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The Effect of Prodigiosin Extract from *Serratia rubidea* against *Citrobacter freundii* Infection in Mice

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Abstract

This study was prepared due to a lack of studies on the immune modulatory effects of prodigiosin in mice against bacterial infection and the increased demand for natural resources. This study aimed to extract prodigiosin from *Serratia rubidaea* against *Citrobacter freundii* in mice by evaluating its immunomodulatory activity and histological alterations. A total of twenty-four Swiss mice were divided up into four groups of six mice each. *C. freundii* (1×10^6 cfu/ml) was administered orally to groups (2, 3 and 4) as an infectious dosage, and one milliliter was administered to the first group as a negative control. Following 24 hours from *C. freundii* infection, the Group 3 and Group 4 groups were given crude prodigiosin extract in the following dosage amounts: the third and fourth groups received (500 and 1000 µg/ml Intraperitoneal) respectively. ELISA test was performed to assess IgM, IL-6, and IL-10 on days 3, 7 and 14. IgM and IL-6 findings demonstrated a significant increase in Group2 and Group4 with differences ($P < 0.05$), however, G3 showed a significant decrease when compared to the negative control. The results of IL-10 concentration revealed that Group 2 and Group 4 had significant decreases with differences ($P < 0.05$), with Group 3 having the highest titer. In conclusion, the study's findings showed that while prodigiosin's high concentration can boost the immune system and help laboratory animals resist bacterial infection, its low concentration acts as an immune suppressant.

Keywords

Prodigiosin, IL-6, IL-10, IgM, Immunomodulator

1. Introduction

Citrobacter species are opportunistic invasive bacterium and possible zoonotic

pathogens that can cause encephalitis, septicemia, as well as respiratory tract infections in both humans and animals [1]-[3]. Due to their accessibility and availability, natural active substances with bacteriostatic characteristics are now being extracted. When it comes to addressing the problem of bacterial resistance, they are seen to be promising alternatives to traditional antibiotics. *Serratia rubidaea* are members of the large Enterobacteriaceae family of bacteria that produce prodigiosin or PG, a dark red pigment [4]. Prodigiosin has a tripyrrole ring structure [5]. It functions as a bioactive compound with anticancer [6], antimalarial [7], antibacterial, and immunomodulatory activity [8] [9]. Previous studies on prodigiosin have focused on vitro studies that inhibit the growth of a wide spectrum of Gram-positive and Gram-negative bacteria using disc diffusion [8] [9].

The *in vivo* results of certain studies suggest that prodigiosin administered orally improves the intestinal microbiota of mice and propose that prodigiosin is a promising candidate medication to treat intestinal inflammation since it enhances the intestinal microbiota of mice and is not harmful to their internal organs [10]. According to other studies, prodigiosin and undecyl prodigiosin altered inflammatory signals and potentially reduced the risk of atherosclerotic plaque in mice [11]. T cells may be impacted by the immunomodulatory actions of PG [12]. Furthermore, by promoting leukocyte recruitment and polarizing T cells, PG was able to decrease the levels of circulating IL-2 and TNF, two important variables in regulating the chronic immune response [13]. To clarify prodigiosin's potential biological function in microbial infection during the assessment of immune modulatory effect and histopathological changes for that, the aim of this research study the effect of prodigiosin extract from *Serratia rubidea* against *Citrobacter freundii* infection in mice.

2. Material and Method

2.1. Ethical Approval

Ethical approval was granted through the local committee of animal care and use at the College of Veterinary Medicine within the University of Baghdad (Number P-G/649, 24/3/2024) during this study.

2.2. Isolation and Identification

S. rubidea was isolated from one hundred fecal cattle samples in Baghdad, each sample was inoculated onto the chrome agar, MacConkey agar, and Nutrient agar, then incubated at 37˚C for 24 - 48 hrs. [14]. The Serratia isolates were identified at the species level using the traditional morphological, and biochemical tests, vitek2 compact system, and PCR [15]. *Citrobacter freundii* was obtained from the University of Baghdad's College of Veterinary Medicine's microbiology department accession No. OR766039.

2.3. Preparation of Crud Prodigiosin Extract

Prodigiosin preparation was extracted by [16]. The *Serratia rubidea* was cultured

on ten nutrient agar plates and incubated at room temperature (28˚C) for 2 days. Observe the colonies growing in confluence with the bright red color. Only the colonies with the bright red color were harvested from all plates in sterile saline and collected in centrifuge tubes. The cells were centrifuged at 3000 rpm for 20 min. The suspension was then digested in a glass test tube by adding 1 N (NaOH) twice the volume of the suspension and then put in a water bath at 100˚C for 1 hour, the pigment was removed from the suspension by adding the equal volume of ethanol, the tubes were centrifuged for 20 min at 3000 rpm. To obtain a color solution free of turbidity, petroleum ether was added and thoroughly mixed with the colored solution. The mixture was then vigorously shaken and blended using a vortex mixer. For layer separation, the tubes were allowed to settle for 10 minutes. In an evaporating dish, the top layer was collected. By heating a water bath to 100˚C, an acquired solvent was evaporated till dry residue was left. Put the leftovers in 5 ml of acidified ethanol. Crud prodigiosin concentration is estimated using spectrophotometer absorbance fixed at 535 nm [16].

2.4. Experimental Design

Twenty-four healthy Swiss mice of both sexes aged between 7 - 8 weeks, weighted 13 - 17 g were divided into 4 groups (each group 6 mice). Groups [3] [4] were inoculation with an infectious dose of *Citrobacter freundii* (1×10^6 cfu/ml orally [17]. 2nd group positive control inoculation with infectious dose *Citrobacter freundii* (1×10^6 cfu/ml orally) [17]. 1st group negative control was injected with (1ml PBS). After 24 hours post-infection with *C. freundii*, the Group 3 and Group 4 groups administered crud prodigiosin extract as follows: the 4th group administered (1000 µg/ml I.P). 3rd group administered (500 µg/ml I.P). Blood samples were collected on days 3, 7 and 14 from all mice groups, and sera were separated for estimating IL-6, IL-10, and IgM concentration by ELISA kits (Elabscience, China, for mice). Two mice from each group were used for histopathological changes at day 7, specimens were taken from the liver, kidney, spleen, and intestine. The tissues were fixed in a 10% formalin solution immediately after removal [18].

