

Assessment of Genotoxicity of Marcescin Produced by Clinical Isolates of *Serratia marcescens* in vivo

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Abstract

Background: Marcescin, a proteinaceous or antimicrobial peptide produced by *Serratia marcescens*, is a Gram-negative bacterium, that uses ribosome synthesis to inhibit the growth of closely related and nonrelated bacterial strains. **Objectives:** In this research, the genotoxic effects produced by *S. marcescens* were identified on the bone marrow cells of male albino mice using the comet assay. **Materials and Methods:** *S. marcescens* was isolated from samples of urine, blood, and stool from patients at the Private Nursing Home Hospital and Baghdad Teaching Hospital. The most effective marcescin producers were selected after the cup assay was used to identify which isolates produced marcescin. By inducing the productive isolates with mitomycin-C (2 µg/mL), marcescin was obtained. The well diffusion method was employed to measure marcescin activity, and the Bradford method was used to measure protein concentration. About 2.5, 5, 10, 20, 40, and 80 mg/kg of the extract were given as intraperitoneal injection to the mice for 24 h. **Results:** According to the results, the crude marcescin has a dose-dependent harmful effect. Damage to deoxyribonucleic acid (DNA) increased significantly ($P \leq 0.01$) with increasing doses of marcescin in comet length, head diameter, %DNA in tail, and olive tail moment, respectively. While tail length showed nonsignificant differences between control and treatment groups. **Conclusion:** Marcescin causes damage to DNA in bone marrow cells of albino mice, and its damage increases with increasing dose. The comet assay is appropriate for determining this.

Keywords: Bone marrow cells, comet assay, genotoxicity, marcescin, *Serratia marcescens*

INTRODUCTION

Serratia marcescens is a bacterium belonging to the Enterobacteriaceae family that is, frequently present in water, soil, animals, insects, and plants. In critically ill or immunocompromised patients, it leads to nosocomial infections and outbreaks.^[1] By producing the three unique enzymes DNAase, lipase, and gelatinase, *Serratia* spp. can be identified from other genera.^[2]

S. marcescens causes nosocomial diseases like wound infections, catheter-associated bacteremia, and urinary tract infections.^[3] *S. marcescens* can colonize the nasopharynx and the stomach, and it occasionally causes noninvasive infections such as urinary sepsis and conjunctivitis in newborns as well as invasive infections including bloodstream infection, pneumonia, and meningitis.^[4] Marcescins are proteinaceous substances produced by ribosomes or antimicrobial peptides that are produced by generating *S. marcescens* to prevent the

propagation of unrelated or closely associated strains. They identify particular receptors on the surface of vulnerable bacterial cells in the extracellular medium after being secreted there.^[5,6] A variety of living organisms create more than 2000 distinct antimicrobial peptides (AMPs). The AMPs with the most complex AMPs are bacteriocins, which are produced by bacteria. Currently, around 280 different bacteriocins have been identified at this time.^[7] Bacteriocins were initially identified by Gratia in 1925, while trying to find methods for killing bacteria and noticed that *Escherichia coli* V could stop *E. coli* S. They were initially known as colicins. Later,

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Fredericq determined in 1946 that they were protein-containing substances. The inhibitory activity was caused by the presence of specific receptors on the surface of susceptible cells.^[8,9] Certain researchers would rather differentiate “real” bacteriocins, which include colicins and colicin-like bacteriocins, from alleged bacteriocin-like inhibitory compounds.^[10] Additionally, the wide range of bacteriocins, as well as their origins, difficulties in production, and modes of action, support the necessity for subclassifications for several other bacteriocin groups.^[11] Numerous earlier research studies supported the conclusion that bacteriocins had a lethal effect on susceptible bacterial cells, including *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, and others.^[12,13] Marcescin has various targets for sensitive cells, including the machinery of biosynthesis of the cells and membranes of bacteria, because prokaryotic organisms need particular receptors to attach the protein to the target cells.^[14] This information prompted the researchers to look into how marcescin affects eukaryotic cells, like mammalian cells without a cell wall.^[15] These marcescin may have a lethal effect on eukaryotic cells by affecting the genetic material [deoxyribonucleic acid (DNA)], the plasma membrane, or the mechanism of protein synthesis via affecting ribonucleic acid.^[16] Depending on the cell type, the sort of bacterium, the exposure period, and the dose utilized, marcescin can be hazardous to mammalian cells. Due to changes in cancer cells’ shape and size that result in them carrying specific receptors for marcescin binding, normal cells are typically less sensitive to the effects of marcescin compared with cancerous cells.^[17,18]

This study aims to assess the genotoxicity of marcescin *in vivo* by using the comet assay, which determines DNA damage.

