is the primary causative agent of bovine mastitis (Abdel-Rady and Sayed, 2009; Rahman *et al.*, 2010) [4, 3]. Mastitis frequently results from *S. aureus* colonization in high yielding dairy cattle which can provoke clinical mastitis but more frequently causes subclinical infections that tend to become chronic. The resistance of bacterial pathogens to antibiotic treatment has caused a massive problem in therapy of mastitis. Thus, these Multidrug-resistant (MDR) pathogens, particularly methicillin-resistant staphylococcal strains are considered to have serious effect on the livestock production leading to huge economic losses for livestock farmers.

Nanotechnology is an emerging approach involving use of nano sized particles with unusual biological properties. Particles which have a diameter of less than 100 nm are considered to be nanoparticles (Garcés *et al.*, 2021) [5]. Due to their size, shape and surface chemistry, they have been demonstrated to be efficient antimicrobial agent (Tariq *et al.*, 2022) [6]. This antimicrobial effect, in particular, is helpful in treatment of various diseases including those caused due to multi-drug resistant pathogens.

Among the various nanoparticles, metal-based nanoparticles show the most promising broad-spectrum bioactivity. Thus, they have been demonstrated to be efficient against bacteria, fungi and viruses (Tariq *et al.*, 2022)^[6]. These antimicrobial properties have led to application of nanoparticles in the treatment of mastitis. There are multiple possible methods for the synthesis of nanoparticles. On one hand, there is chemical reduction, (Khatoun *et al.*, 2023)^[7] and on other hand there are physical methods (Vishwanath and Negi, 2021)^[8] involved in the synthesis of nanoparticles. The drawback of these methods is high cost input and environmental hazard (Sharma *et al.*, 2022)^[10]. Alternatively, biological method using plant parts is an environmentally friendly approach (Garg *et al.*, 2022)^[9] to produce nanoparticles.

Therefore, in the present study the green synthesised silver and zinc oxide nanoparticles from *Thespesia populnea* leaves were used to treat the *Staphylococcus aureus* induced mastitis in mice by observing the deviation and restoration of haematological indices levels.

Materials and Methods

Synthesis of TPE mediated nano silver particles (TPNS) and nano ZnO (TPNZ) particles

Thespesia populnea solution (2%) was prepared by dissolving *T. populnea* methanolic leaf extract in the distilled water. Silver nanoparticles were synthesised by using 0.1 M silver nitrate solution, which was added 2% TPE at 95 °C with vigorous stirring and the size of particles obtained is 95nm zinc oxide nanoparticles were synthesised by adding zinc acetate to TPE at pH 12 and the size of particles obtained is 85nm

Experimental Design

Permission was obtained before starting the experiment from the Institutional Animal Ethics Committee (I/2018-3/IAEC/C.V.Sc., Hyd). Forty-eight swiss albino female mice between 10-15 days of lactation were randomly divided into six groups (n=8) as following

Group I: Normal lactating Mice treated with 20 µl of Phosphate buffer saline

Group II: Mastitis induced mice

Group III: Mastitis mice treated with 20 µl of *T. populnea* methanolic extract (TPE) dissolved in 1% aqueous DMSO solution which equates to 12.5 mg/kg body weight.

Group IV: Mastitis mice treated with 20 µl of *T. populnea* methanolic extract mediated nano silver (TPNS) that equates to 1.06 mg/kg body weight.

Group V: Mastitis mice treated with 20 µl of *T. populnea* methanolic extract mediated nano ZnO (TPNZ) which equates to 2.5 mg/kg body weight

Group VI: Mastitis mice treated with Ceftriaxone that equates to 1.25 mg/kg body weight

Induction of Mastitis in MICE

Mastitis was induced experimentally in mice as per Chandler, (1970a) with slight modifications. Lactating female albino mice of 10-15 days post-partum typically

weighing 35-40 g were used. Around 20 µl of bacterial inoculum containing 4.0×10^4 organisms were injected through the orifice of L4 abdominal mammary gland. The guidelines of the CPCSEA were followed during the procedure.

Animals were anaesthetized with ketamine and blood was collected via cardiac puncture into EDTA coated vacutainers for blood cell profile. Fully automated hematology analyzer (Medsorce ozone Bio chemicals Pvt. Ltd.) was used for haematological parameters.

The data were statistically analysed using the statistical programme for social sciences (SPSS) version 25.0 and one-way ANOVA. Duncan's multiple comparison test was used to assess differences between means, with a significance level of $p < 0.05$.

Results and Discussion

A significant ($p < 0.05$) decrease in haemoglobin, RBC, MCHC, eosinophils and monocytes values were observed in group II compared to control group as seen in table 1. Restoration of haemoglobin values is observed in different treatment groups in the increasing order of group III, V, VI and IV. However, no significant difference was observed in group V and VI with respect to haemoglobin, RBC and MCHC values. There was no significant difference was observed among the treated groups with respect to eosinophils value.

There was a significant ($p < 0.05$) increase in PCV, MCV, WBC count, neutrophils and lymphocytes in group II when compared to control group and a significant ($p < 0.05$) restoration of these values were seen in the decreasing order of III, V, VI and IV. However, no significant difference was observed between the groups IV, V and VI with respect to PCV and MCV.

The decrease in RBCs and haemoglobin group II might be due to the inflammation of lupus glands leading to increased release of oxidizing factors causing plasma membrane destruction and breakdown of haemoglobin ultimately causing decreased in the levels of RBCs and haemoglobin in group II (Sayhood *et al.*, 2018)^[11].