2.5. Statistical Analysis

Data were subjected to analysis using SAS (Statistical Analysis System—version 9.1). Two-way ANOVA with interaction and least significant differences (LSD) was performed to assess significant differences among means. Results are expressed as mean ± standard error. $P < 0.05$ is considered statistically significant [19].

3. Results

3.1. Isolation and Identification

Thirty isolates of *S. rubidea* out of one hundred fecal samples from cattle and the colonies of *S. rubidea* were observed in red colonies on nutrient (**Figure 1**). While dark pink to red on MacConkey agar and Chrome agar.

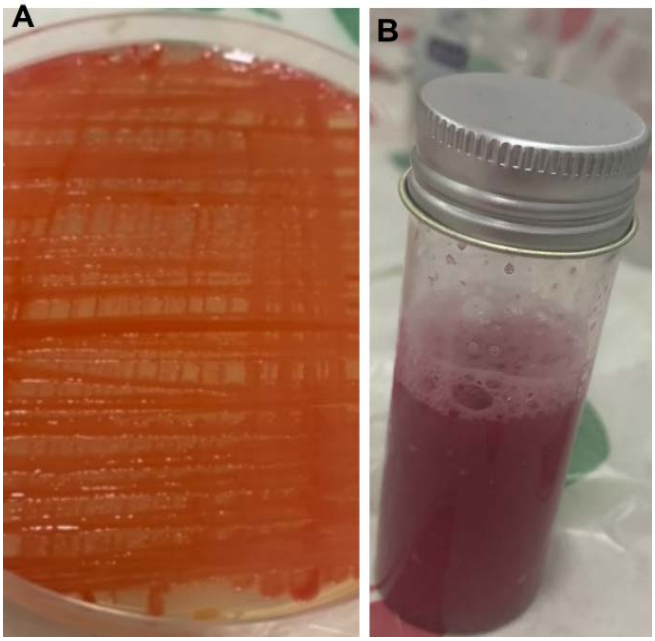

Figure 1. *Serratia rubidaea* (A) colonies on N.A. (B) crud PG.

To confirm the identification of *S. rubidea* vitek 2 compact system was done and gave 99% Probability to *Serratia rubidae*. The isolate of *Serratia rubidea* was registered by GenBank under Accession No. (OR757107.1). This isolate was used for crud prodigiosin extract, the prodigiosin production was high at 28˚C for 48 h yielding a final concentration of 130 mg/L, and two concentrations were prepared (1000 and 500 µg/ml).

3.2. Immune Response to Prodigiosin

3.2.1. IL-6 Concentration for Mice

The IL-6 concentration results for groups Group 2 and Group 4 showed a significant increase on days 7 and 14, with significant differences ($P < 0.05$) compared to the negative control group. Group 3 showed a moderate increase at day 7, relative to Group 1 (the control group); Group 2 (Positive control) showed the highest IL-6 concentration at days 3 and 7, with 824.71 pg/ml and 955.28 pg/ml, followed by Group 4 at days 3 and 7, with 817.73 pg/ml at day 7 and 952 pg/ml, as indicated in Figure 2.

3.2.2. IL-10 Concentration for Mice

Comparing the IL-10 concentration data for groups Group 2 and Group 4 to the negative control group on days 3, 7, and 14 revealed no significant variations. Figure 3 shows that, in comparison to the other groups, Group 4 had the lowest level of IL-10 concentration on days 3, 7, and 14 (64.83, 52.41, and 41.48 pg/ml), followed by Group 2 which also had the lowest concentration of IL-10 on the same days (64.81, 55.20, and 42.08 pg/ml). Group 3 had the highest IL-10 concentration on days 3, 7, and 14 (129.91, 450.06, and 835.54 pg/ml) (Figure 3).

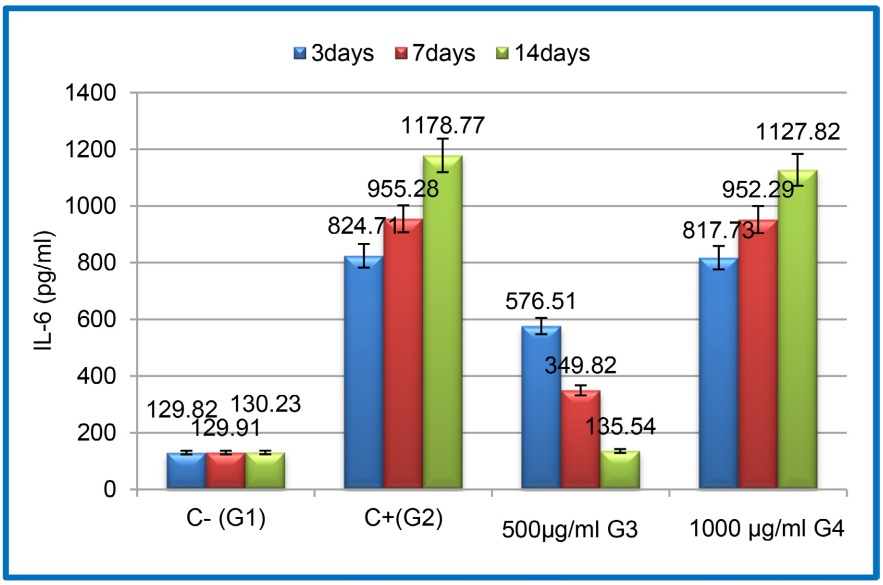

Figure 2. IL-6 concentration administered with different doses by ELISA test.

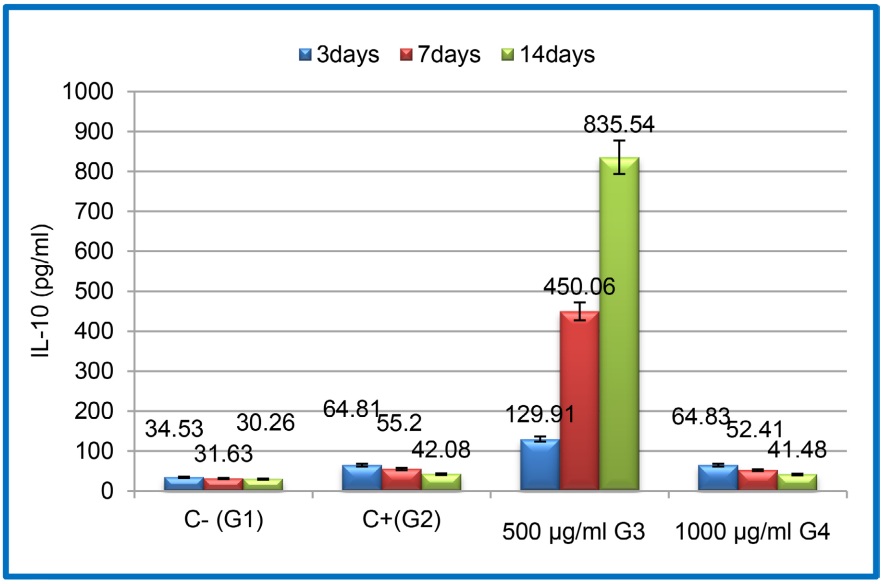

Figure 3. IL-10 concentration administered with different doses by ELISA test.

