Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Pharmacology and Toxicology

ISSN 1816-496X
DOI: 10.3923/jpt.2025.21.31

Research Article
Direct Effects of Various Bacterial Toxins (LPS & LTA) on Membrane Potential in a *Drosophila* Model

[1,3]Shelby McCubbin, [2,3]Maya O. Abul-Khoudoud and [3]Robin L. Cooper

[1]University of Kentucky College of Medicine, Lexington, KY 40536-0298, United State of America
[2]College of Physicians and Surgeons, Columbia University, New York, NY 10032, United State of America
[3]Department of Biology, University of Kentucky, Lexington, Kentucky 40506, United State of America

Abstract

Background and Objective: The physiological responses to Gram-positive and negative bacterial toxins are diverse and complex. The rapid effects of cells within seconds to minutes are also diverse depending on the type of Lipopolysaccharides (LPS) and Lipoteichoic acid (LTA). This research aims to elucidate how these microbial components influence neurophysiological responses at the cellular level, thereby contributing to a better understanding of host-pathogen interactions and innate immune signaling in invertebrate systems. **Materials and Methods:** The early third-instar larval body wall muscle m6 was used to monitor transmembrane potentials with sharp intracellular electrodes. The membrane potential was measured while exposing the preparation to different forms of LPS and LTA. Evoked and synaptic excitatory synaptic potentials were monitored before and during exposure to the compounds. Statistical analysis was conducted using paired t-test and Sign test, considering $p<0.05$ as significant. **Results:** Strains of LPS from *Serratia marcescens* and *Pseudomonas aeruginosa* result in rapid and acute hyperpolarization of muscle, while LPS from *E. coli*, commercially obtained *Salmonella enterica,* and ultra-pure LPS from *Salmonella enterica*, as well as LTA from *Staphylococcus aureus* have little to no acute response on membrane potential on the same cell type. **Conclusion:** These novel findings in the differential effects of strains of LPS and LTA on membrane potential may aid in selective treatments for bacterial septicemia depending on the bacterial strain.

Key words: Lipopolysaccharides, *drosophila*, lipoteichoic acid, membrane potential, synapse

Citation: McCubbin, S., M.O. Abul-Khoudoud and R.L. Cooper, 2025. Direct effects of various bacterial toxins (LPS & LTA) on membrane potential in a drosophila model. J. Pharmacol. Toxicol., 20: 21-31.

Corresponding Author: Robin Lewis Cooper, Department of Biology, University of Kentucky, Lexington, Kentucky 40506, United States of America

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bacterial septicemia is a worldwide problem for animals, including humans[1,2]. To a large extent, bacteria are not the direct cause of the pathological responses but rather the host's immune response to the toxins released from bacteria. A focus on treating septicemia depends on the severity and the organs affected, and there is primarily an emphasis on dampening the effects of the cytokines triggered by the immune response. However, if the initial reaction to the toxins could be blocked, then the amplified immune response would be lessened. This is the crux of the problem because there are no selective blockers to the various toxins released by bacteria.

The majority of bacterial septicemic cases are from Gram-negative bacteria, and Gram-negative strains of bacteria release various compounds such as Lipopolysaccharides (LPS) and repeats-in-toxin (RTX)[3,4]. Low levels of LPS are normally detected in the blood of mammals after the consumption of high-fat meals because of the microbiota in the intestine. However, there are also increased levels of LPS in disease states where the gastrointestinal tract is compromised. Other forms of bacterial infection, such as entry via the pulmonary system (a common route for *Pseudomonas aeruginosa*) or a compromised skin barrier, allow whole bacteria to enter and proliferate, releasing LPS systemically. This can result in the therapeutic use of high doses of antibiotics, leading to bacterial lysis, which can cause a surge of LPS, potentially producing a cytokine storm[5-9]. In such cases, if the receptors to LPS could be temporarily blocked before a bolus of antibiotics, this could be advantageous in preventing a cytokine storm as well as any direct effects of LPS on the cells.

Similarly, bacterial septicemia caused by Gram-positive bacteria is also due to a compound (i.e. to lipoteichoic acid- LTA) from the bacterium leading to an immune and cytokine response. Like for Gram-negative bacteria, lysing due to antibiotics will result in a systemic surge of LTA. Recently, it has been shown that LPS from *P. aeruginosa* and *S. marcescens* can result in rapid hyperpolarization followed by a slower depolarization of the membrane in *Drosophila* larval muscle[10-12]. In addition, purified LPS from *S. marcescens* had a more pronounced effect than *P. aeruginosa* for the given concentrations. It was also previously shown that LPS from *P. aeruginosa* and *S. marcescens* resulted in a reduction in the postsynaptic glutamatergic excitatory junction potentials (EJPs) as well as smaller spontaneous quantal events. This was previously demonstrated to be due to blocking of glutamate receptors on the muscle fiber[10-13]. Even glutamatergic synaptic responses in hippocampal slices of rodents are rapidly blocked by LPS from *S. marcescens*, which likely involves a mix of AMPA, kainate, and NMDA glutamate receptor subtypes[10,14,15]. The precise mechanism resulting in the synaptic depression in the hippocampal slice has not been established, as the responses have not been isolated from indirect glial involvement (i.e., astrocytes, oligo). Responses also have not been isolated from the potential release of cytokines (TNF-α and IL-1) through activating Nuclear Factor Kappa B (NF-κB), as well as neurons themselves[16-19]. The receptor complex for LPS binding in mammals involves TLR4, and TLR4 proteins are located on microglia, astrocytes, and oligodendrocytes, as well as neurons[20-24]. Hippocampal neurons in culture without the presence of glia have shown a response to LPS (1 µg/mL, *E. coli*), such as increased neuronal cytosolic Ca^{2+} leading to cell death[25].

The immune response in *Drosophila* to Gram-positive and Gram-negative bacteria is different than that for mammals. The response to Gram-positive bacteria is nicely reviewed by Bangham *et al.*[26]. Gram-negative bacteria are known to mediate cellular response through the PGRP-LE/PGRP-LC receptors and the Imd cascade through the NF-κB factor Relish for the genomic response to produce antimicrobial peptides (AMPs). However, the use of RNAi expression for PGRP-LC and PGRP-LE did not alter the acute responses to LPS on the larval *Drosophila* body wall muscles[10]. Thus, the rapid cellular responses, <1 second, on membrane potential by LPS from *P. aeruginosa* and *S. marcescens* are mediated by a different mechanism.