MATERIALS AND METHODS

Isolation and identification of bacteria

A total of 200 urine, blood, and stool specimens from patients at the Private Nursing Home Hospital and the Baghdad Teaching Hospital were used to isolate *S. marcescens*. For primary identification, the specimens were cultured on eosin methylene blue agar and MacConkey agar for 18–24 h at 37°C. Microscopical, cultural, and biochemical tests (oxidase catalase, indole, citrate, methyl red, urease, Kligler iron agar, Voges–Proskauer, and lactose fermentation).^[19] The Vitek 2 system (bioMérieux, Marcy-l’Étoile, France) was used to confirm the identification of *S. marcescens*.

Detection of marcescin-producing isolates by cup assay

A screening procedure was used to examine the competitive activities of the *S. marcescens* isolates to prevent the propagation of susceptible isolates (*E. coli*). Using the cup assay, the capacity of these isolates to produce marcescin

was evaluated. The isolates were incubated with brain-heart infusion (BHI) broth (BHIB) containing 5% glycerol for 24 h at 37°C. The expanding isolates of bacteria were thickly streaked on BHI agar containing 5% glycerol and incubated at 37°C for 24 h after the initial incubation period. A 0.5 McFarland standard solution was compared with the indicator isolate suspension. The indicator isolate suspension was applied to the nutrient agar surface and 10 min of drying time at 37°C. A duplicate plate of each isolate was prepared. Discs were made on the BHI agar, which included the marker isolates, using a cork borer (5 mm in diameter). The discs were taken out of the agar medium and carefully transferred onto the nutrient agar surface that had been inoculated with the indicator isolate, followed by an overnight incubation at 37°C. The effective marcescin-producing isolates were selected based on the size of the inhibition zone surrounding the agar disk compared with that created by the marker isolate.^[20]

Extraction of crude marcescin

Each test tube containing the effective isolates was filled with 2.5 mL of sterile BHIB. Then, the test tubes were incubated at 37°C for 18 h. The appropriate flasks were filled with a liquid medium consisting of BHIB (100 mL) and 5% glycerol. The bacterial cells were initially inoculated (2.5 mL) and then incubated in a shaking incubator (150–200 cycles/min) at 37°C until the cells reached around 3×10^8 cells/mL after 14 h. To stimulate the production of marcescin after the incubation period, mitomycin-C was added to a final concentration of 2 µg/mL. This was followed by an additional 3 h of shaking incubation. The culture was centrifuged for 30 min at 7000 rpm using a cooling centrifuge. To ensure that any cells present in the supernatant were destroyed, 5 mL of chloroform was added. BHI agar was used to culture all supernatants. Then, until they were used, they were then stored at 4°C.^[21] The supernatant was used to assess marcescin activity utilizing the well method^[22] and the protein concentration was measured by the Bradford method.^[23]

Genotoxicity effects of marcescin in albino mice

Animal treatment

Male albino mice that were 6–8 weeks old, and weighed 25–30 g were used. The Iraqi Center for Cancer and Medical Genetics Research provided the mice. Under carefully controlled settings of $25 \pm 5^\circ\text{C}$ and 12 ± 2 h of light/dark cycles, all living necessities were provided, including food and drinking water. Various marcescin dosages (2.5, 5, 10, 20, 40, and 80 mg/kg) were generated according to the previously estimated LD₅₀ of *S. marcescens* marcescin.^[24] Three mice were used for each dose and 0.3 mL of each dose was administered intraperitoneally to the animals. The control mice received 0.3 mL of regular saline as their injection. The mice were killed after 18 h by cervical dislocation and dissected to obtain the femur.^[25]