3.2.3. IgM Concentration in Mice

Results revealed that the differences among groups within each period were significant ($P < 0.05$). The concentration of IgM at all periods in the Group 4 (60.01 pg/ml) and Group 2 (59.35 pg/ml) did not differ significantly but they were significantly ($P < 0.05$) higher than other groups. Concerning the differences among periods within each group, results showed that the IgM in Group 2 and Group 4 increased significantly with advanced period while the Group 3 showed significant decreasing (62.46 pg/ml) at 14 days as compared with 7 days (95.43 pg/ml). On the other hand, the differences among periods in G1 were not significant (Figure 4).

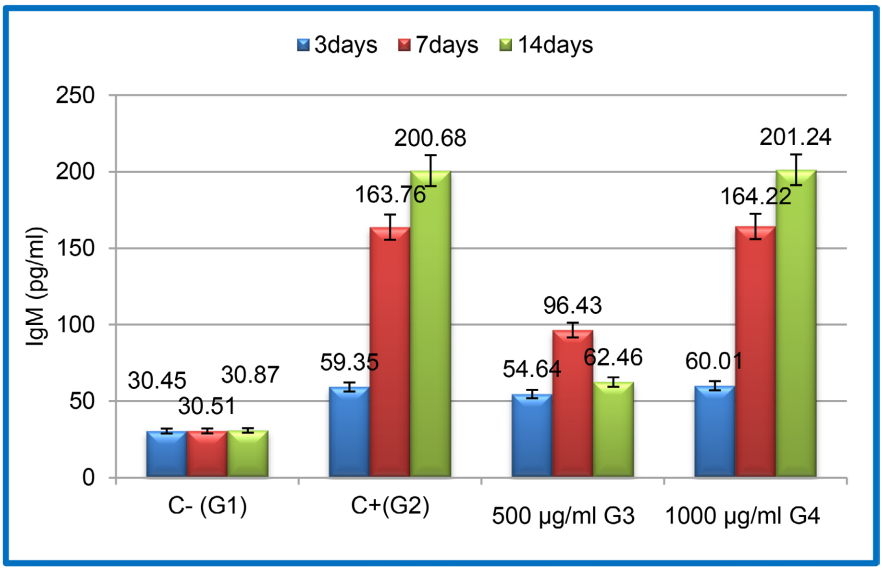

Figure 4. IgM concentration in the administered groups with different doses by ELISA test.

3.3. Histopathological Changes

Following a 7-day histological examination revealed that all groups under investigation had varying histopathological alterations. Group 2: the liver shows marked portal fibrosis with marked bridge formation, proliferation of choanocytes, and degeneration with necrosis of hepatocytes (Figure 5). Also, the liver shows marked vascular degeneration of hepatocytes, aggregation of MNCs, and portal congestion (Figure 6). The liver of G2 shows marked deterioration with damage to the cytoarchitecture of the hepatic (Figure 7). Kidney shows marked focal nephritis characterized by local necrosis of renal tubules with aggregation of MNCs (Figure 8). kidney marked interstitial nephritis with necrosis of renal tissue and aggregation of MNCs and tubular dilation (Figure 9). spleen shows marked sinusoidal congestion with distention, and slight atrophy of lymphoid follicles with marked irregular shapes (Figure 10). enteritis marked thickening of villi with marked hyperplasia of lining cells with severe infiltration of mononuclear leukocytes (Figure 11). Group 4: liver marked disarrangement of hepatic cords with sinusoidal dilation and degeneration of hepatocytes (Figure 12). also marked disarrangement of hepatic cords with atrophy of hepatocytes and sinusoidal congestion (Figure 13). kidney marked nephritis with severe tissue damage and aggregation of MNCs, degeneration with necrosis of lining cells of renal tubules, degeneration of podocytes of glomerulus (Figure 14). Spleen showed marked lymphoid hyperplasia within follicles of splenic white pulp and congestion with dilation of splenic sinuses with proliferation of megakaryocytes (Figure 15). The intestine showed hypernulcation of enterocytes (Figure 16). Group 3: liver severe per central cellular swelling with necrosis of hepatocytes (Red arrows) and per central vein (V) aggregation of MNCs (Figure 17). kidney marked dilation of bowman capsule with tubular dilation with accumulation of non-cellular eosinophilic substance (Figure 18). spleen shows splenic sinus dilation with the

proliferation of megakaryocytes (Figure 19). The intestine shows hyperplasia of lining cells of intestinal glands involving paneth cells (Figure 20).

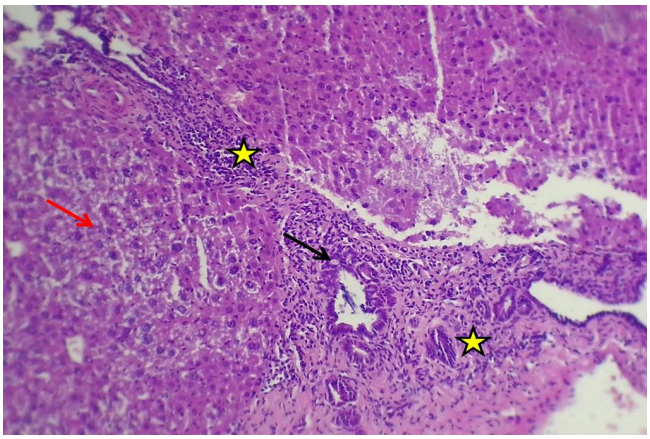

Figure 5. G2 the liver shows marked portal fibrosis with marked bridge formation (Asterisks) proliferation of cholangiocytes (Black arrow) & degeneration with necrosis of hepatocytes (Red arrow).