Thus, it is essential to develop an understanding of the mechanisms behind the rapid effects in membrane potential in larval *Drosophila* muscle, as well as whether LPS and LTA from various strains of bacteria have similar effects as for LPS from *P. aeruginosa* and *S. marcescens.* There are no prior reports examining the different effects on membrane potential for the various forms of LPS compared to the responses from *P. aeruginosa* and *S. marcescens*. Thus, in this brief report, the effects previously observed for *P. aeruginosa* and *S. marcescens* and compared to the responses obtained when LPS from *Escherichia coli*, *Salmonella enterica*, ultra-pure LPS from *Salmonella enterica*, as well as LTA from *Staphylococcus aureus* are exposed to the same types of cells. As demonstrated in this investigation, the most prominent responses on membrane potential occur for LPS from *S. marcescens*. Thus, a companion manuscript specifically addresses the mechanistic details in the responses caused by LPS from *S. marcescens*[12]. This brief report compares the differences in the effects on membrane potential, evoked glutamatergic transmission as well as the observations in occurrences in spontaneous quantal events.

MATERIALS AND METHODS

Study area: This research was conducted at the University of Kentucky in Lexington, Kentucky, USA, between May, 2023 and November, 2023.

Animals: *Drosophila melanogaster* Canton S (CS) flies were used in physiological assays. This strain has been isogenic in the laboratory for several years after being originally obtained from the Bloomington Drosophila Stock Center (BDSC). Early third-instar *Drosophila* CS larvae were used (50-70 hrs) post-hatching. The CS larvae were maintained at room temperature, ~21°C, in vials partially filled with a cornmeal-agar-dextrose-yeast medium.

The technique of dissecting larvae and measuring membrane potential[27], with the exception that all segmental nerves were transected close to the larval brain to prevent spontaneous evoked contractions induced from the CNS of the larvae.

Physiological recordings: The early third-instar larval body wall muscle m6 was used to monitor transmembrane potentials with sharp intracellular electrodes (30 to 40 Ω) filled with 3 M KCl. An Axon clamp 2B (Molecular Devices, Sunnyvale, CA, USA) amplifier and 1 X LU head stage were used. The EJPs and spontaneous mEJPs were collected and analyzed with LabChart 7.0 (ADInstruments, USA) as previously detailed[28]. Standard saline is HL3 saline (in mM):1.0 $CaCl_2 \cdot 2H_2O$, 70 NaCl, 20 $MgCl_2$, 5 KCl, 10 $NaHCO_3$, 5 trehalose, 115 sucrose, 25 5N, N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) at pH of 7.1[29]. All experiments were performed at room temperature (20-21°C). Exchanges in saline bathing media are shown within the figures. All chemicals for saline were obtained from Sigma-Aldrich, St. Louis, MO, USA.

The compounds used are presented in Table 1 and highlighted with each representative trace of the changes in the membrane potentials for the paradigm, as well as the percent change from the initial saline for each compound examined in the Results section.

Statistical analysis: Statistical analysis was performed as a paired t-test for changes in membrane potential and amplitudes of the EJPs. Since some data sets were used to examine a direct change in the membrane potential, the non-parametric Sign test was used to compare percent changes from initial saline to a time in the paradigm. A significant difference is considered as $p<0.05$.

Ethical consideration: Invertebrate animal care was approved by the Institutional Animal Care and Use Committee.

RESULTS

In order to compare the effects of LPS for the different strains, the responses from *P. aeruginosa* and *S. marcescens* are reviewed and then compared to the responses by LPS from *Escherichia coli*, *Salmonella enterica*, ultra-pure LPS from *Salmonella enterica*, as well as LTA from *Staphylococcus aureus*.

Application of 100 µg/mL of *S. marcescens*, the membrane rapidly hyperpolarized, followed by a gradual depolarization (Fig. 1a). The concentration was then increased to 500 µg/mL of *S. marcescens* for different sets of preparations to see whether the same effect was noted and whether the hyperpolarization remained consistent or occurred on a greater scale (Fig. 1b). The effect of 750 µg/mL of *S. marcescens* was very pronounced in a hyperpolarization followed by rapid depolarization and muscle contraction (Fig. 1c). Due to the strong contractions and inability to maintain intracellular recordings for three consecutive preparations, this high concentration was not further examined. The blue boxes in each trace indicate the exchange of bath solution (and potentially artifacts accompanying the bath exchanged).

The 500 µg/mL of *P. aeruginosa* also caused rapid hyperpolarization (Fig. 2a). The 750 µg/mL concentration also resulted in a notable hyperpolarization (Fig. 2b). The figure on the right of each trace displays the change in membrane potential for each preparation.

After reviewing the effects of LPS from *S. marcescens* and *P. aeruginosa* on *Drosophila* membrane potential, the responses induced by LPS from *E. coli*, *S. enterica* ultra-pure LPS from, as well as LTA from *S. aureus* were compared.

Table 1: Compounds used for assessment of the responses to LPS or LTA

Conditions used
LPS 1 *Serratia marcescens* (Product number L6136; Sigma)
LPS 2 *Pseudomonas aeruginosa* 10 (product number L9143; Sigma)
LPS 3 *E. coli* O111:B4 (product number L2630; Sigma)
LPS 4 *Salmonella enterica* Minnesota (product number 437632; Sigma)
LPS 5 Ultra-pure *salmonella enterica* Minnesota S R595(product number 437628; Sigma)
LTA *Staphylococcus aureus* (catalog from InvivoGen (10515 Vista Sorrento Pkwy, San Diego, CA 92121 USA)

