JJBS

Volume 18, Number 1,March 2025
ISSN 1995-6673
Pages 73 – 78
https://doi.org/10.54319/jjbs/180108

Jordan Journal of Biological Sciences

# Immunomodulatory Activities of *Cnidoscolus aconitifolius* Leaves Extract via Modulation of TLR4 Expression and Neutrophil Cell Infiltration in Infected Mice

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*Received: January 25, 2024; Revised: July 25, 2024; Accepted: August 29, 2024*

**Abstract**

Toll Like Receptor (TLR) plays a pivotal role in activating innate and adaptive immune responses Modulating TLR4 activation and signaling is crucial in the discovery of immunomodulatory drugs. *Cnidoscolus aconitifolius* leaf extract (CAE) exhibits potent antioxidant properties, suggesting its potential as an immunomodulatory agent. This study aimed to explore the immunomodulatory potential of CAE by assessing neutrophil infiltration and TLR4 expression in Babl/C mice infected with *Salmonella typhimurium*. Thirty *Babl/C* mice were used as experimental animals and divided into 6 groups (n=5). Five days post *Salmonella typhimurium* induction, infected mice were orally treated once daily for 7 days. Evaluation included assessment of neutrophil infiltration in small intestine tissue and TLR4 expression using flow cytometric analysis of lymphoid organs. Results demonstrated that administration of CAE at a dose of 400 mg/kgbw significantly increased neutrophil count (10.00 ± 0.84) compared to the infection-only group (5.00 ± 0.32), with no significant difference compared to the positive control group (10.60 ± 1.36). Evaluation of TLR4 expression revealed that CAE at a dose of 200 mg/kgbw significantly upregulated TLR4 expression (8419.80 ± 276.745) compared to the negative control group (7237.20 ± 162.17), and was comparable to the positive control group (8037.00 ± 206.40). All data showed statistical significance ($p<0.05$). CAE demonstrated the ability to modulate TLR4 expression and enhance neutrophil activity, thereby potentially serving as an immunomodulatory agent.

**Keywords**: *Cnidoscolus aconitifolius*, immunomodulators; leaves extract; neutrophil; TLR4

## 1. Introduction

Infectious diseases rank second globally in terms of mortality rates, following cardiovascular disease. Typhoid fever is notably prevalent in several Asian countries (Fitrya et al., 2020). This acute illness is primarily caused by Salmonella enterica, particularly its variant *Salmonella typhimurium* (Cordero-Alba et al., 2016). Salmonella infection poses a public health challenge worldwide and imposes a substantial economic burden on health systems. It ranks as the second most frequently reported gastrointestinal infection and is the primary cause of foodborne outbreaks in Europe cause of foodborne outbreaks in Europe. In 2020, there were 53,169 laboratory-confirmed cases of salmonellosis, resulting in 61 fatalities. According to WHO data, Indonesia has a relatively high incidence of typhoid fever, affecting 81,000 out of 100,000 people (Rahmasari & Lestari, 2018). Individuals with compromised immune systems are particularly vulnerable to typhoid fever (Kalia et al., 2016). The innate immune system, comprising phagocytic cells, plays a critical role in defending against microorganisms and cancerous cells (Venkatalakshmi & Brindha, 2016). Activation of the immune system is essential for aiding the body in combating antigenic substances, which can be facilitated through the use of immunomodulators.

Toll Like Receptor (TLR) is a receptor that plays a role in activating the innate immune response and adaptive immunity. TLRs consists of TLR 1, TLR 2, TLR 4, TLR 5, TLR 6 which are present in the extracellular part of the immune cell and respond to extracellular microorganisms. On the other hand, TLR3, TLR7, TLR8, and TLR9 are located in the endosomal part of immune cells and respond to intracellular viral and bacterial infections (Firmal et al., 2020). TLR4 is an innate immune receptor found on the surface of cells that recognizes patterns of pathogen-associated molecules (PAMPs), including viral proteins. It triggers the production of type I interferons and proinflammatory cytokines to combat infections (Aboudounya & Heads, 2021). TLR4 is expressed not only on the surface of immune cells such as macrophages and dendritic cells, which regulate acute inflammation, but also