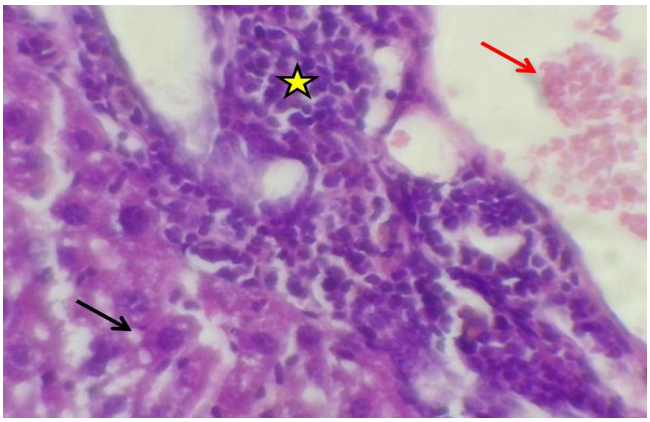

Figure 6. G2 the liver shows marked vacular degeneration of hepatocytes (Black arrow), aggregation of MNCs (Asterisk) & portal congestion (Red arrow). H&E. 400×.

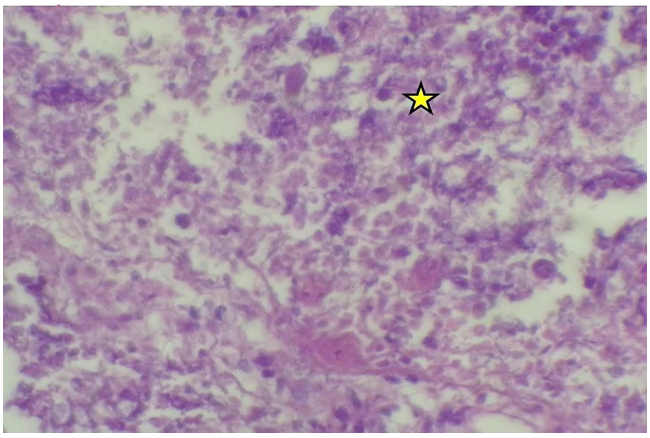

Figure 7. G2 the liver shows marked deterioration with damaged of cytoarchitecture of the hepatic (Asterisk) H&E. 400×.

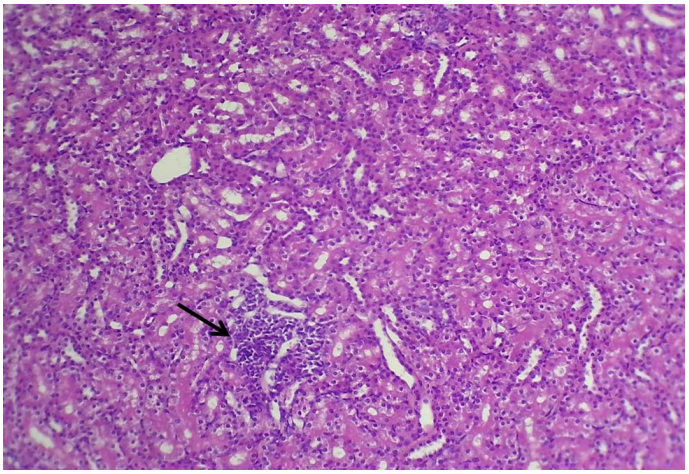

Figure 8. G2: kidney shows marked focal nephritis characterized by local necrosis of renal tubules with aggregation of MNcs. H&E stain. 100×.

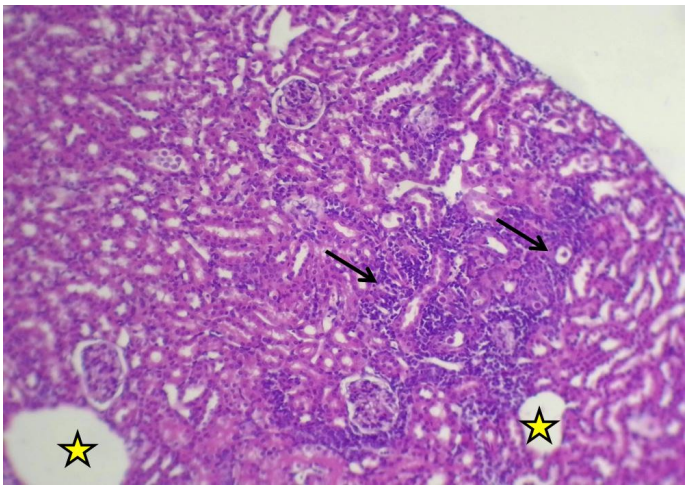

Figure 9. G2 kidney marked interstitial nephritis with necrosis of renal tissue and aggregation of MNCs (Arrows) and tubular dilation (Asterisks). H&E. 100×.

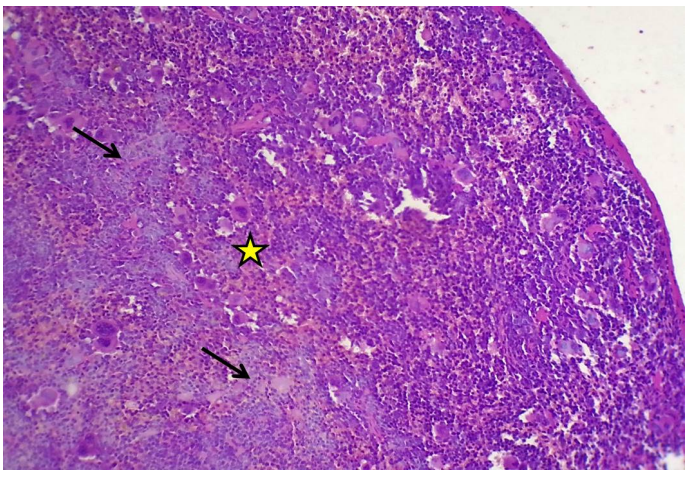

Figure 10. G2 spleen shows marked sinusoidal congestion with distention (Asterisk), slight atrophy of lymphoid follicles with marked irregular shapes (Arrows).

