

In vivo Assessment of Toxicity and Histopathological studies of bacteriocin in Wistar albino rats

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Abstract

Drug-resistant infectious organisms like MRSA are causing deadly infections to spread throughout the world. In response, several methods have lately been developed to treat them for bacteriocin development. Bacteriocins are ribosome-safe antimicrobial peptides produced by a variety of bacteria that are shown to inhibit either related or unrelated organisms. Clinically significant sensitive and drug-resistant bacteria can be controlled by bacteriocins. Their pharmacological properties and biosafety can be modified and improved with the help of bacteriocin research. The goal of the current investigation was to determine the toxic effect and histological evaluation of bacteriocin supplied orally via gastric intubation to Wistar albino rats. Both the experimental and control groups showed no deaths during the three-week experiment. Haematological measures, serum biochemical indicators, and histopathological analyses did not show any significant alterations. These results demonstrate that bacteriocin is safe, non-immunogenic, and non-toxic and may be a possibility. This work ends the argument for improving the antimicrobial efficacy of bacteriocins and figuring out their potential for toxicity and side effects. This is important for figuring out how well they could work as a potential treatment for infections caused by microorganisms that are resistant to multiple drugs.

Keywords: MRSA, bacteriocin, acute toxicity, histopathology, biosafety

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I. Introduction

Staphylococcus aureus, a methicillin resistant is a serious bacterium that affects people all over the world. MRSA infections are linked to higher rates of morbidity and mortality. 1 The efficiency of standard antibiotics has decreased due to the multi-drug resistance bacteria' quick spread. 2 New antibacterial agents required to be created. 3 There are many uses for the significant class of antimicrobial peptides known as bacteriocins in human health. 4 Relevant pathogenic microorganisms can be killed by bacteriocins. 5,6,7,8. The discovery of novel medications or therapeutic agents for use in pre-clinical trials involves extensive study utilising animal models. 9 Those mice utilized in in vivo bacteriocin effectiveness research are the most well-known animal models.

It has been challenging to choose the best bacteriocin and delivery methods. The bioavailability, stability, solubility, and sensitivity to enzyme proteolysis in blood circulatory system are crucial pharmacokinetic characteristics that affect how effective bacteriocins are. 10 The method of administration also has a significant role in determining the efficacy, duration, and side effects of a drug's pharmacological action. In murine models, the common injection methods for bacteriocin^{11,12}, including intra-nasal, intra-gastric¹³, intra-peritoneal^{14,15}, subcutaneous^{16,17,18}, and topical^{19,20}, have shown to be more effective. Soon, bacteriocin may be regarded a pharmabiotic contender.

II. Methods And Materials

Bacterial strains and culture condition:

After screening and molecular techniques including plasmid profile and plasmid cures to show the bacteriocin was plasmid-mediated, MRSA strains were collected from clinical samples. Positive bacterial strains were grown in new MRS broth for 24 to 48 hours at 37 degrees Celsius, and then the cultures were preserved using 50% glycerol at -200 degrees Celsius.

Extraction of bacteriocin (“Staphylococin”):

The synthesis of Staphylococin was chosen from the Methicillin-Resistant *Staphylococcus aureus* isolates with the broadest inhibition zone in the antibiogram test. After being grown in tryptic soy broth, obtained isolates

were inoculated with 6X10⁸ MRSA cells/ml and at 37 degrees Celsius for 3–4 days. Centrifugation was used to separate the cells for 10 min. at 5000 rpm. To purify the crude bacteriocin, the supernatant was collected and put through an ammonium salt precipitation process before being dialyzed.

Toxicity assessment:

According to the Organization of Economic Co-operation & Development's recommendations (423 (OECD, 1987), acute toxicity investigations were carried out (OECD). The experiment was carried out on male Wistar albino type rats that were ten weeks old and weighed 250–2 g. The rats were purchased from Kerala University of Agriculture in Thrissur's small animal breeding facility. The rats were divided into groups I through V and kept in polyacrylic cages at a temperature of 22 C–20 C with a maximum of six animals per cage. For the trial, they were given a conventional pelleted diet and free access to water. Before the trial began, the rats spent 7–10 days becoming used to the lab environment. According to the regulations for facilities using laboratory animals (659/02/a/CPCSEA), the investigations were carried out by the Committee for the Control and Supervision on Animal Experiments (CPCSEA).