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in various tissue cells for defense against infection and regulation of fibrotic phenotype during tissue damage (Rosadini & Kagan, 2017). TLR4 plays a crucial role in inducing host immune responses against infectious diseases such as bacterial, fungal, viral infections, malaria (Mukherjee et al., 2016). It is present on the plasma membranes of neutrophils, macrophages, dendritic cells, and endothelial cells. Modulating TLR4 activation and signaling is vital in regulating the immune system and developing immunomodulators (Romerio & Peri, 2020). Activation of the immune system is essential to help the body eliminate antigenic substances, which can be stimulated with immunomodulators.

The development of immunomodulators focuses on natural products, which are considered safer and more effective. Plant extracts are widely regarded as potential immunomodulatory agents due to their minimal side effects (Alamgir & Uddin, 2010). Plant-based immunomodulatory activity involves enhancing the function and efficiency of macrophages, granulocytes, complement, natural killer cells, and the production of effector molecules (Jayathirtha & Mishra, 2004). Plant metabolites such as sterols, polysaccharides, alkaloids, flavonoids, lectins, and glycoproteins exhibit immunomodulatory agents (Harun et al., 2015).

*Cnidoscolus aconitifolius* belongs to the family Euphorbiaceae, which also includes plants like *Phyllanthus niruri* developed as immunomodulatory drugs. Studies on *Cnidoscolus aconitifolius* leaves have identified various activities, such as antibacterial, hepatoprotective, anti-inflammatory (Pérez-González et al., 2018), antidiabetic and anti-hypertension effects (Somade et al., 2021). The 70% ethanol extract of *Cnidoscolus aconitifolius* leaves contains a total flavonoid content of 418.46 ± 3.28 mg QE/g extract. Furthermore, antioxidant activity tests indicate that the extract shows potent antioxidant potential with an IC50 value of 34.3149 µg/ml (Hidayati et al., 2023).

Building upon this background, this study aims to investigate the immunomodulatory activity of *Cnidoscolus aconitifolius*. Specifically, it evaluates the impact of administering *Cnidoscolus aconitifolius* leaf extract on neutrophil cell infiltration and TLR4 expression in Babl/C mice induced by *Salmonella typhimurium* bacteria.

## 2. Materials and Methods

### *2.1. Materials*

*Salmonella typhimurium* bacteria were obtained from Brawijaya University, Malang. *Cnidoscolus aconitifolius* leaves were sourced from the Patrang area, Jember, Indonesia. The materials used included ethanol (Merck), TLR4 antibodies (BioLegend, USA), hematoxylin-eosin staining kit (Sigma-Aldrich, USA) and aquadest (Brataco, Indonesia). Tools utilized included a mouse sonde, surgical instruments, a flow cytometric instrument (BD FACS-Calibur, USA).

### *2.2. Preparation of Cnidoscolus aconitifolius Leaf Extract*

A certificate of determination was obtained from the Biology Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University with number 489/Lab. Bio/B/XII/2022. The collected leaves were wet sorted to select only those with a green color, then cleaned and washed using aquadest. Subsequently, they were dried in an oven until completely dry and powdered using a blender. The extraction process began by sorting the *Cnidoscolus aconitifolius* leaves, followed by airing for 3 days and oven-drying at 40°C until dry simplisia formed, which was then ground into powder. Approximately, 500 g of powder was obtained, and the extraction was carried out using the maceration method with 70% ethanol for 1 hour, totaling 600 ml. Remaceration was performed for 2 cycles with 200 ml each. The resulting liquid extract was concentrated using a rotary evaporator (Hidayati et al., 2023).