Alkaline comet assay

Through an injection of phosphate-buffered saline (37°C), bone marrow was extracted from a femur bone. The final cell concentration in the suspension was 1×10^5 cells/mL. After being melted for 5 min in a beaker of boiling water, the low-melting (LM) agarose was cooled for at least 20 min in a water bath at 37°C. A suspension of cells was mixed at 1×10^5 with molten LM agarose at 37°C at a ratio of 1:10 (v/v), and 50 μ L of the resulting mixture was pipetted directly onto a comet slide. For 10 min, flat slides were maintained at 4°C in the dark. Later, a clear ring with a diameter of 0.5 mm appeared at the comet's slide edge. Slides were submerged in lysis solution at 4°C between 30 and 60 min, after that in an alkaline unwinding solution for 20 h at room temperature or 1 h at 4°C, in the dark. For the comet assay electrophoresis, 850 mL of 4°C Alkaline electrophoresis solution was added (ES-unit), and the electrophoresis slide tray was then filled with the slides. For 30 min, the power source was set to 21 V. The samples were carefully drained of excess electrophoresis solution before being submerged twice for 5 min each in distilled water (after sodium hydroxide has been dissolved) and then once for 5 min in 70% ethanol. The samples were allowed to dry for 10–15 min at 37°C. Every circle of dried agarose was incubated with 100 L of diluted SYBR green for 30 min (room temperature) in the dark. Slides were examined using fluorescence microscopy after being fully dried at 37°C. Quantification was carried out by utilizing the comet score image analysis program, which determines several parameters for every comet: comet length, head diameter, tail length, %DNA in tail, and olive tail moment.^[26,27]

Ethical approval

The study was conducted following ethical principles. It was carried out with patients' verbal and analytical approval from patients before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by the University of Baghdad College of Science's local ethics committee according to document number CSEC/0922/0089 on September 26, 2022.

Statistical analysis

The Statistical Analysis System^[28] program was used to detect the effect of different factors on study parameters (SAS institute, North Carolina, USA). The least significant difference test (analysis of variation) was used to compare between means in this study.^[28]

RESULTS

Marcescin-producing isolates

Marcescin producers were identified through cup assay, which had an average inhibition zone of 30 mm. On the other hand, isolated marcescin produced 2300 μ g/mL of protein and 320 AU/mL of activity.

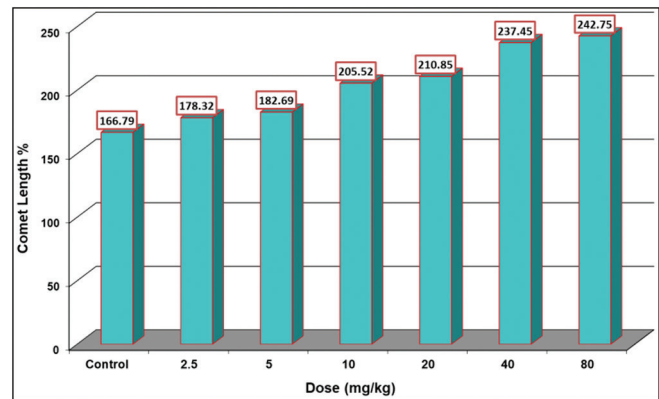


Figure 1: Effect of marcescin on DNA damage (comet length)

DNA damage caused by marcescin

Comet length

Comet length demonstrates a substantial increase in comet length value for the doses (10, 20, 40, and 80 mg/kg) in the treated mice ($205.52 \pm 5.98\%$, $210.85 \pm 6.03\%$, $237.45 \pm 10.11\%$, and $242.75 \pm 10.89\%$), respectively, compared with the control ($166.79 \pm 11.49\%$). However, no significant differences were seen between the control and doses of 2.5 and 5 mg/kg. While the results of the comparison between the doses (2.5, 5, 10, 20, 40, and 80 mg/kg) revealed a significant increase in comet length value ($242.75 \pm 10.89\%$) at the dose of 80 mg/kg ($P \leq 0.01$) [Figure 1].

The damage in DNA can be seen in Figure 2.