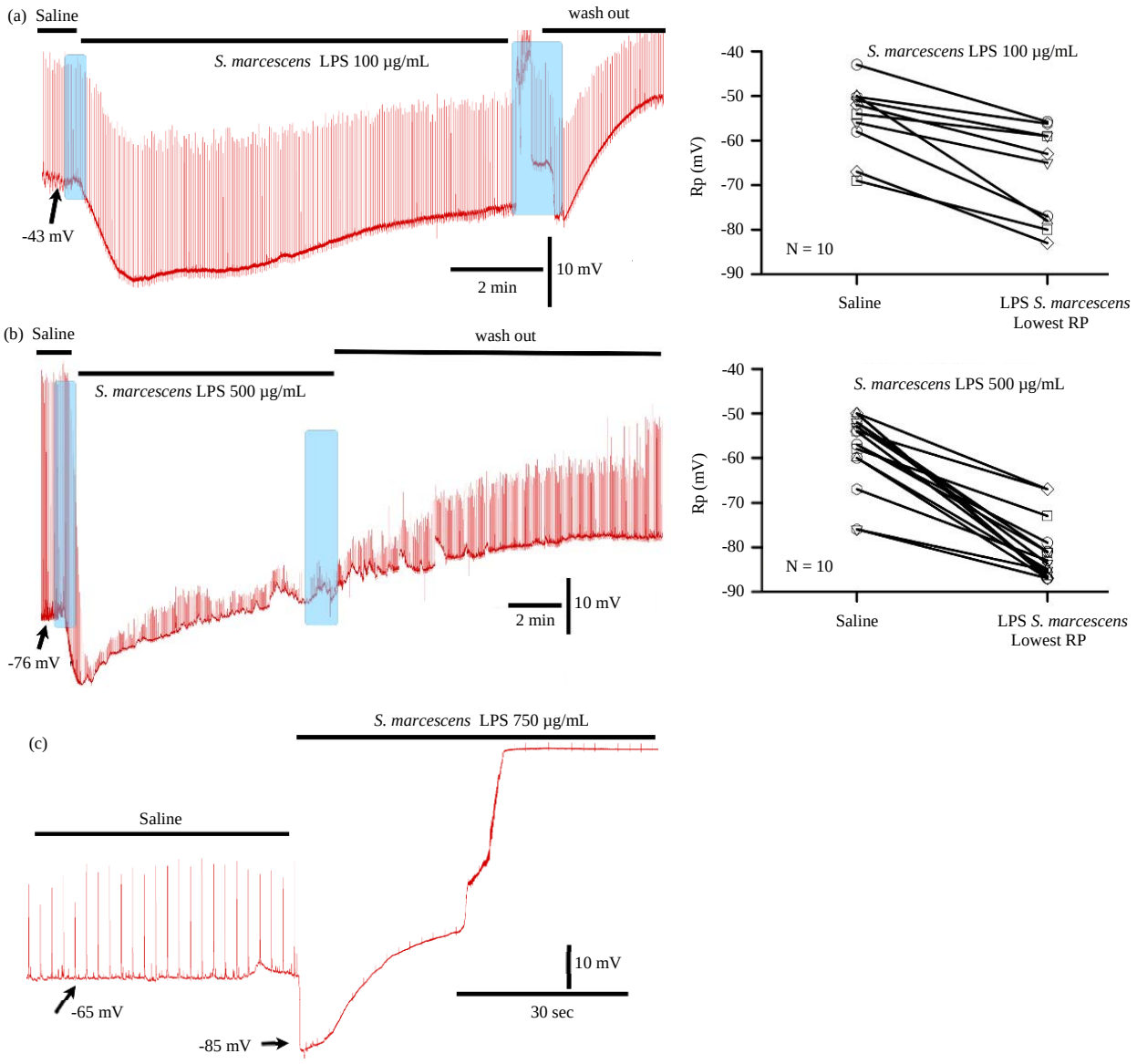

Fig. 1(a-c): Acute effects of *Serratia marcescens* on membrane potential, (a) On the left, 100 µg/mL of *S. marcescens* caused rapid hyperpolarization upon application, (b) Trace of a higher concentration of *S. marcescens* (500 µg/mL) is shown on the left and (c) Trace of a concentration at (750 µg/mL) of *S. marcescens*

(a) Representative preparation began to gradually depolarize with time and washout with saline caused further depolarization. The figure on the right shows the change in each preparation from membrane potential at the time of saline to the most negative potential after the application of 100 µg/mL of *S. marcescens*, (b) Rapid hyperpolarization is again seen with the application of LPS, followed by a gradual depolarization. A decrease in evoked EJP amplitude was also noted, which reversed upon saline washout. Individual preparations are again shown on the right. Individual preparations had a larger hyperpolarization in comparison to the 100 µg/mL *S. marcescens* and (c) Note the very strong hyperpolarization and rapid depolarization, which resulted in the loss of the recording due to the electrode being dislodged. Due to the large contractions, this high concentration was not further pursued for analysis. The blue boxes indicate where the bathing solution was exchanged.

The blue boxes in each trace indicate the exchange of bath solutions, which sometimes lasted longer due to individual variation in preparations. The application of 750 µg/mL of LPS from *E. coli* showed no significant change in membrane potential or activity (Fig. 3a). Commercial *S. enterica* (750 µg/mL) also did not cause a significant hyperpolarization (Fig. 3b). This led to examining the effects by ultrapure *S. enterica* (750 µg/mL), but there was again no notable change in membrane potential or activity (Fig. 3c). Finally, LTA from *S. aureus* (750 µg/mL) was applied, but again, no notable change was seen (Fig. 3d). The figure on the right of each trace displays the change in membrane potential for each preparation.