### *2.3. Animal Preparation*

Thirty male Babl/C mice weighing 20-30 g were acclimatized for 14 days in the experimental animal laboratory of the Faculty of Health Sciences, Universitas dr. Soebandi. Ethical clearance was obtained from the Health Research Ethics Committee of Universitas dr. Soebandi with number 096/KEPK/UDS/III/2023. The mice were divided into 6 groups: a negative control group of healthy mice without infection, a negative control group of mice infected with *Salmonella typhimurium* bacteria and administered a placebo of CMC-Na solution, a positive control group of mice infected with *Salmonella typhimurium* bacteria and treated with the standard immunomodulator Stimuno containing standardized *Phyllanthus niruri L*. extract (Dexa Medica, Indonesia), and treatment groups 1, 2, and 3 consisting of mice infected with *Salmonella typhimurium* bacteria and administered *Cnidoscolus aconitifolius* leaf extract (CAE) at doses of 100 mg/kg bw, 200 mg/kg bw, and 400 mg/kg bw, respectively. Infection induction was performed orally using *Salmonella typhimurium* bacteria at a concentration of 1x10^8 cfu (Destiawan et al., 2023). After 5 days post-induction, mice were evaluated for fecal texture, and tail blood smears were examined with Giemsa staining to detect. The treatment was administered orally once daily for 7 days.

### *2.4. TLR4 Analysis using Flow cytometer*

The TLR4 analysis involved lymphoid organs. The organs were washed and homogenized in 5 ml of phosphate buffer solution. Homogenization was continued until complete organ disruption using the base of a 3 ml syringe plunger, followed by filtration through a mesh and transfer into a 15 ml propylene tube with a 1:3 ratio. The sample was then centrifuged at 2500 rpm for 5 minutes at 10°C, and the supernatant was discarded. The pellet was resuspended in 1 ml of phosphate buffer solution.

Fifty microliters of the suspension were transferred to a 1.5 ml microtube, centrifuged at 2500 rpm for 5 minutes at 10°C, and the supernatant was discarded. Fifty microliters of TLR4 antibody solution were added and incubated for 20 minutes at 4°C in a dark room. Four hundred microliters of PBS were added, and the mixture was transferred to a cuvette for flow cytometry analysis (Djati et al., 2017).

### *2.5. Histological analysis*

Following the post-treatment period, all mice were euthanized, and their intestinal organs were collected and fixed in 4% formalin solution in phosphate buffer for 24 hours. Tissues were subsequently embedded in paraffin,

sectioned at a thickness of 4 µm, deparaffinized in xylene, dehydrated in graded alcohol concentrations, and stained with Hematoxylin-Eosin to assess neutrophil infiltration. Evaluation was performed using a light microscope with a 40X objective and 10X field of view, and the observed areas were selected randomly. The number of neutrophil cells within each tissue area in the images was counted and compared as a percentage relative to the control.

### *2.6. 2.6 Analysis of Results*

The neutrophil count data and TLR4 expression analysis were analyzed using a one-way ANOVA test with a significance level set at $p<0.05$, using the SPSS software for Windows. Post-hoc comparisons were conducted using the LSD test to determine differences between groups.

## 3. Results

### *3.1. Neutrophil cell infiltration effect of Cnidoscolus aconitifolius leaves extract*

Neutrophils were visualized using histopathological methods with hematoxylin-eosin staining (figure 1). Neutrophils constitute the initial innate immune defense against invasive infections caused by pathogens (Desai & Lionakis, 2018). Administration of CAE at doses of 100 mg/kgbw, 200 mg/kgbw, and 400 mg/kgbw resulted in increased neutrophil infiltration into the infected tissue.