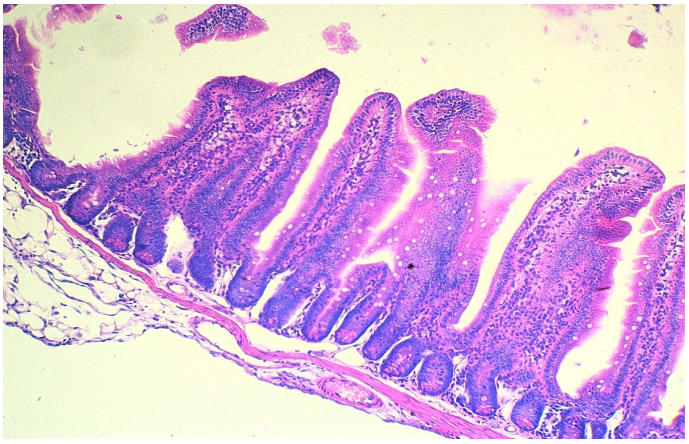

Figure 11. G2 enteritis marked thickening of villi with marked hyperplasia of lining cells with sever infiltration of mononuclear leukocytes. H&E. 100×.

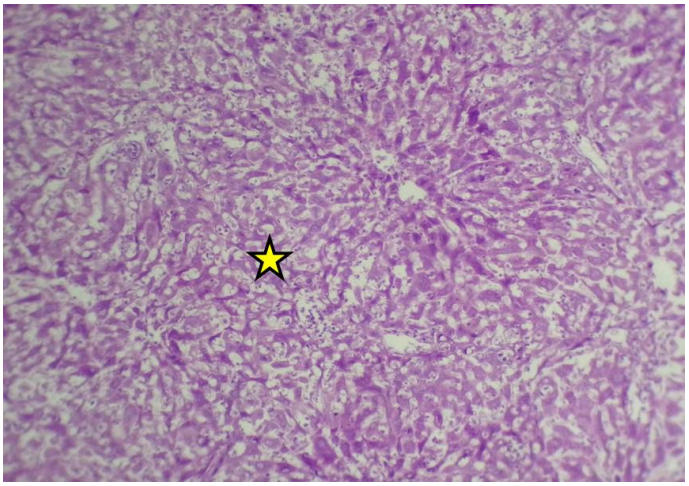

Figure 12. G4 liver marked disarrangement of hepatic cords with sinusoidal dilation and degeneration of hepatocytes (Asterisk). H&E. 100×.

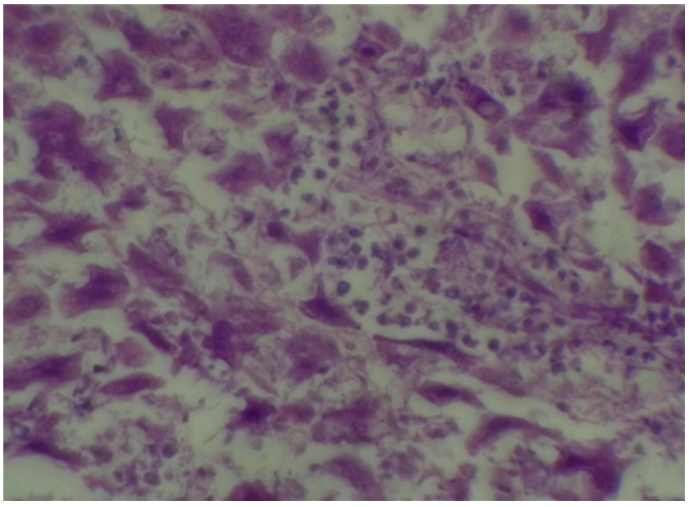

Figure 13. G4 liver marked disarrangement of hepatic cords with atrophy of hepatocytes and sinusoidal congestion. H&E. 100×.

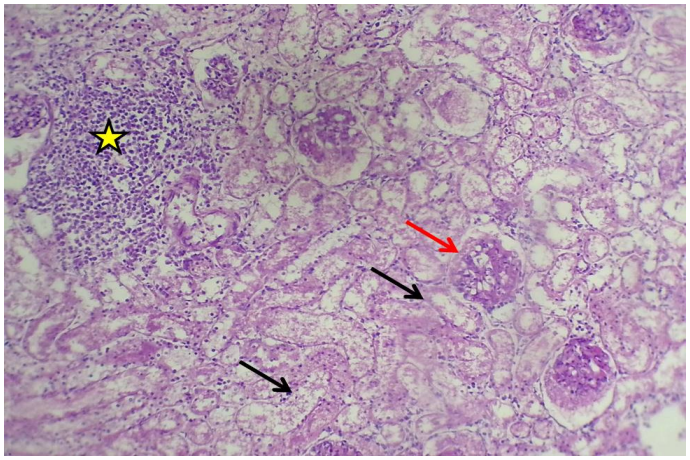

Figure 14. G4 kidney marked nephritis with sever tissue damage and aggregation of MNCs (Asterisk), degeneration with necrosis of lining cells of renal tubules (Black arrows), degeneration of podocytes of glomerulus (Red arrow). 100×.

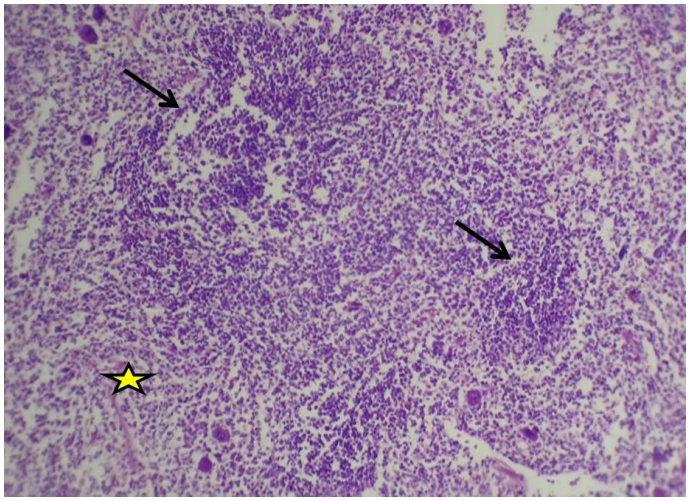

Figure 15. G4 spleen showed marked lymphoid hyperplasia within follicles of splenic white pulp (Arrows) and congestion with dilation of splenic sinuses with proliferation of megakaryocytes (Asterisk). H&E. 40×.