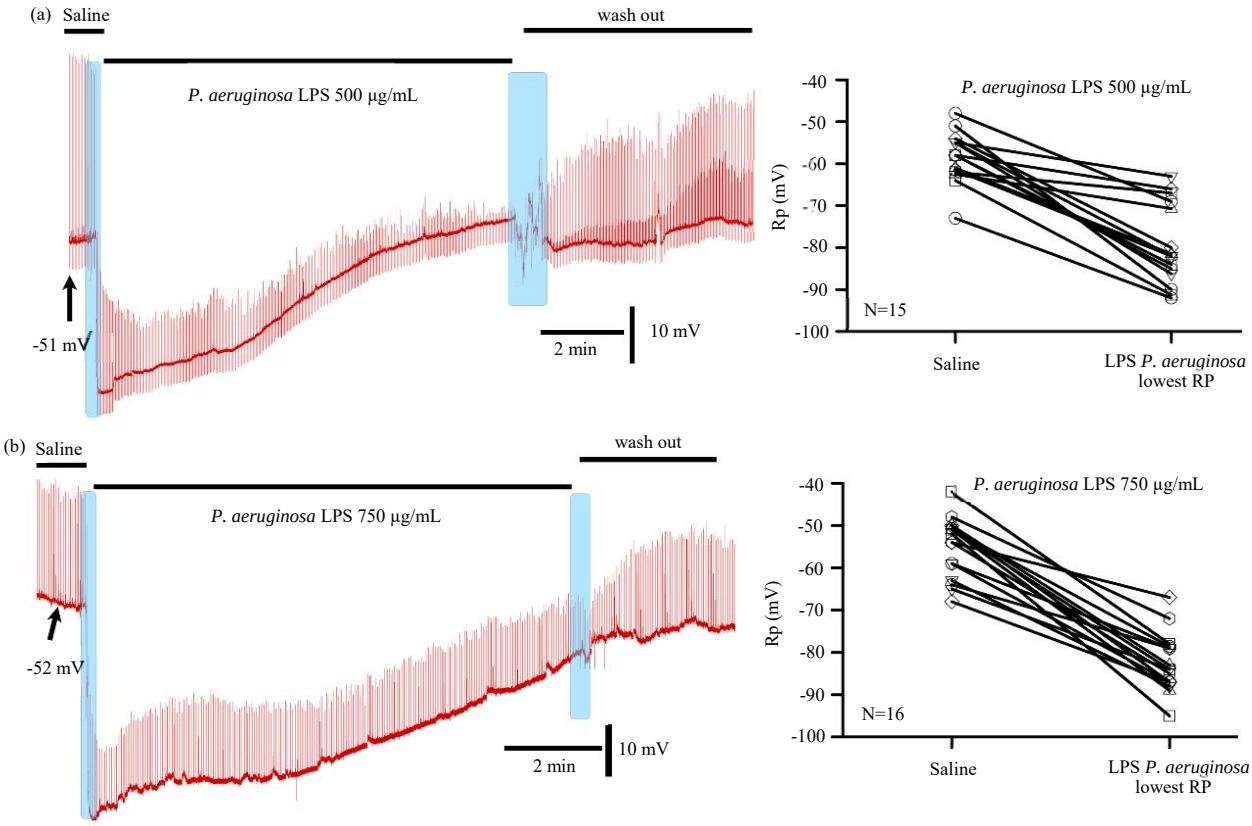

Fig. 2(a-b): Acute effects of *Pseudomonas aeruginosa* on membrane potential, (a) Trace on the left show's rapid hyperpolarization upon application of 500 µg/mL *P. aeruginosa* and (b) Trace of a higher concentration of *P. aeruginosa* (750 µg/mL)

(a) One can also note decreased evoked EJP amplitudes. The preparation gradually depolarized and activity returned with a saline washout. Figures on the right show hyperpolarization upon application to each preparation and (b) As with the others, rapid hyperpolarization was seen immediately upon LPS application. There is an even greater change with this concentration than with 500 µg/mL. Gradual depolarization was still noted and membrane potential continued to rise after saline washout. Figures on the right show the hyperpolarization of each preparation. The blue boxes indicate where the bathing solution was exchanged and Rp (mV): Resting membrane potential measured in millivolts

The percent change for each condition was determined by comparing the initial membrane potential value during the saline condition at the start of the experiment to the most negative potential of each preparation after switching to the compound being tested (Fig. 4). A percent change was determined for each preparation, and the mean percent change for each compound is presented. A negative percent change indicates hyperpolarization. There was a significant difference between 100 and 500 µg/mL for *S. marcescens* (two-tailed t-test, p-value=0.0072). Also, the 500 and 750 µg/mL for *P. aeruginosa* were significantly different (two-tailed t-test, p-value=0.0397). However, a two-tailed ANOVA revealed only a significant difference between *S. marcescens* at 100 µg/mL and *P. aeruginosa* at 750 µg/mL (p=0.004, alpha=0.050: 0.816).

The histogram illustrates the change in membrane potential as measured in initial saline prior to and after the application of LPS/LTA. The negative values indicate an average hyperpolarization of the membrane. Hyperpolarization was prominent for *S. marcescens* and *P. aeruginosa* exposures. It is also of interest to notice that there was a difference in response between the varying concentrations of these two. The asterisk each bar on ±SEM indicate a statistically significant change ($p<0.05$, paired t-test from saline to the compound; the bar between groups and an asterisk was t-test between compound types; # for a significant difference for an ANOVA).

In addition to membrane potential, changes in the amplitude of the evoked synaptic responses and changes in the occurrences of spontaneous quantal events were observed. Representative traces for the effects produced by each compound examined are shown in Fig. 5. The traces show the response before (left side) and during exposure to each compound. The larger defection to the left of the traces depicts evoked EJPs. The smaller deflections are miniature EJPs ("minis"). The right side shows the potential change in the