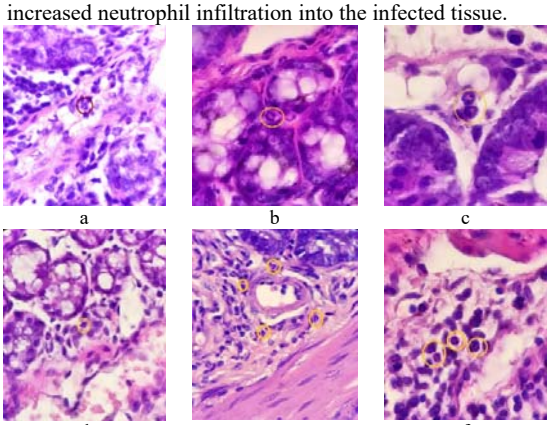
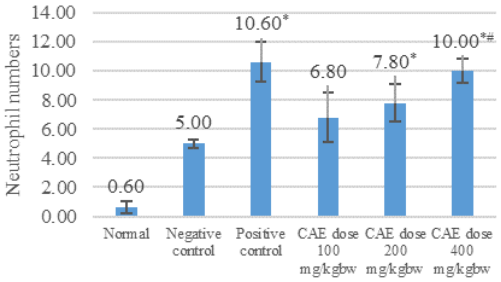



**Figure 1.** Neutrophil Description in Histological Preparations with Hematoxylin-Eosin Staining. (a) Normal groups, (b) Negative control, (c) Positive control, (d) CAE dose 100 mg/kgbw, (e) CAE dose 200 mg/kgbw, (f) CAE dose 400 mg/kgbw.

In this study, an evaluation of the number of neutrophils was carried out which showed that in the infection group the number of neutrophils in the tissue increased (Figure 2), which was shown by an increase in the number of neutrophil infiltration in the normal group of 0.60±0.40 significantly different from the negative control group 5.00±0.32 ($p<0.05$). Neutrophils recognize viruses and cells infected with the virus and infiltrate the site of infection. At the beginning of viral infection, neutrophils are rapidly recruited from the blood at the site of infection and mobilized to differentiate and migrate out of the bone marrow (Rawat et al., 2021).

Treatment of CAE dose administration of 400 mg/kgbw was able to increase the number of neutrophils significantly (10.00±0.84) compared to the untreated infection group (5.00±0.32), and this increase was not significantly different when compared to the positive control group (10.60±1.36) ($p<0.05$). Bioactive compounds are secondary metabolites found in plants and provide many health benefits if consumed over a long period of time in certain amounts. *Cnidoscolus aconitifolius* contains many bioactive compounds such as phenolic acids, alkaloids, saponins, flavonoids and terpenoids with a unique structure (Panghal et al., 2021a). Flavonoids have been declared therapeutic agents to avoid such damage, since these compounds exhibit anti-inflammatory activity, that is, through modulating the oxidative explosion of neutrophils. The catechol group on the B ring, together with the presence of 3-OH on the C ring and the double bond between C2–C3, is a determinant of activity (Ribeiro et al., 2018).



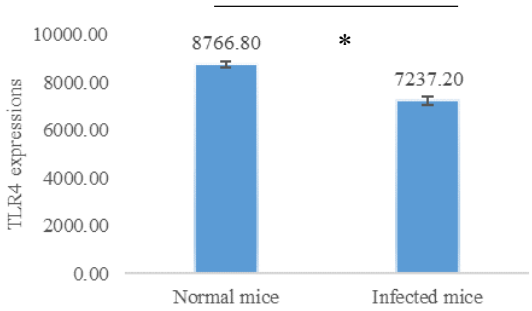

*Significant difference with negative control group ($p<0.05$)

#No significant different with positive control group ($p<0.05$)

**Figure 2.** Profile of the number of neutrophils in mice with *Salmonella thypimurium* faction with administration of CAE

### *3.2. TLR4 expressions in mice infected with Salmonella thpymurium bacteria*

In this study, it was found that TLR4 expression from lymph organs in mice infected with *Salmonella thpymurium* bacteria decreased significantly after acute infection in post treatment from 8766.80±172.73 to 7237.20±162.17 (Figure 3). Research using pigs with lipopolysaccharide administration showed that TLR4 expression in lymph on day 7 decreased significantly (Ghosh et al., 2016; Qin et al., 2020).