In Table 1, a significant ($p < 0.05$) increase in WBC count in group II is observed which might be due to the action of white blood cells as body's first line of defence against inflammatory process after infection with pathogenic bacteria in the udder (Sayhood *et al.*, 2018)^[11]. Significant increase in neutrophils in group II might be due to role of neutrophils as first line of immunological defence of the bovine mammary gland in order to phagocytose invading bacteria (Paape *et al.*, 2003)^[12]. The increased lymphocytes in blood of group II can be attributed to invasion of *S. aureus* suggesting development of a humoral response (Grönlund *et al.*, 2006)^[13].

Among the treated groups, the restoration of haematological values in group IV and V treated with TPNS and TPNZ were significantly higher compared to group III treated with TPE alone, indicating herbal mediation with nanoparticles is more effective than using herbal extract alone.

The bacterial surface adherence of silver nanoparticles, their penetration into the cell and subsequent destruction of bacterial macromolecules and intracellular structures, and their generation of ROS and free radicals that lead to oxidative stress and cellular damage cause antibacterial effect by silver nanoparticles (Peron *et al.*, 2021)^[14]. Apart from these the DNA damage in the bacterial cell resulting

from the oxidative stress induction by Zinc oxide nanoparticles causes apoptosis of bacteria (Mishra *et al.*, 2017) [15]. The antimicrobial effect of the *Thespesia populnea* is due to the presence of bioactive compounds. All these factors might have caused the reduction of inflammation due to bacterial infection thereby restoring the

blood parameters nearer to their normalcy.

Further, greater improvement in the haematological parameters by TPE mediated nanoparticles when compared to TPE extract alone might be attributed to the combined antimicrobial action of bioactive compounds of TPE and the nanoparticles.

Table 1: Mean \pm SE values of Haematological parameters of different experimental groups

Groups	Haemoglobin (g/dl)	RBC ($\times 10^6$ / μ l)	PCV (%)	MCV (fL)	MCHC (g/dl)	WBC ($\times 10^3$ / μ l)	Neutrophils ($\times 10^2$ / μ l)	Lymphocytes ($\times 10^2$ / μ l)	Eosinophils ($\times 10^2$ / μ l)	Monocytes ($\times 10^2$ / μ l)
1	12.70 \pm 0.12 ^a	9.44 \pm 0.19 ^a	39.72 \pm 0.11 ^d	53.48 \pm 0.44 ^d	32.17 \pm 0.90 ^a	6.13 \pm 0.02 ^f	2.08 \pm 0.02 ^f	7.92 \pm 0.01 ^f	3.93 \pm 0.03 ^a	3.92 \pm 0.37 ^a
2	10.93 \pm 0.07 ^c	7.07 \pm 0.12 ^e	49.40 \pm 0.75 ^a	61.27 \pm 0.64 ^a	27.12 \pm 0.51 ^c	10.66 \pm 0.07 ^a	4.14 \pm 0.01 ^a	9.04 \pm 0.01 ^a	3.72 \pm 0.06 ^b	2.47 \pm 0.01 ^f
3	11.31 \pm 0.10 ^d	7.87 \pm 0.05 ^d	46.75 \pm 0.61 ^b	59.65 \pm 0.88 ^b	28.04 \pm 0.25 ^c	9.14 \pm 0.02 ^b	3.91 \pm 0.04 ^b	8.81 \pm 0.02 ^b	3.84 \pm 0.06 ^{ab}	2.86 \pm 0.02 ^e
4	12.34 \pm 0.06 ^b	9.04 \pm 0.09 ^b	42.87 \pm 0.97 ^c	55.00 \pm 1.08 ^c	31.89 \pm 0.86 ^a	7.51 \pm 0.11 ^e	2.83 \pm 0.06 ^c	8.08 \pm 0.01 ^c	3.87 \pm 0.04 ^a	3.56 \pm 0.04 ^b
5	11.66 \pm 0.16 ^c	8.54 \pm 0.08 ^c	43.62 \pm 1.05 ^c	57.87 \pm 1.44 ^c	29.90 \pm 0.43 ^b	8.83 \pm 0.02 ^c	3.70 \pm 0.03 ^c	8.52 \pm 0.007 ^c	3.95 \pm 0.02 ^a	3.21 \pm 0.01 ^d
6	11.88 \pm 0.08 ^c	8.92 \pm 0.04 ^c	42.62 \pm 0.77 ^c	56.10 \pm 1.10 ^c	30.13 \pm 0.06 ^b	8.15 \pm 0.03 ^d	3.10 \pm 0.02 ^d	8.29 \pm 0.01 ^d	3.84 \pm 0.05 ^{ab}	3.48 \pm 0.01 ^c

Means with different superscripts in column are significantly ($p < 0.05$) different

Conclusion

Bovine mastitis is a major disease in dairy cattle whose treatment is mainly complicated by the multi drug resistant organisms such as *Staphylococcus aureus*. Newer research has found that nanotechnology-based treatment is an alternative therapy for treating various pathogenic diseases including multi drug resistant organisms. Therefore, in the present study the green synthesised metal-based nanoparticles like silver and zinc oxide nanoparticles have found to be effective in treating *Staphylococcus aureus* induced mastitis in mice. These nanoparticles have restored the haematological values more efficiently than the *Thespesia populnea* extract alone confirming their increased potency due to the combined effect of the nanoparticles as well as bioactive compounds of the plant leaf extract. However, further studies are also required for their application as potential antibiotic in the treatment of diseases like mastitis

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