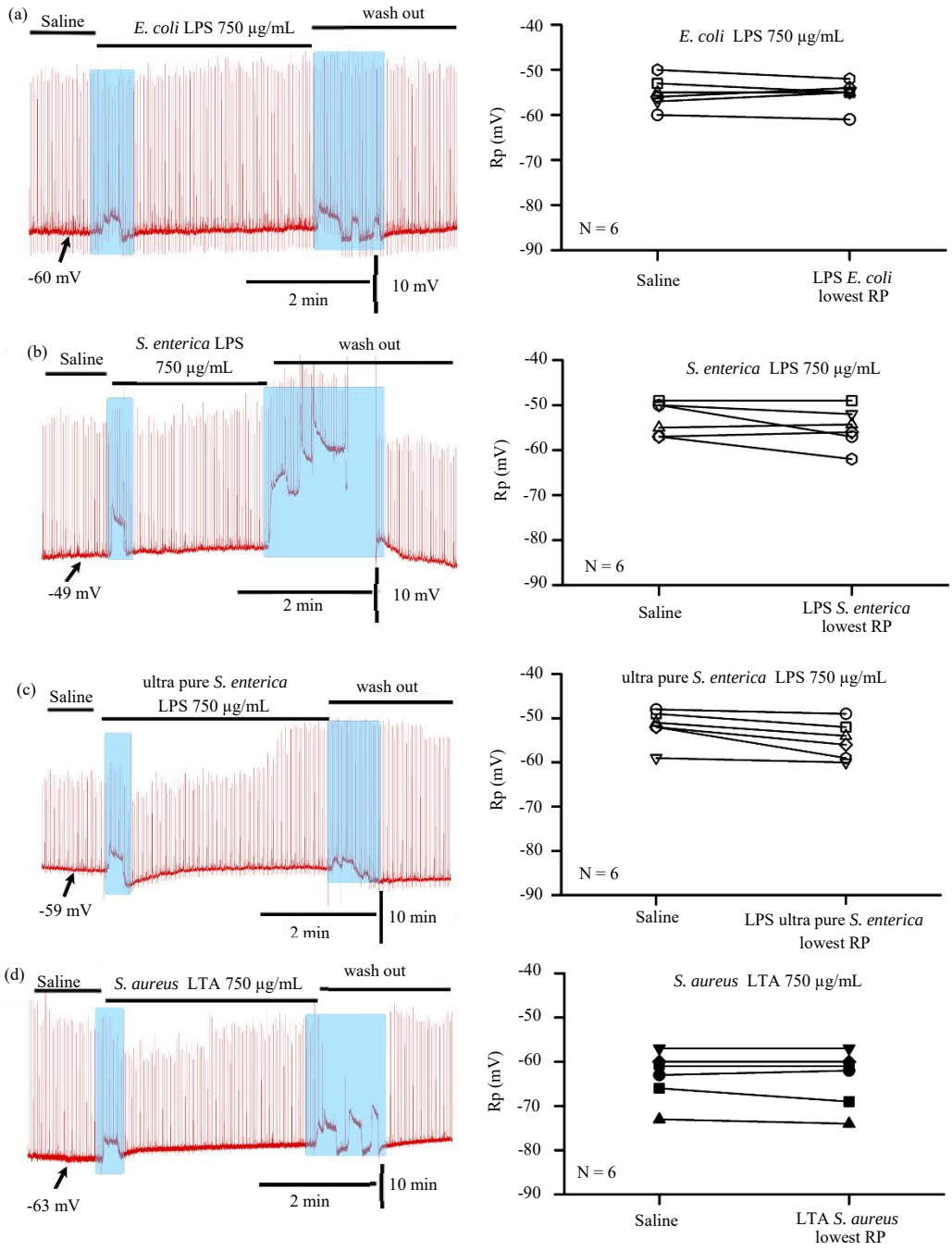

Fig. 3(a-d): Acute effects of LPS from *Escherichia coli*, *Salmonella enterica*, ultra-pure LPS from *Salmonella enterica*, as well as LTA from *Staphylococcus aureus*, (a) On the left, the application of 750 µg/mL of LPS from *E. coli* had little to no effect on membrane potential. This is again reflected in the graph on the right, where none of the individual preparations had a notable change, (b) The addition of 750 LPS from µg/mL *S. enterica* also had no significant effect on membrane potential or the amplitudes of the EJPs. The graph on the right shows a slight variation between preparations, but still, there was no notable change, (c) Application of 750 µg/mL ultrapure LPS from *S. enterica* revealed no notable difference in membrane potential. The line graphs of individual preparations on the right reflect the membrane potential before and during exposure to the compound without any significant changes and (d) 750 µg/mL of LTA from *S. aureus* was applied to the *Drosophila* preparations

Once again, there was no significant change in membrane potential as seen in both the figure on the left and on the right. The blue boxes indicate where the bathing solution was exchanged, and Rp (mV): Resting membrane potential measured in millivolts

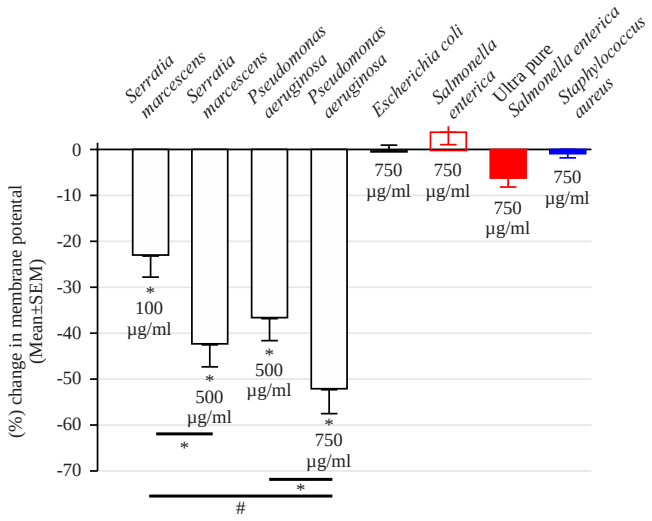

Fig. 4: Direct comparison between different forms of LPS and of LTA

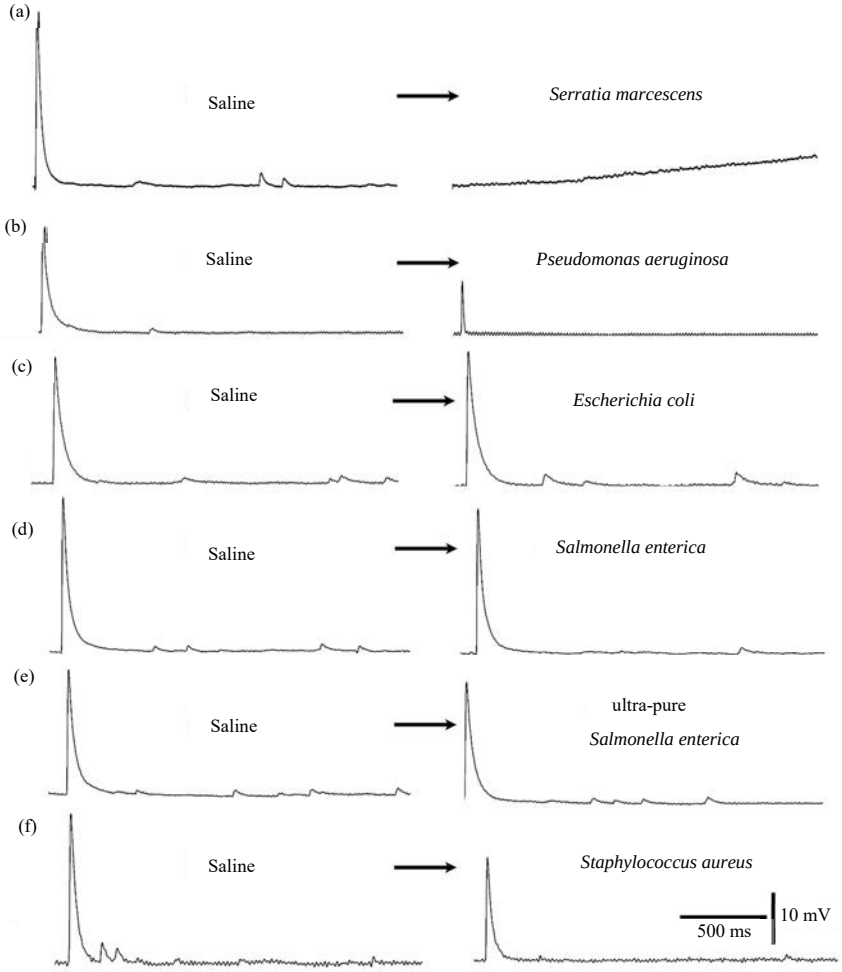

Fig. 5(a-f): Amplified traces showing evoked excitatory junction potentials (EJPs) and miniature EJPs ("minis") before and during exposure to various bacterial components (LPS or LTA)