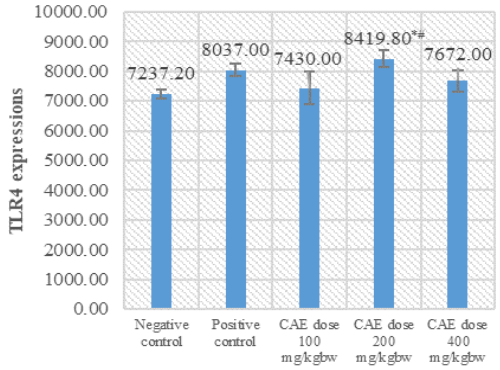

* Significant differences between groups ($p<0.05$)

**Figure 3.** TLR4 expression profile in normal mice and mice infected with *Salmonella thypimurium* as shown at the the decreased TLR4 expression after induction

### *3.3. TLR4 expressions of Cnidoscolus aconitifolius leaves extract*

In figure 4 and 5, we can see that after treatment for 7 days orally, CAE dose administration of 200 mg/kgbw showed a significant increase in TLR4 expression

(8419.80± 276.745) compared to the negative control group (7237.20± 162.17) and did not differ significantly from the positive control group (8037.00± 206.40). TLR4 activation is associated with infection repair. TLR4 overexpression can counteract the invasion of Salmonella typhimurium as well as fight gut inflammation in sheep by regulating gut microbiota composition and increasing anti-inflammatory metabolites (Xu et al., 2023).




*Significant difference with negative control group (p<0.05)

#No significant different with positive control group (p<0.05)

**Figure 4.** Effect *Cnidoscolus aconitifolius* leaves extract to TLR4 expressions in mice infected with *Salmonella thypimurium*.

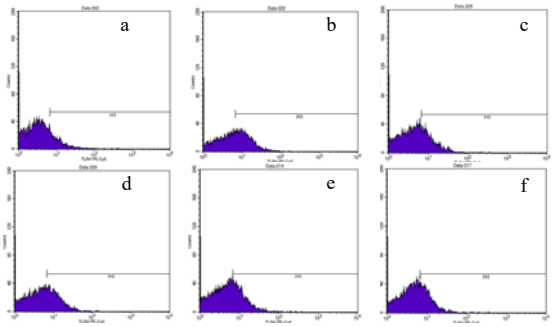



**Figure 5.** Results of flow cytometric analysis of TLR4 expression in mice infected with *Salmonella thypimurium*. (a) Normal groups, (b) Negative control, (c) Positive control, (d) CAE dose 100 mg/kgbw, (e) CAE dose 200 mg/kgbw, (f) CAE dose 400 mg/kgbw.

## 4. Discussion

The immune system encompasses a complex network of cells, chemicals, and processes aimed at protecting various body sites from foreign antigens such as microbes (e.g. bacteria, fungi, parasites), viruses, and cancer cells (Marshall et al., 2018). Apart from physical and chemical barriers, the immune system operates through two main defense mechanisms: innate immunity and adaptive immunity. While innate immunity acts as the initial defense against invading pathogens, it works in conjunction with adaptive immunity to provide comprehensive protection against disease (Prabhu, 2023).

Neutrophils, also known as polymorphonuclear leukocytes, develop from blast cells and mature into terminally differentiated cells in the bone marrow before being released into peripheral blood. Under physiological conditions, the number of neutrophil production is $10^{11}$ per day, which is influenced by cell death and production rate. Neutrophils are relatively short-lived. It is estimated that neutrophils have an age of 8-12 hours in peripheral blood (Ma et al., 2021). During acute infections, neutrophils migrate from the blood into infected tissues, where they execute vital functions including binding, phagocytosis, and the oxidative and non-oxidative killing of intracellular microorganisms. They also release inflammatory mediators extracellularly (Desai & Lionakis, 2018).

In this study, it was determined that CAE at a dose of 200 mg/kgbw and 400 mg/kgbw significantly increased neutrophil infiltration compared to the negative control group ($p<0.05$). Specifically, only the CAE at 400 mg/kgbw achieved neutrophil levels comparable to the positive control group (10.60±1.36) without significant difference ($p>0.05$). Neutrophils play a crucial role as the body's primary defense mechanism against bacterial and fungal pathogens, operating swiftly before the adaptive immune responses come into play (Kubes, 2018). The ability of CAE to enhance neutrophils numbers underscores its potential importance in bolstering initial defenses post-infection.