Experimental design for toxicity studies:

Six animals each made comprised each of the five groups into which the animals were split. Overnight, just water was given to the animals while they were fasting. Rats in groups II, III, IV, and V received oral bacteriocin at dosages of 20, 40, 60, and 80 mg/kg/BW, respectively, for 21 days whereas albino rats in group I functioned as the normal control group. During the adaptation period, the body weight of each rat was determined using a sensitive balance. For 4 hours, they were strictly followed to look for any change in their posture, attitude, or motor activity.

Sample Collection:

The animals were slain using a light anesthetic solution of diethyl ether following the experimental protocol. Heart puncture blood was collected in an EDTA-containing centrifuge tube, as well as the serum, and was separated by centrifuging at 1000 for ten minutes before being used for various biochemical tests. Instantly after being immediately removed, the liver was completely cleaned with cold sterile saline and dried. A portion of the tissue samples were taken out and preserved in 10% formalin for further histological analysis.

Hematological assay:

Hematological parameters such as haemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), packed cell volume (PCV) and platelet counts were estimated from the whole blood sample. 21,22,23

Biochemical analysis:

After the toxicity tests, serum samples were used for laboratory biochemical parameter analysis. Serum glucose levels, protein concentrations, albumin estimations, and tests for liver function markers such alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and acid phosphatase all were crucial biochemical measures. 24,25,26

Histopathological analysis:

Histopathological research was done on the liver tissue samples from all of the rat groups. They were divided into tiny pieces (about 1 mm x 1 mm x 1 mm), stored in 10% normal saline for 48 hours, dehydrated by treatment in a series of various ethyl alcohol-water solution mixes (50%,80%,95%) of the respondents and in alcohol), cleaned with xylene, then embedded in paraffin. For microscopic examination of the cells, each sample was divided into very thin slices using a microtome, stained in hematoxylin and eosin, and mounted in neutral deparaffinization xylene (DPX) media. The micrographs were taken with an Axiostar plus microscope and a Canon 10.1-megapixel camcorder from Japan (Zeiss- Germany).

Statistical analysis:

Five groups' means and standard deviations were used to express the results, which were then subjected to a one-way variance analysis (ANOVA) test and a post hoc Dennett's test to assess their statistical significance. When P 0.05 and P 0.01 the values were deemed statistically significant. The software Graph Pad Prism was used to do the statistical analysis (graph pad 9.4.0).

III. Results

At various bacteriocin concentrations, the rats are subjected to an acute toxicity test. None of the concentrations tested (20, 40, 60, or 80 mg/kg/BW) resulted in a fatality; the LD 50 was determined to be greater than 2502 mg/kg/BW.

Body weight:

Table 1 displays the weight gain of the rats used in the toxicity investigation. According to the data on body weight, there was no discernible change during the course of the toxicity study.

Table 1. Mean body weight of the albino rats before and after 21 days of treatment with bacteriocin in different concentration (20,40,60 &80 mg/kg/bw

Groups	Body weight (g)			
	'0' DAY	7 th DAY	14 th DAY	21 st DAY
Group I	283.3±2.88	283.3±2.88	291.6±2.88	315.0±5.00
Group II	280.0±0.00 ^{ns}	280.0±0.00 ^{ns}	293.33±2.88 ^{ns}	315.0±5.00 ^{ns}
Group III	281.6±2.88 ^{ns}	281.6±2.88 ^{ns}	291.6±2.88 ^{ns}	318.3±2.88 ^{ns}
Group IV	281.6±2.88 ^{ns}	281.6±2.88 ^{ns}	293.3±2.88 ^{ns}	308.3±11.5 ^{ns}
Group V	281.6±2.88 ^{ns}	281.6±2.88 ^{ns}	295.0±0.00 ^{ns}	321.6±2.88 ^{ns}

Values are expressed as mean ± SD of six animals in each group

*p< 0.05 significantly different when compared group I vs group II, III, IV, V.ns - not significant

Hematological assay:

When compared with the control group, the haematological parameters from table 2, including haemoglobin, white blood cells, packed volume, RBCs, and platelets, were determined to be close to normal. The outcomes show that the bacteriocin is not harmful.