Head diameter

Head diameter reveals a substantial increase in head diameter value for the doses in the treated animals (2.5, 5, 10, 20, 40, and 80 mg/kg) were (156.12 ± 5.2 , $168.81 \pm 5.10\%$, $198.42 \pm 5.81\%$, $202.24 \pm 6.03\%$, $223.85 \pm 9.39\%$, and $226.009 \pm .85\%$), respectively, in comparison with the control ($147.24 \pm 7.97\%$). However, when the doses of 2.5, 5, 10, 20, 40, and 80 mg/kg were compared, the results showed a significant rise in head diameter value ($295.08 \pm 234.58\%$) at the dose of 80 mg/kg ($P \leq 0.01$) [Figure 3].

Tail length

Compared with the control ($12.43 \pm 1.34\%$), there were no significant variations at tail length ($P \leq 0.01$) between the whole doses ($13.27 \pm 1.4\%$, $13.55 \pm 1.39\%$, $13.60 \pm 3.16\%$, $13.87 \pm 1.40\%$, $16.75 \pm 4.15\%$, and $19.55 \pm 4.67\%$). Additionally, there were no discernible differences between the lower (2.5 and 5 mg/kg) and higher doses with the value nearing $19.55 \pm 4.67\%$ at the 80 mg/kg dose [Figure 4].

Percentage of DNA in tail

At dosages of 10, 20, 40, and 8 mg/kg, there were appreciable increases ($P \leq 0.01$) compared with the control ($2.40 \pm 0.41\%$) of $5.14 \pm 0.51\%$, $6.58 \pm 1.46\%$,

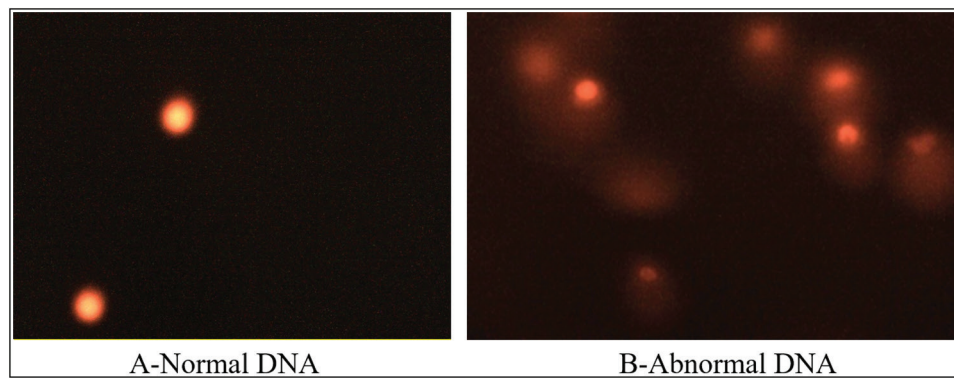


Figure 2: DNA from bone marrow cells of albino mice exposed to marcescin. (A) Normal DNA: This describes DNA that is, undamaged. (B) Abnormal DNA: This describes DNA that is, damaged

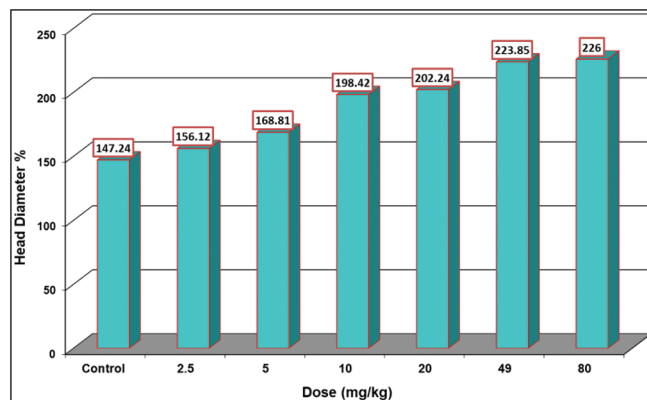


Figure 3: Effect of marcescin on DNA damage (head diameter)