*Cnidoscolus aconitifolius* leaves are rich in phytochemical compounds, including 9-Octadecenoic (Z) acid and its esters, n-Hexadecanoic acid, n-Octadecanoic acid, n-Octacosane, 1,2,3-Propanetriol derivatives, and l-(+)-Ascorbic acid-2,6-dihexadecanoate, which offer diverse therapeutic benefits (Abayomi et al., 2014).

Flavonoids isolated from *Cnidoscolus aconitifolius* include procyanidin B1, procyanidin B2, catechin, rutin, gallocatechin gallate, epigallocatechin gallate, epicatechin-3-O-gallate, quercetin-3-O-galactoside, quercetin-3-O-glycoside, quercetin-3-O-rhamnoside, trans-reverseratol, quercetin and Kaemferol (Panghal et al., 2021b). Another study reported Japanese papaya leaves had ten carotenoids detected consisting mainly of carotene (43.7-46.1%), lutein (20.8-22.5%) and neo-xanthin (10.92-12.99%). Beta-amyrin (52.2–66.3%), alpha-amyrin (18.5–31.6%) and lupeol (14.8–15.9%) were the most abundant detected phytosterols. These results show that *Cnidoscolus aconitifolius* leaves are a good source of nutrients and bioactive phytochemicals that can support human health and nutrition (Mercy et al., 2019). Plant extracts containing active phytochemicals and various bioactive ingredients such as flavonoids, alkaloids, saponins, quinones, triterpenoids, tannins and phenolics have been claimed to have benefits in various treatments (Nugroho et al., 2020).

The Toll like receptor (TLR) family serves as vital pattern recognition receptors (PRRs) capable of identifying various bacteria and viruses, triggering the secretion of inflammatory cytokines and chemokines. TLR4, predominantly expressed on macrophages, dendritic cells, and neutrophils, plays a critical role in inflammation, autophagy, and oxidative stress during pathogenic infections (Zhang et al., 2021). TLR4 predominantly expressed on macrophages, dendritic cells, and neutrophils plays a critical role in inflammation, autophagy, and oxidative stress during pathogenic infections (Deng et al., 2020). Specifically, TLR4 recognizes lipopolysaccharide endotoxins (LPS), a major component of Gram-negative bacteria, stimulating immune cells to produce proinflammatory cytokines like interleukins (IL)-8, IL-6,

IL-1β, IL-12, and tumor necrosis factor α (TNFα) to combat invading pathogens (Ciesielska et al., 2021).

Research indicates that TLR4 and its downstream genes are expressed across gut and immune tissues at various developmental stages, with tissue-specific variations possibly reflecting differential functional responses to pathogenic stimuli. Notably, TLR4 expression is more pronounced in the spleen, the body's largest immune organ comprising 25% of lymphoid tissue, central to both cellular and humoral immunity (Qin et al., 2020).

## 5. Conclusion

From this study, it can be concluded that the administration of CAE at a dose of 200 mg/kgbw effectively modulates TLR4 expression and enhances neutrophil activity. This modulation contributes to the activation of the immune response, suggesting that CAE holds promise as a candidate immunomodulatory agent.

## Acknowledgment

This research received funding from the Kemenristekdikti through a beginner lecturer research 2023.

## References

Abayomi O, Olorunfemi EO and Mikailu S. 2014. Phytochemical Analysis of *Cnidoscolus aconitifolius* (Euphorbiaceae) leaf with Spectrometric Techniques. *Niger J Pharm Appl Sci Res*., ***3*(31)**: 38–49.

Aboudounya MM, & Heads RJ. 2021. COVID-19 and Toll-Like Receptor 4 (TLR4): SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating Entry and Causing Hyperinflammation. *Mediators Inflamm.,* **2021(8874339):** 1-18

Alamgir M and Uddin SJ. 2010. **Recent advances on the ethnomedicinal plants as immunomodulatory agents**. Research Signpost, pp. 227-244.