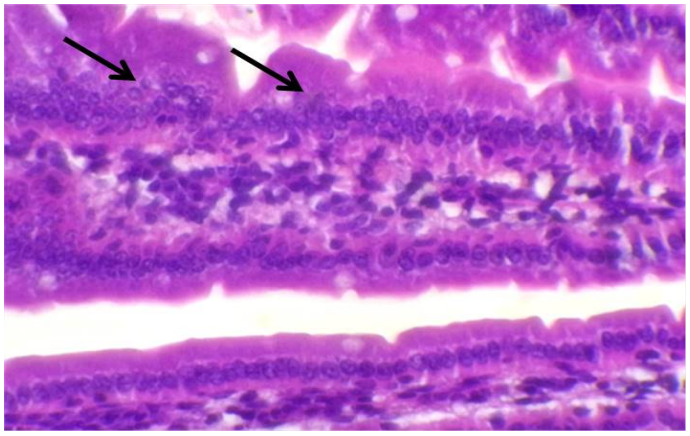

Figure 16. G4 Intestine hypernulcation of enterocytes (Arrows). H&E. 400×.

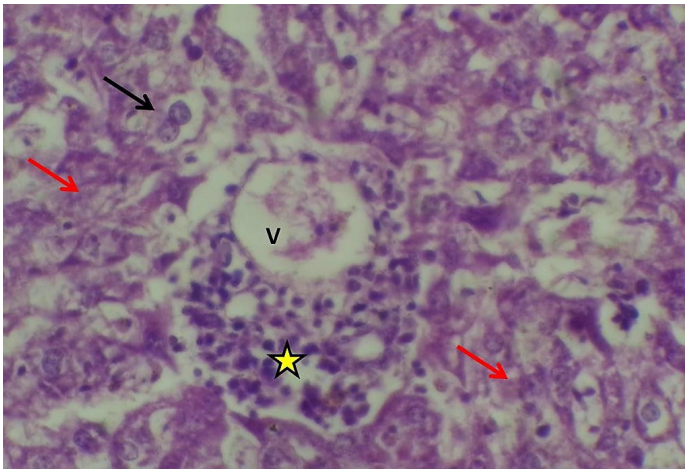

Figure 17. G3 liver severe per central cellular swelling (Black arrow) with necrosis of hepatocytes (Red arrows) and per central vein (V) aggregation of MNCs (Asterisk). H&E. 400×.

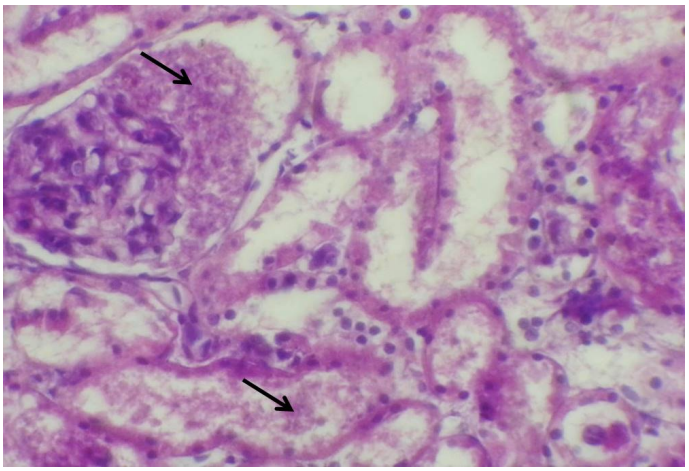

Figure 18. G3 kidney marked dilation of bowman capsule with tubular dilation with accumulation of non-cellular eosinophilic substance (Arrows). H&E. 400×.

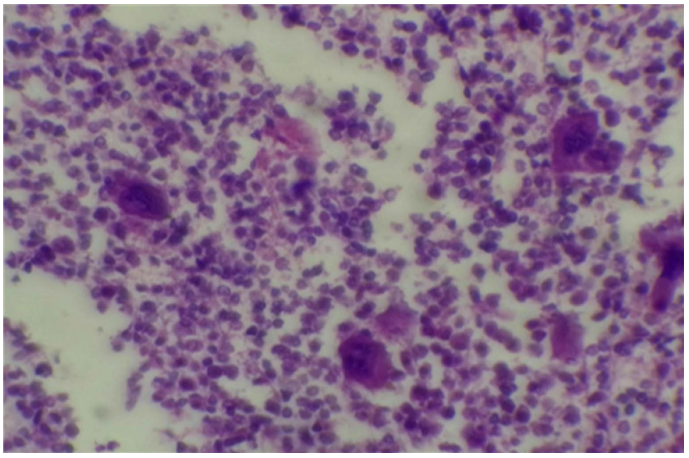

Figure 19. G3 spleen shows splenic sinuses dilation with proliferation of megakaryocytes. H&E. 400×.

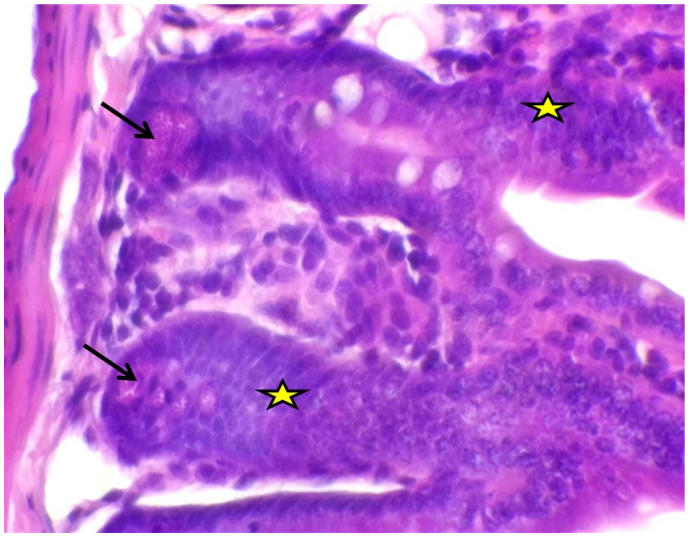

Figure 20. G3 intestine shows hyperplasia of lining cells of intestinal glands (Asterisks) involve Paneth cells (Arrows). H&E. 400×.