(a) *S. marcescens* application eliminated detectable EJPs and minis, indicating strong suppression of synaptic activity, (b) *P. aeruginosa* reduced the amplitude of evoked EJPs, though minis remained faintly observable, (c) *E. coli* treatment caused no significant change; both evoked EJPs and minis persisted, (d-e) Commercial and ultra-pure *S. enterica* had no detectable effect on membrane activity and (f) *S. aureus* slightly reduced EJP amplitude and minis frequency, but the change was not statistically significant due to variability

evoked EJPs as well as the potential for the occurrences of minis during exposure for each compound, Fig. 5 presents amplified electrophysiological traces illustrating the evoked excitatory junction potentials (EJPs) and miniature EJPs ("minis") recorded before and during the application of various bacterial components, specifically lipopolysaccharide (LPS) or lipoteichoic acid (LTA). In Fig. 5a, exposure to *Serratia marcescens* led to a complete suppression of synaptic activity, as evidenced by the absence of both evoked EJPs and minis, indicating a profound inhibitory effect on neurotransmission. In Fig. 5b, the application of *Pseudomonas aeruginosa* resulted in a noticeable reduction in the amplitude of evoked EJPs; however, occasional minis were still detectable, suggesting partial synaptic disruption. In contrast, Fig. 5c shows that treatment with *Escherichia coli* did not induce any statistically significant changes in either evoked EJPs or minis, implying minimal or no effect on synaptic transmission. Fig. 5(d-e) illustrate the effects of commercial and ultra-pure preparations of *Salmonella enterica*, respectively, both of which failed to produce any discernible alterations in membrane activity or synaptic potential amplitude. Finally Fig. 5f demonstrates that exposure to *Staphylococcus aureus* led to a mild decrease in evoked EJP amplitude and a reduced frequency of minis; however, these changes were not statistically significant due to variability across experimental preparations. Collectively, these traces highlight species-specific effects of bacterial components on neuromuscular synaptic activity.

DISCUSSION

This brief report compared various forms of LPS and a strain of LTA to examine the acute actions (seconds to 3 min) of LPS on membrane potential and synaptic transmission. The larval *Drosophila* neuromuscular junction was used as an assay for this study since earlier studies have addressed some of the mechanisms of action by LPS from *S. marcescens* and *P. aeruginosa* in this same experimental model, and thus, direct comparisons could be made to other forms of LPS. Since commercially available LPS is processed differently and reported to have differences in some responses from ultra-pure LPS[30-33], both LPS forms from *S. enterica* Minnesota were directly compared. No differences were detected on the effect of membrane potential. However, there might be a trend to enhance synaptic transmission by the ultra-pure form LPS. Future studies specifically addressing effects on synaptic efficacy with quantal analysis are needed to determine if there are consistent effects between commercially available and ultra-pure LPS forms and how they may play out over various incubation times and concentrations. However, neither form showed a significant hyperpolarization of the membrane within 3 min of exposure. The LPS from *E. coli* also had no noticeable acute effects on membrane potential or synaptic transmission. Since LPS from *S. marcescens* and *P. aeruginosa* did show pronounced acute effects on membrane potential and synaptic transmission, the isolated LTA from *S. aureus* was examined at the same concentration, but no noticeable differences were noted within the 3 min exposure.

It was noted by Hardy and White[34] that non-purified preparations of LPS from *E. coli* were contaminated with other biologically active molecules, and it was stressed that great care should be taken when interpreting results obtained with low-purity LPS preparations. RTX toxins are created by Gram-negative bacteria and can directly form pores in membranes as well as alter cellular cascades such as adenylate cyclase[4,35]. The RTX toxin is, therefore, possible as a contaminant, but it is unlikely to be in the commercial samples differentially. Since no effects were noted on the membrane potential for commercially available *E. coli* and *S. enterica* as well as ultra-pure *S. enterica*, if there was contamination in the samples, they did not influence membrane potential. This may be more of a concern for longer-term studies than the short time frame used in this study.

As for the LPS from *S. marcescens* and *P. aeruginosa,* there could be a difference in the levels of contaminated agents in comparison to these other commercially available forms of LPS. If so, this could potentially account for the transient hyperpolarization and depression of synaptic transmission, but it is unlikely Sigma-Aldrich used different isolation procedures for LPS from these bacterial forms than for *E. coli* and *S. enterica.* Data sheets for all the forms state a phenol extraction procedure to isolate the LPS. These studies were also all conducted in the same freshly made physiological saline.

Thus, it appears the LPS from *S. marcescens* and *P. aeruginosa* are unique and similar in their biological action on the larval *Drosophila* NMJ. Both forms of LPS produce a notably rapid onset of hyperpolarization that is transitory over 1 to 2 min, followed by a gradual depolarization. If the LPS is not rinsed away, the depolarization continues to a greater extent than the initial resting membrane potential. Both forms at 500 to 750 µg/mL will produce muscle contractions. However, after exposure to 750 µg/mL of *S. marcescens,* the body wall muscles undergo waves of contraction, making

it difficult to maintain an intracellular recording electrode. Thus, the reason not to test higher concentrations of *S. marcescens.* It is unusual that the contractions exist in a saline devoid of Ca^{2+} and even in the presence of Cd^{2+} [13]. This also suggests that Cd^{2+} can bind to troponin to induce skeletal muscle contraction, as is reported in other studies[36,37]. However, LPS from *S. marcescens* is more potent in initiating contractions at 500 µg/mL than *P. aeruginosa* is. It is also known that *S. marcescens* is more potent than *P. aeruginosa* in inducing an immune response in mammals[38-40]; however, this is by a different mechanism than the acute responses reported herein.

It is potentially possible that the structural differences in the LPS forms can account for the actions on the membrane potential as well as blocking glutamatergic synaptic function. The chemical structure of the forms of LPS used in this study have previously been reported[41-46].