Ciesielska A, Matyje M and Kwiatkowska K. 2021. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. *Cell Mol Life Sci.,* **78(4)**: 1233–1261.

Cordero-Alba M, García-Gómez JJ, Aguilera-Herce J and Ramos-Morales F. 2016. Proteomic insight into the effects of the Salmonella ubiquitin ligase SlrP on host cells. *Biochem Biophy Res Comm.*, **472(3)**: 539–544.

Deng JS, Jiang WP, Chen CC, Lee LY, Li PY, Huang WC, Liao JC, Chen HY, Huang SS and Huang GJ. 2020. Cordyceps cicadae mycelia ameliorate cisplatin-induced acute kidney injury by suppressing the TLR4/NF-κB/MAPK and activating the HO-1/Nrf2 and Sirt-1/AMPK pathways in mice. *Oxid Med Cell Longev*., **2020(7912763):** 1-17

Desai JV and Lionakis MS. 2018. The Role of Neutrophils in Host Defense Against Invasive Fungal Infections. *Curr Clin Microbiol Rep.,* **5(3)**: 181–189.

Destiawan RA, Rahmawati SE, Wijaya AF, Muflihah AI, Nurjanah MH, Hidayati S, and Sari NKY. 2023. Effect Of Black Grape Extract On Cd4+ And Cd8+ Expression In Mice Infected With *Salmonella typhimurium*. *Jurnal Biosains Pascasarjana.*, **24(1SP)**: 54–63.

Djati MS, Habibu H, Jatiatmaja NA and Rifa'I M. 2017. *Elephantopus scaber* L extract-induced CD4+ and CD8+ differentiation from hematopoietic stem cells and progenitor cell proliferation in mice (Mus musculus L). *AIP Conference Proceedings*., **1908(2)**: 2–8.

Firmal P, Shah VK and Chattopadhyay S. 2020. Insight Into TLR4-Mediated Immunomodulation in Normal Pregnancy and Related Disorders. *Front Immunol*., **11(5):** 1–16.

Fitrya F, Amriani A, Novita RP, Elfita and Setiorini D. 2020. Immunomodulatory effect of Parkia speciosa Hassk. pods extract on rat induced by Salmonella typhimurium. *J Pharm Pharmacog Res*., **8(5):** 457–465.

Ghosh D, Wikenheiser DJ, Kennedy B, McGovern KE, Stuart JD, Wilson EH and Stumhofer JS. 2016. An Atypical Splenic B Cell Progenitor Population Supports Antibody Production during Plasmodium Infection in Mice. *J Immunol*., **197(5):** 1788–1800.

Harun NH, Septama AW and Jantan I. 2015. Immunomodulatory effects of selected Malaysian plants on the CD18/11a expression and phagocytosis activities of leukocytes. *Asian Pac J Trop Biomed*., **5(1)**: 48–53.

Hidayati S, Susanti DA, Faizah N and Purwanti A. 2023. *Optimization of Solvent Concentration on Flavonoid Levels of Cnidoscolus aconitifolius Leaf Extract*. Katalisator., **8(2):** 324–333.

Jayathirtha MG and Mishra SH. 2004. Preliminary immunomodulatory activities of methanol extracts of Eclipta alba and *Centella asiatica*. *Phytomedicine*. **11(4):** 361–365.

Kalia P, Kumar NR and Harjai K. 2016. Effect of propolis extract on hematotoxicity and histological changes induced by Salmonella enterica serovar Typhimurium in balb/C mice. *Archiv Biol Sci*., **68(2):** 385–391.

Kubes, P. 2018. The enigmatic neutrophil: what we do not know. *Cell Tiss Res*., **371**: 399–406.

Ma Y, Zhang Y and Zhu L. 2021. Role of neutrophils in acute viral infection. *Immun, Inflamm Dis*., **9(4):** 1186–1196).