4. Discussion

A strain of *S. rubidaea* that produced red pigment was identified in this investigation. The isolated strain Accession No. (OR757107.1) was used to extract prodigiosin from the bacterium and it was found that the crude prodigiosin conc. had 130 mg/ml. These findings concur with those of [20] who discovered that 120 mg/ml of prodigiosin conc. was isolated from Serratia ssp. This study looked at the immunological characteristics linked to PG in the context of bacterial infection because PG is a powerful immunomodulator that affects lymphoreticular cells, including B cells, T cells, and macrophages. The results of IL-6 concentration give the highest significant increase in G2 (824.71 pg/ml and 955.28 pg/ml) and G4 (817.73 pg/ml and 952 pg/ml) at days 3 and 7. while, G3 showed a decrease in IL-6 concentration all day compared to other groups, as well as the result of IgM concentration in G2 and G4 increased significantly with advanced period while the G3 showed significant decreasing (62.46 pg/ml) at 14 days as compared with 7 days (95.43 pg/ml). The results may return to activate the immune system and, at high concentrations, function as a mitogen, numerous studies have demonstrated that biosurfactants can promote cell differentiation and the cellular immune response rather than just cell proliferation. Additionally, they observed that biosurfactants inhibited macrophage cells' capacity to create pro-inflammatory cytokines and stimulated lymphocytes' production of anti-inflammatory cytokines, thereby acting as an anti-inflammatory [21]-[23]. This is consistent with [24], who viewed this as an effort to show how some biological components might boost the immune system's effectiveness. Research has demonstrated that prodigiosin family chemicals can specifically control T lymphocyte proliferation [25] and macrophage responsiveness to inflammatory stimuli [26]. Prodigiosin exhibits potent antimicrobial properties against a variety of microorganisms, including oxacillin-resistant *S. aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*,

Streptococcus pyogenes, *Acinetobacter* sp., and *Escherichia coli*. All of these results support prodigiosin's anti-inflammatory and immune-boosting properties [27]. The results of the G3 decrease in the concentration of IL-6 and IgM may be due to low concentration and prodigiosin has been discovered to have immunosuppressive effects on immunological responses, both humoral and cellular, it prevents T lymphocytes, natural killer cells, macrophages, and lymphocytes from proliferating [28]. The results of IL-10 concentration showed a significant decrease in G2 and G4, while a significant increase in G3, these results revealed that pigmentation can regulate the production of cytokines to have immunomodulatory effects: it promoted the expression of IL-10 while downregulating TNF-α and IL-6 expression [29]. These results of the histopathological study demonstrated the presence of severe histopathological effects in the specimens of mice caused by their infection with *C. freundii*, compared to the administered group. The histological sections matched the findings of [30] who reported that the presence of lipopolysaccharide receptors on the surfaces of various cells, whether free or enveloping, proteins bound to LPS binding protein represent the germ and these receptors. The histopathological examination of infected to gram-negative bacteria is represented by tissue necrosis and the breakdown of blood vessels which in turn leads to death through its association with immune system cells as it works to induce them to release inflammatory mediators (proinflammatory mediators), including cytokines, which can cause physiological and pathological changes [31].

5. Conclusion

Prodigiosin is a significant medical and therapeutic chemical. The study's findings showed that while prodigiosin's high concentration can operate to boost the immune system and help laboratory animals resist bacterial infection, its low concentration acts as an immune suppressant.

Acknowledgements

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Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication and or funding of this manuscript.

References

[1] Yimer, N., Asseged, B. (2017) Aerobic Bacterial Flora of the Respiratory Tract of Healthy Sheep Slaughtered in Dessie Municipal Abattoir, Northeastern Ethiopia. *Revue de Medecine Veterinaire*, **158**, 473-478.

[2] Liu, H., Zhao, Z., Xue, Y., Ding, K. and Xue, Q. (2017) Fatal Cases of *Citrobacter freundii* Septicemia and Encephalitis in Sheep. *Journal of Veterinary Diagnostic Investigation*, **30**, 245-248. https://doi.org/10.1177/1040638717731090

[3] Raphael, E. and Riley, L.W. (2017) Infections Caused by Antimicrobial Drug-Resistant Saprophytic Gram-Negative Bacteria in the Environment. *Frontiers in Medicine*, **4**, Article 183. https://doi.org/10.3389/fmed.2017.00183

[4] Pereira, R.F.S. and de Carvalho, C.C.C.R. (2024) Improving Bioprocess Conditions for the Production of Prodigiosin Using a Marine *Serratia rubidaea* Strain. *Marine Drugs*, **22**, 142. https://doi.org/10.3390/md22040142

[5] Zhou, W., Li, J., Chen, J., Liu, X., Xiang, T., Zhang, L., *et al.* (2016) The Red Pigment Prodigiosin Is Not an Essential Virulence Factor in Entomopathogenic *Serratia marcescens*. *Journal of Invertebrate Pathology*, **136**, 92-94. https://doi.org/10.1016/j.jip.2016.03.011

[6] Lazic, J., Skaro Bogojevic, S., Vojnovic, S., Aleksic, I., Milivojevic, D., Kretzschmar, M., *et al.* (2022) Synthesis, Anticancer Potential and Comprehensive Toxicity Studies of Novel Brominated Derivatives of Bacterial Biopigment Prodigiosin from *Serratia marcescens* ATCC 27117. *Molecules*, **27**, Article 3729. https://doi.org/10.3390/molecules27123729

[7] Papireddy, K., Smilkstein, M., Kelly, J.X., Shweta Salem, S.M., Alhamadsheh, M., *et al.* (2011) Antimalarial Activity of Natural and Synthetic Prodiginines. *Journal of Medicinal Chemistry*, **54**, 5296-5306. https://doi.org/10.1021/jm200543y

[8] Yip, C., Mahalingam, S., Wan, K. and Nathan, S. (2021) Prodigiosin Inhibits Bacterial Growth and Virulence Factors as a Potential Physiological Response to Interspecies Competition. *PLOS ONE*, **16**, e0253445. https://doi.org/10.1371/journal.pone.0253445

[9] Gohil, N., Bhattacharjee, G. and Singh, V. (2020) Synergistic Bactericidal Profiling of Prodigiosin Extracted from *Serratia marcescens* in Combination with Antibiotics against Pathogenic Bacteria. *Microbial Pathogenesis*, **149**, Article ID: 104508. https://doi.org/10.1016/j.micpath.2020.104508

[10] Li, X., Tan, X., Chen, Q., Zhu, X., Zhang, J., Zhang, J., *et al.* (2021) Prodigiosin of *Serratia marcescens* ZPG19 Alters the Gut Microbiota Composition of Kunming Mice. *Molecules*, **26**, Article 2156. https://doi.org/10.3390/molecules26082156

[11] Cuevas, A., Saavedra, N., Salazar, L.A., Cavalcante, M.F., Silva, J.C. and Abdalla, D.S.P. (2020) Prodigiosin Modulates the Immune Response and Could Promote a Stable Atherosclerotic Lession in C57bl/6 *Ldlr*-/- Mice. *International Journal of Molecular Sciences*, **21**, Article 6417. https://doi.org/10.3390/ijms21176417

[12] Oppi, S., Lüscher, T.F. and Stein, S. (2019) Mouse Models for Atherosclerosis Research—Which Is My Line? *Frontiers in Cardiovascular Medicine*, **6**, Article 46. https://doi.org/10.3389/fcvm.2019.00046

[13] Ait-Oufella, H., Taleb, S., Mallat, Z. and Tedgui, A. (2011) Recent Advances on the Role of Cytokines in Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **31**, 969-979. https://doi.org/10.1161/atvbaha.110.207415

[14] Quinn, P.J. (2011) Veterinary Microbiology and Microbial Disease.