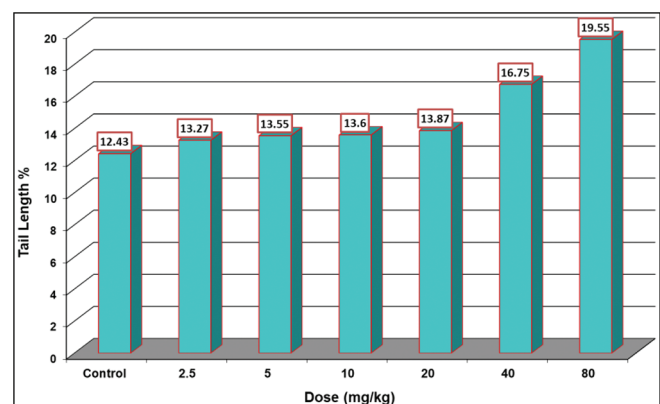


Figure 4: Effect of marcescin on DNA damage (tail length)

8.44 $\pm$ 0.79%, and 9.99 $\pm$ 1.19%, respectively. Additionally, there were no discernible differences with the doses of 2.5 and 5 mg/kg, however, a considerable rise was observed at the dose of 10 mg/kg (5.14 $\pm$ 0.51%). In addition, there were significant differences between doses (2.5, 5, 10, and 2mg/kg) and the higher dose, with a value nearing 9.99 $\pm$ 1.19% on the dose of 80 mg/kg [Figure 5].

Olive tail moment

The results revealed a substantial rise ($P \leq 0.01$) in the values of the olive moment in mice given marcescin at dosages of 10, 20, 40, and 80 mg/kg with 4.09 $\pm$ 0.99%, 5.4 $\pm$ 10.53%, 5.54 $\pm$ 1.44%, and 6.96 $\pm$ 1.38%, respectively, compared with the control (1.43 $\pm$ 0.28%). However, there were no notable variations between the lower doses (2.5 and 5 mg/kg) and the control (1.43 $\pm$ 0.28%). The comparison of the doses (2.5, 5, 10, 20, 40, and 80mg/kg) revealed significant differences between the doses 2.5, 5, and 10 with the dose of 80 mg/kg having a value of 6.96 $\pm$ 1.38% [Figure 6].

DISCUSSION

In comet length ($P \leq 0.01$), when mice were treated with doses (10, 20, 40, and 80mg/kg with 205.52 $\pm$ 5.98%,

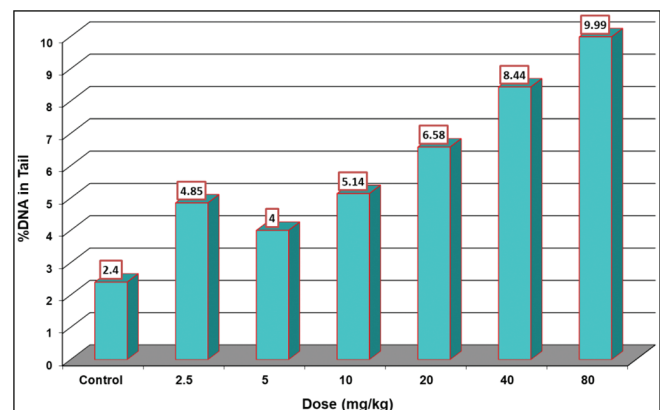


Figure 5: Comparison between difference doses in %DNA in tail

210.85 $\pm$ 6.03%, 237.45 $\pm$ 10.11%, and 242.75 $\pm$ 10.89%), significant differences appeared when compared with the control (166.79 $\pm$ 11.49%), whereas the lower doses (2.5 and 5mg/kg) showed nonsignificant differences when compared with the control. In addition, the head diameter showed significant differences ($P \leq 0.01$) when treating the animals with doses (2.5, 5, 10, 20, 40, and 80mg/kg with 156.12 $\pm$ 5.2%, 168.81 $\pm$ 5.10%, 198.42 $\pm$ 5.81%, 202.24 $\pm$ 6.035, 223.85 $\pm$ 9.39%, and 226.009 $\pm$ 0.85%), and comparing the results with the control (147.24 $\pm$ 7.97%). As for %DNA, a significant