Considering septicemia induced by a perforated intestine and even a wound would potentially be due to a mixture of bacterial strains, it would also be of interest to examine the effects of cocktails of LPS strains to determine whether they may block the acute action of each other or have additive or even synergic acute effects on membrane potential and synaptic transmission.

Since reports on the rapid actions of various forms of LPS are scant, and some forms produce prominent acute responses within seconds, it is of interest to survey more forms and concentrate on a single model system for direct comparison. Perhaps genetic and physiological examination of larva *Drosophila* can continue to aid in such future studies despite the mechanisms of the immune systems being different than for the direct acute action of LPS on the membrane potential. It now appears that the hyperpolarization induced by *S. marcescens* is due to activation of the K^+ leak channels (i.e., K2P channels) in the membrane followed by a Na^+ leak promoting a delayed depolarization[12].

CONCLUSION

In conclusion, commercial LPS purified in the same manner *P. aeruginosa, S. marcescens, Escherichia coli*, and *Salmonella enterica* produced different direct effects on membrane potential and reduction of glutamatergic synaptic transmission. Both *P. aeruginosa* and *S. marcescens* produced similar effects with membrane hyperpolarization and blocking of glutamate receptors, with *S. marcescens* producing a stronger effect than *P. aeruginosa*. LPS from *Escherichia coli* and *Salmonella enterica,* as well as LTA from *Staphylococcus aureus,* had no significant direct effects on membrane potential or glutamatergic transmission.

SIGNIFICANCE STATEMENT

These findings are significant since researchers use commercial LPS for various studies in examining an immune response min to hrs after exposure, but the general one does not address the direct, within seconds, effect on membrane potential and synaptic transmission. In addition, the varied responses in the rapid and direct effects among strains of LPS that were purified in the same commercial process indicate that the structures of LPS from the various strains are activating K2P channels and blocking glutamate receptors differentially.

REFERENCES

1. Reinhart, K., R. Daniels, N. Kissoon, F.R. Machado, R.D. Schachter and S. Finfer, 2017. Recognizing sepsis as a global health priority-A WHO resolution. N. Engl. J. Med., 377: 414-417.
2. Rudd, K.E., S.C. Johnson, K.M. Agesa, K.A. Shackelford and D. Tsoi *et al.*, 2020. Global, regional, and national sepsis incidence and mortality, 1990-2017: Analysis for the Global Burden of Disease Study. Lancet, 395: 200-211.
3. Osborn, M.J., S.M. Rosen, L. Rothfield, L.D. Zeleznick and B.L. Horecker, 1964. Lipopolysaccharide of the gram-negative cell wall: Biosynthesis of a complex heteropolysaccharide occurs by successive addition of specific sugar residues. Science, 145: 783-789.
4. Linhartová, I., L. Bumba, J. Mašín, M. Basler and R. Osicka *et al.*, 2010. RTX proteins: A highly diverse family secreted by a common mechanism. FEMS Microbiol. Rev., 34: 1076-1112.
5. Singer, M., C.S. Deutschman, C.W. Seymour, M. Shankar-Hari and D. Annane *et al.*, 2016. The third international consensus definitions for sepsis and septic shock (Sepsis-3). J. Am. Med. Assoc., 315: 801-810.
6. Lin, E. and S.F. Lowry, 1998. The human response to endotoxin. Sepsis, 2: 255-262.
7. Tang, X.D., T.T. Ji, J.R. Dong, H. Feng and F.Q. Chen *et al.*, 2021. Pathogenesis and treatment of cytokine storm induced by infectious diseases. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms222313009.
8. Gupta, K.K., M.A. Khan and S.K. Singh, 2020. Constitutive inflammatory cytokine storm: A major threat to human health. J. Interferon Cytokine Res., 40: 19-23.
9. Mangalmurti, N. and C.A. Hunter, 2020. Cytokine storms: Understanding COVID-19. Immunity, 53: 19-25.