[15] Ahmed, R.N. and Al-Samarraae, I.A.A. (2021) Molecular and Biochemical Study of *Serratia marcescens* Isolated from Sheep in Baghdad Province. *Natural Volatiles and Essential Oils*, **8**, 12647-12657. https://www.nveo.org/index.php/journal/article/view/2669

[16] Maurya, K.K., Tripathi, A.D., Kumar, D. and Srivastava, S.K. (2020) Production, Purification and Characterization of Prodigiosin by Serratia Nematodiphilia (NCIM 5606) Using Solid-State Fermentation with Various Substrate. *Annals of Phytomedicine: An International Journal*, **9**, 302-306. https://doi.org/10.21276/ap.2020.9.2.30

[17] Mohammed, R.J. (2021) Isolation and Identification of *Citrobacter freundii* from

Sheep with Investigation the Immunological Responses in Rabbits. Ph.D. Thesis, University of Baghdad.

[18] Al-Eqabi, S.R.S. and Al-Abedi, G.J.K. (2021) Pathological, Immunological, and Hematological Parameters Associated with Experimental Infection of *Citrobacter freundii* in Rabbits. *DOAJ*: *Directory of Open Access Journals*, **76**, 1607-1615. https://doi.org/10.22092/ari.2021.356801.1911

[19] SAS/STAT (2010) Users Guide for Personal Computer. Release 9. 1. SAS Institute, Inc.

[20] Song, M., Bae, J., Lee, D., Kim, C., Kim, J., Kim, S., *et al.* (2006) Purification and Characterization of Prodigiosin Produced by Integrated Bioreactor from *Serratia* sp. Kh-95. *Journal of Bioscience and Bioengineering*, **101**, 157-161. https://doi.org/10.1263/jbb.101.157

[21] Stipcevic, T., Knight, C.P. and Kippin, T.E. (2013) Stimulation of Adult Neural Stem Cells with a Novel Glycolipid Biosurfactant. *Acta Neurologica Belgica*, **113**, 501-506. https://doi.org/10.1007/s13760-013-0232-4

[22] Tang, J., Zhao, F., Gao, H., Dai, Y., Yao, Z., Hong, K., *et al.* (2010) Characterization and Online Detection of Surfactin Isomers Based on HPLC-MS^n Analyses and Their Inhibitory Effects on the Overproduction of Nitric Oxide and the Release of TNF-α and IL-6 in LPS-Induced Macrophages. *Marine Drugs*, **8**, 2605-2618. https://doi.org/10.3390/md8102605

[23] Park, S.Y. and Kim, Y. (2009) Surfactin Inhibits Immunostimulatory Function of Macrophages through Blocking NK-κB, MAPK and Akt Pathway. *International Immunopharmacology*, **9**, 886-893. https://doi.org/10.1016/j.intimp.2009.03.013

[24] Al-Wazni, W.S. (2018) Immunological Effects of Biosurfactant Extract from *Serratia marcescens* in Mice. *Al-Kufa University Journal for Biology*, **10**, 147-159. https://core.ac.uk/download/pdf/235693224.pdf

[25] Lee, M.H., Kataoka, T., Honjo, N., Magae, J. and Nagai, K. (2000) *In Vivo* Rapid Reduction of Alloantigen-Activated $Cd8^+$ Mature Cytotoxic T Cells by Inhibitors of Acidification of Intracellular Organelles, Prodigiosin 25-C and Concanamycin B. *Immunology*, **99**, 243-248. https://doi.org/10.1046/j.1365-2567.2000.00961.x

[26] Huh, J., Yim, J., Lee, H., Moon, E., Rhee, D. and Pyo, S. (2007) Prodigiosin Isolated from *Hahella chejuensis* Suppresses Lipopolysaccharide-Induced NO Production by Inhibiting P38 MAPK, JNK and NF-κB Activation in Murine Peritoneal Macrophages. *International Immunopharmacology*, **7**, 1825-1833. https://doi.org/10.1016/j.intimp.2007.09.002

[27] Lapenda, J.C., Silva, P.A., Vicalvi, M.C., Sena, K.X.F.R. and Nascimento, S.C. (2014) Antimicrobial Activity of Prodigiosin Isolated from *Serratia marcescens* UFPEDA 398. *World Journal of Microbiology and Biotechnology*, **31**, 399-406. https://doi.org/10.1007/s11274-014-1793-y

[28] Huh, J., Koo, H., Kim, K., Yim, J., Lee, H., Sohn, E., *et al.* (2008) Immunosuppressive Effect of Prodigiosin on Murine Splenocyte and Macrophages. *Biomolecules and Therapeutics*, **16**, 351-355. https://doi.org/10.4062/biomolther.2008.16.4.351

[29] Verinaud, L., Lopes, S.C.P., Prado, I.C.N., Zanucoli, F., Alves da Costa, T., Di Gangi, R., *et al.* (2015) Violacein Treatment Modulates Acute and Chronic Inflammation through the Suppression of Cytokine Production and Induction of Regulatory T Cells. *PLOS ONE*, **10**, e0125409. https://doi.org/10.1371/journal.pone.0125409

[30] Gutsmann, T., Müller, M., Carroll, S.F., MacKenzie, R.C., Wiese, A. and Seydel, U. (2001) Dual Role of Lipopolysaccharide (LPS)-Binding Protein in Neutralization of

LPS and Enhancement of LPS-Induced Activation of Mononuclear Cells. *Infection and Immunity*, **69**, 6942-6950. https://doi.org/10.1128/iai.69.11.6942-6950.2001

[31] Luchi, M. and Morrison, D.C. (2000) Comparable Endotoxic Properties of Lipopolysaccharides Are Manifest in Diverse Clinical Isolates of Gram-Negative Bacteria. *Infection and Immunity*, **68**, 1899-1904. https://doi.org/10.1128/iai.68.4.1899-1904.2000