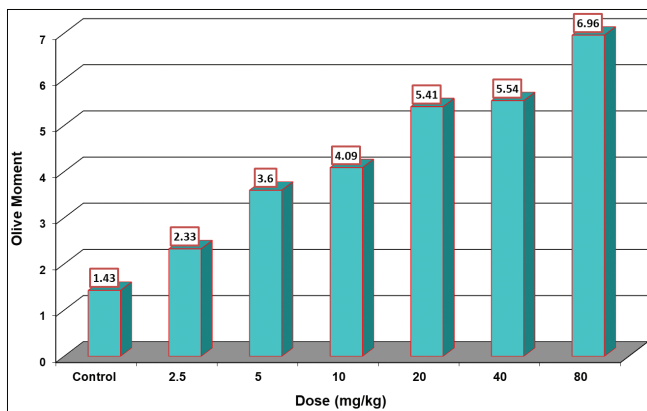


Figure 6: Comparison between different doses in olive moment

increase was shown ($P \leq 0.01$) for the elevated doses (10, 20, 40, and 80 mg/kg with $5.14 \pm 0.51\%$, $6.58 \pm 1.46\%$, $8.44 \pm 0.79\%$, and $9.99 \pm 1.19\%$), respectively, when compared with the control ($2.40 \pm 0.41\%$), whereas for the lower doses, no significant differences were shown.

In olive, the results showed significant differences ($P \leq 0.01$) from the doses (10, 20, 40, and 80 mg/kg with $4.09 \pm 0.99\%$, $5.4 \pm 10.53\%$, $5.54 \pm 1.44\%$, and $6.96 \pm 1.38\%$) compared with the control ($1.43 \pm 0.28\%$). The mice were treated with, but the small doses (2.5 and 5 mg/kg) did not show significant differences. The four-parameter results were compatible with the study conducted by Hammad and Obaid^[29] showed that the higher doses of *Citrobacter freundii* bacteriocin given to mice caused significant differences, whereas low doses caused nonsignificant differences. Tail length results appear to show non-significant differences ($P \leq 0.01$) between all doses (2.5, 5, 10, 20, 40 and 80 mg/kg with $13.27 \pm 1.4\%$, $13.55 \pm 1.39\%$, $13.60 \pm 3.16\%$, $13.87 \pm 1.40\%$, $16.75 \pm 4.15\%$, and $19.55 \pm 4.67\%$) and the control group ($12.43 \pm 1.34\%$), which disagree with the same study. Another study by Hammad^[30] with three bacteriocins, which are colicin, proticine, and *C. freundii* bacteriocin, showed that the high doses of these bacteriocins given to mice led to significant differences when compared with the control group. These results were consistent with the findings for four parameters but disagreed with the parameter tail length.

Using the alkaline approach, single-cell gel is used for identifying damage to the DNA. This method makes it possible to recognize broken double-stranded and single-stranded DNA as well as alkali-labile spots. Due to its wide range of DNA damage detection capabilities, it has become the most common method. A broken DNA strand in cells that are in interphase or mitosis can be identified by using the comet assay. These breaks can be seen in an increase in the migration of free DNA segments, which produces comet-like patterns. By detecting the exchanges between the nucleus's genetic material and the ensuing tail, a lot of researchers employed this technique to

identify DNA flaws and estimate the amount of DNA.^[31] To show the effect of chromosomal damage on DNA integrity, an alkaline comet assay was used to examine the DNA content in the comet tail. Even though it is uncertain how these effects on DNA integrity work, the effect was likely observed because the animals did not adapt to the chemosignal utilized at the stage of the bone marrow interphase nuclei. Additionally, it is probable that after 24h, the affected cells will assemble because they were unable to enter mitosis.^[32,33] Due to its simple setup and inexpensive cost, the comet test was utilized to assess DNA damage in apoptotic cells. The presence of hedgehog comets, distinguished by a short head and a lengthy or nonexistent tail, is not believed to be an indication of apoptosis, even if apoptosis was predicted using the comet assay. Numerous studies have shown a connection between necrosis and DNA fragmentation.^[31,34-36]

CONCLUSION

The comet assay has proven to be the most reliable and sensitive *in vivo* method for assessing the induction of broken DNA strands caused by marcescin.

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Conflicts of interest

There are no conflicts of interest.

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