10. Ballinger-Boone, C., O. Anyagaligbo, J. Bernard, S.M. Bierbower and E.E. Dupont-Versteegden *et al*., 2020. The effects of bacterial endotoxin (LPS) on cardiac and synaptic function in various animal models: Larval *Drosophila*, crayfish, crab, and rodent. Int. J. Zool. Res., 16: 33-62.
11. Cooper, R.L. and R.M. Krall, 2022. Hyperpolarization induced by lipopolysaccharides but not by chloroform is inhibited by doxapram, an inhibitor of two-P-domain K^+ channel (K2P). Int. J. Mol. Sci., Vol. 23. 10.3390/ijms232415787.
12. Hadjisavva, M.E. and R.L. Cooper, 2025. The biphasic effect of lipopolysaccharide on membrane potential. Membranes, Vol. 15. 10.3390/membranes15030074.
13. Potter, R., A. Meade, S. Potter and R.L. Cooper, 2021. Rapid and direct action of lipopolysaccharide (LPS) on skeletal muscle of larval *Drosophila*. Biology, Vol. 10. 10.3390/biology10121235.
14. Khakpai, F., M.R. Zarrindast, M. Nasehi, A. Haeri-Rohani and A. Eidi, 2013. The role of glutamatergic pathway between septum and hippocampus in the memory formation. EXCLI J., 12: 41-51.
15. Royo, M., B.A. Escolano, M.P. Madrigal and S. Jurado, 2022. AMPA receptor function in hypothalamic synapses. Front. Synaptic Neurosci., Vol. 14. 10.3389/fnsyn.2022.833449.
16. Pérez Otaño, I., M.K. McMillian, J. Chen, G . Bing, J.S. Hong and K.R. Pennypacker, 1996. Induction of NF-κB-like transcription factors in brain areas susceptible to kainate toxicity. Glia, 16: 306-315.
17. Friedman, W.J., S. Thakur, L. Seidman and A.B. Rabson, 1996. Regulation of nerve growth factor mRNA by interleukin-1 in rat hippocampal astrocytes is mediated by NFκB. J. Biol. Chem., 271: 31115-31120.
18. Nomura, Y., 1998. A transient brain ischemia- and bacterial endotoxin-induced glial iNOS expression and NO-induced neuronal apoptosis. Toxicol. Lett., 102: 65-69.
19. Hartlage-Rübsamen, M., R. Lemke and R. Schliebs, 1999. Interleukin-1β, inducible nitric oxide synthase and nuclear factor-κB are induced in morphologically distinct microglia after rat hippocampal lipopolysaccharide/interferon-γ injection. J. Neurosci. Res., 57: 388-398.
20. Aravalli, R.N., P.K. Peterson and J.R. Lokensgard, 2007. Toll-like receptors in defense and damage of the central nervous system. J. Neuroimmune Pharmacol., 2: 297-312.
21. Bowman, C.C., A. Rasley, S.L. Tranguch and I. Marriott, 2003. Cultured astrocytes express toll-like receptors for bacterial products. Glia, 43: 281-291.
22. Okun, E., K.J. Griffioen and M.P. Mattson, 2011. Toll-like receptor signaling in neural plasticity and disease. Trends Neurosci., 34: 269-281.
23. Olson, J.K. and S.D. Miller, 2004. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J. Immunol., 173: 3916-3924.
24. Poltorak, A., X. He, I. Smirnova, M.Y. Liu and C. van Huffel *et al*., 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in *Tlr4* gene. Science, 282: 2085-2088.
25. Calvo-Rodriguez, M., C. de la Fuente, M. Garcia-Durillo, C. Garcia-Rodriguez, C. Villalobos and L. Nunez, 2017. Aging and amyloid β oligomers enhance TLR4 expression, LPS-induced Ca^{2+} responses and neuron cell death in cultured rat hippocampal neurons. J. Neuroinflamm., Vol. 14. 10.1186/s12974-017-0802-0.
26. Bangham, J., F. Jiggins and B. Lemaitre, 2006. Insect immunity: The post-genomic era. Immunity, Vol. 25. 10.1016/j.immuni.2006.07.002.
27. Brock, K.E. and R.L. Cooper, 2023. The effects of doxapram blocking the response of gram-negative bacterial toxin (LPS) at glutamatergic synapses. Biology, Vol. 12. 10.3390/biology12081046.
28. Stewart, B.A., H.L. Atwood, J.J. Renger, J. Wang and C.F. Wu, 1994. Improved stability of *Drosophila* larval neuromuscular preparation in haemolymph-like physiological solutions. J. Comp. Physiol. A, 175: 179-191.
29. de Castro, C., J. Titlow, Z.R. Majeed and R.L. Cooper, 2014. Analysis of various physiological salines for heart rate, CNS function, and synaptic transmission at neuromuscular junctions in *Drosophila melanogaster* larvae. J. Comp. Physiol. A, 200: 83-92.
30. Dana, N., S.H. Javanmard and G. Vaseghi, 2017. Effect of lipopolysaccharide on toll-like receptor-4 signals in mouse cancer cells. Bratislava Med. J., 118: 598-601.
31. Farhat, K., A.J. Ulmer and T.W. Jungi, 2012. A potential test system for detecting contaminations by bacterial lipoproteins. Vet. Immunol. Immunopathol., 145: 66-73.
32. Sasaki, H. and S.H. White, 2008. Aggregation behavior of an ultra-pure lipopolysaccharide that stimulates TLR-4 receptors. Biophys. J., 95: 986-993.
33. Wang, C., T. Nelson, D. Chen, J.C. Ellis and N.L. Abbott, 2019. Understanding lipopolysaccharide aggregation and its influence on activation of factor C. J. Colloid Interface Sci., 552: 540-553.
34. Hardy, K.W. and T.D. White, 2001. Some commercial preparations of *Escherichia coli* bacterial endotoxin lipopolysaccharide (LPS) are contaminated with biologically active substances. J. Neurochem., 78: 1183-1184.
35. Gray, M., G. Szabo, A.S. Otero, L. Gray and E. Hewlett, 1998. Distinct mechanisms for K^+ efflux, intoxication and hemolysis by *Bordetella pertussis* AC toxin. J. Biol. Chem., 273: 18260-18267.
36. Chao, S., C.H. Bu and W.Y. Cheung, 1990. Activation of troponin C by Cd^{2+} and Pb^{2+}. Arch. Toxicol., 64: 490-496.
37. Drakenberg, T., S. Forsén, E. Thulin and H.J. Vogel, 1987. The binding of Ca^{2+}, Mg^{2+} and Cd^{2+} to tryptic fragments of skeletal muscle troponin C. Cadmium-113 and proton NMR studies. J. Biol. Chem., 262: 672-678.

38. Kreger, A.S., D.M. Lyerly, L.D. Hazlett and R.S. Berk, 1986. Immunization against experimental *Pseudomonas aeruginosa* and *Serratia marcescens* keratitis. vaccination with lipopolysaccharide endotoxins and proteases. Invest. Ophthalmol. Vis. Sci., 27: 932-939.
39. Lorenzon, S., P. Pasqual and E.A. Ferrero, 2002. Different bacterial lipopolysaccharides as toxicants and stressors in the shrimp *Palaemon elegans*. Fish Shellfish Immunol., 13: 27-45.
40. Luchi, M. and D.C. Morrison, 2000. Comparable endotoxic properties of lipopolysaccharides are manifest in diverse clinical isolates of gram-negative bacteria. Infect. Immun., 68: 1899-1904.
41. Makimura, Y., Y. Asai, A. Sugiyama and T. Ogawa, 2007. Chemical structure and immunobiological activity of lipid A from *Serratia marcescens* LPS. J. Med. Microbiol., 56: 1440-1446.
42. Huszczynski, S.M., J.S. Lam and C.M. Khursigara, 2020. The role of *Pseudomonas aeruginosa* l ipopolysaccharide in bacterial pathogenesis and physiology. Pathogens, Vol. 9. 10.3390/pathogens9010006.
43. Patel, D.S., P. Blasco, G. Widmalm and W. Im, 2020. *Escherichia coli* O176 LPS structure and dynamics: A NMR spectroscopy and MD simulation study. Curr. Res. Struct. Biol., 2: 79-88.
44. Bertani, B. and N. Ruiz, 2018. Function and biogenesis of lipopolysaccharides. EcoSal Plus, Vol. 8. 10.1128/ecosalplus.esp-0001-2018.
45. Ohto, U., K. Fukase, K. Miyake and T. Shimizu, 2012. Structural basis of species-specific endotoxin sensing by innate immune receptor TLR4/MD-2. Proc. Natl. Acad. Sci. U.S.A., 109: 7421-7426.
46. Rietschel, E.T., T. Kirikae, F.U. Schade, A.J. Ulmer and O. Holst *et al*., 1993. The chemical structure of bacterial endotoxin in relation to bioactivity. Immunobiology, 187: 169-190.