Table 2. Determination of hematological parameters from serum alysis of albino rats administered oral bacteriocin at different concentrations in acute toxicity test

Groups	Hb (g/dl)	PCV (%)	WBC (Thousands / mm ³)	RBC (millions/ mm ³)	Platelets (lakhs / mm ³)
Group I	11.56±0.11	34.73±0.40	6.96±0.20	5.63±0.15	5.26±0.20
Group II	11.36±0.15 ^{ns}	34.16±0.35 ^{ns}	7.10±0.26 ^{ns}	5.63±0.15 ^{ns}	5.10±0.30 ^{ns}
Group III	11.46±0.05 ^{ns}	34.40±0.17 ^{ns}	7.26±0.15 ^{ns}	5.53±0.25 ^{ns}	5.23±0.46 ^{ns}
Group IV	11.53±0.30 ^{ns}	34.63±0.92 ^{ns}	6.93±0.23 ^{ns}	5.66±0.15 ^{ns}	5.40±0.10 ^{ns}
Group V	11.50±0.20 ^{ns}	34.50±0.60 ^{ns}	7.16±0.32 ^{ns}	5.63±0.15 ^{ns}	5.33±0.20 ^{ns}

Values are expressed as mean ± SD of six animals in each group

*p< 0.05 significantly different when compared group I vs group II, III, IV, V.ns - not significant

Biochemical analysis:

According to the study, no statistically substantial increase in glucose was seen (84.433.6 to 94.11.53) in between minimum of 20 mg/kg/BW and a maximal of 80 mg/kg/BW, nor was there a significant increase in total protein or albumin content compared to the control group.(Figure 1)

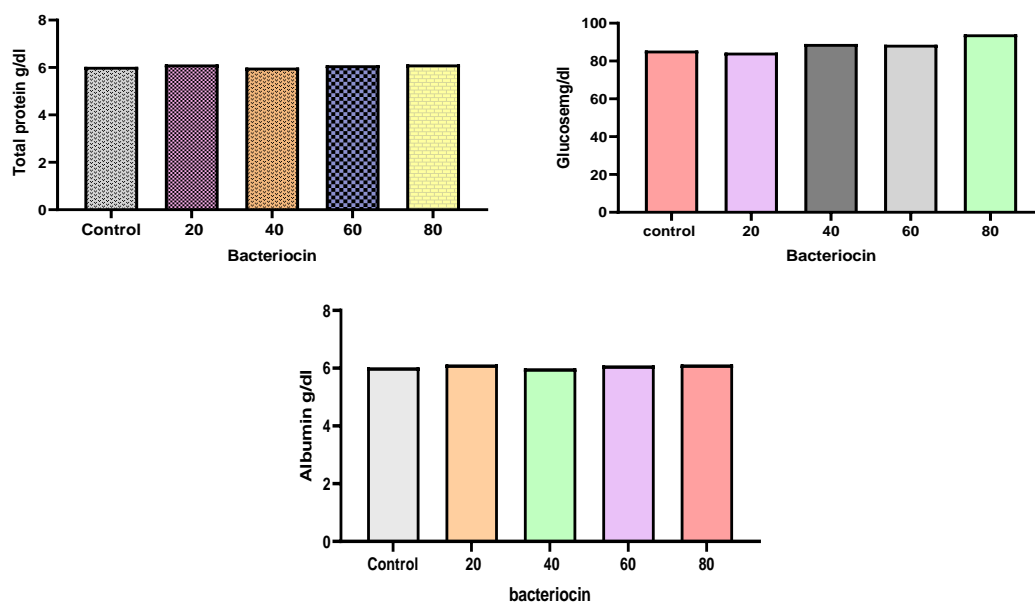


Figure 1. Determination of biochemical parameters (Total protein, glucose & albumin) from serum of Albino rats administered oral bacteriocin mg/kg at different concentrations in acute toxicity test

Histopathological analysis after acute toxicity test:

After 21 days of therapy, there was no discernible alteration in the histopathology of the liver in albino rats given doses of bacteriocin ranging from 20 mg/kg to 80 mg/kg (Figures 2a, 2b, 2c, 2d, & 2e), with the exception of the blood vessels in the hepatocytes. This indicated that bacteriocin had little to no effect on the liver following an acute toxicity test.