Mercy, OI., Catherine, CI and Jude, CI. 2019. Nutrient and bioactive phytochemical compositions of *Cnidoscolus aconitifolius*. *Malay J Biochem Mol Biol*., **22(2):a** 26–36.

Mukherjee S, Karmakar S and Babu SPS. 2016. TLR2 and TLR4 mediated host immune responses in major infectious diseases: a review. *Brazilian J Infec Dis*., **20**: 193–204.

Nugroho RA, Sari YP and Hardi EH. 2020. Inclusion of Myrmecodia pendens bulb Extract in the Diet Stimulates Immune Response in Clarias gariepinus against Aeromonas hydrophila. Jordan J Biol Sci., **13(4):** 463-468

Panghal A, Shaji AO, Nain K, Garg MK and Chhikara N. 2021. *Cnidoscolus aconitifolius*: Nutritional, phytochemical composition and health benefits – A review. *Bioact Compound Health Dis*., **4(11):** 260–286.

Pérez-González MZ, Siordia-Reyes AG, Damián-Nava P, Hernández-Ortega S, Macías-Rubalcava ML and Jiménez-Arellanes MA. 2018. Hepatoprotective and Anti-Inflammatory Activities of the *Cnidoscolus chayamansa* (Mc Vaugh) Leaf Extract in Chronic Models. *Evid Based Complement Alternat Med.,* **2018(3896517):**1-12.

Prabhu SR. 2023. **An Introduction to Immunology and Immunopathology**. In *Textbook of General Pathology for Dental Students*. Springer. pp. 73–80.

Qin W, Gao Z, Cao Y, Wu Z, Bao W and Wu S. 2020. Developmental expression patterns and correlation analysis of TLR4 and its downstream genes in the intestinal and immune tissues of Meishan pigs. *R. Bras. Zootec.,* **49(e20180296):** 1-12.

Rahmasari V & Lestari K. 2018. Article Review: *Management of Typhoid Fever Therapy: A Study of Pharmacological and Non-Pharmacological Therapies*. *Farmaka*. **16(1):** 184–195.

Rawat S, Vrati S and Banerjee A. 2021. Neutrophils at the crossroads of acute viral infections and severity. *Mol Aspects Med.,* **81(100996):**1-16

Ribeiro D, Fernandes E and Freitas M. 2018. **Flavonoids as modulators of neutrophils' oxidative burst: Structure-activity relationship**. In *Polyphenols: Mechanisms of Action in Human Health and Disease,* Elsevier. pp. 261–276.

Romerio A and Peri F. 2020. Increasing the chemical variety of small-molecule-based TLR4 modulators: An overview. *Front Immunol*, **11:** 1210.

Rosadini CV and Kagan JC. 2017. Early innate immune responses to bacterial LPS. *Curr Opinion Immunol*., **44:** 14–19.

Somade OT, Ugbaja RN, Idowu MA and Akinloye OA. 2021. *Cnidoscolus aconitifolius* leaf extract and ascorbate confer amelioration and protection against dimethyl nitrosamine-induced renal toxicity and testicular abnormalities in rats. *Toxicol Rep.*, **8:** 1098–1108.

Venkatalakshmi P and Brindha P. 2016. Antimicrobial activity of aqueous extracts of different parts of Terminalia catappa L. *Int J Curr Microbiol Appl Sci.*, **5(12):** 493–498.

Xu XL, Zhao Y, Chen MM, Li Y, Li Y, Wu SJ, Zhang JL, Zhang XS, Yu K and Lian ZX. 2023. Shifts in intestinal microbiota and improvement of sheep immune response to resist Salmonella infection using Toll-like receptor 4 (TLR4) overexpression. *Front Microbiol*., **14(2023):**1-15.

Zhang K, Huang Q, Deng S, Yang Y, Li J and Wang S. 2021. Mechanisms of TLR4-Mediated Autophagy and Nitroxidative Stress. *Front Cell Infect Microbiol*., **11(766590):**1-11.