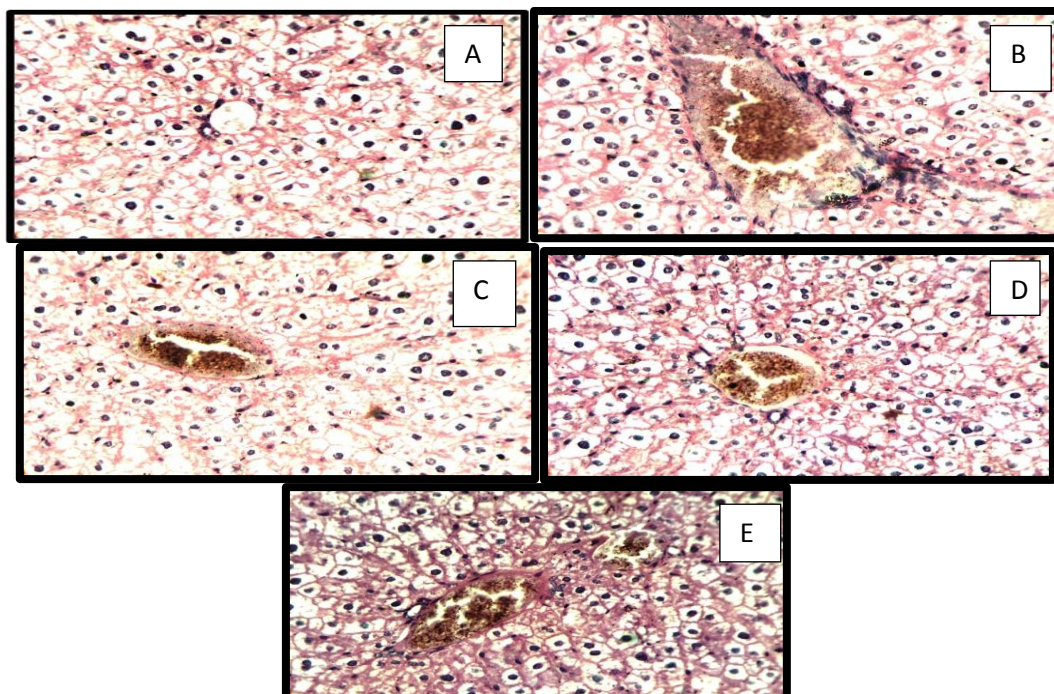


Figure 2. Histopathological analyses of liver after acute toxicity test treated with different doses of bacteriocin (A-Group I, B-Group 2, C-Group 3, D-Group 4, E- Group 5)

V. Discussion

For the discovery of new medications to treat the multi-drug resistance bacteria, the assessment of the therapeutic drugs, the complete characterisation, and requirements including such acute, sub-acute, & chronic toxicity are usually carried out. 27,28,29,30

In dose-fixing trials, daily clinical findings are of critical value.

31 No visible toxicity symptoms, including such weakness, feed avoidance, and hair loss, were present, and no group animals died during the study period. Because the animals that live should lose more than 10percent of total of its initial weight, changes in body weight are a sign of negative impacts. 32,33,34 Hematological characteristics are crucial in determining the functional ability of animals exposed to a toxin since blood serves as a pathological reflection of the entire organism. 35 Damage and the elimination of RBC cells are harmful to the body's proper operation. It serves as a significant indicator of the pathological and physiological condition of both people and animals. The PCV, Hb, WBC, RBC, and platelet count are the parameters that are measured. The consumption of some harmful chemicals can change the typical ranges of these values. 36,37

The biochemical measures assessed during acute toxicity testing, like AST, ALP, & ACP, are thought of as indicators of liver function and are frequently examined to detect bile duct obstruction. 38,39,40 The treated albino rats, who met the parameters set out by the preceding studies, showed no macroscopic alterations in the liver tissue.

VI. Conclusion

An important factor in determining whether or not an antimicrobial peptide may be used safely is the assessment of its acute toxicity. For an acute toxicity studies, the bacteriocin was given orally at doses ranging from 20 mg/kg/BW to 80 mg/kg/BW. During the experimental time, no deaths, illnesses, or abnormalities were noted. None of the biochemical parameter's indicators significantly altered. The liver tissue did not show signs of necrosis during the histological investigation. These results demonstrated the safety and non-toxicity of bacteriocin.

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