JIPHO (Jurnal Ilmiah Perternakan Halu Oleo): Vol: 8, No 2, April 2026 Halaman: 243-249
eISSN: 2548-1908
DOI: 10.56625/jipho.v8i2.924

Andrographis paniculata ethanolic extract affects the spleen's histopathology of *Salmonella*-infected mice

Purnaning Dhian Isnaeni[1*], Fuji Astuty Auza[2], Astriana Napirah[2], Putu Nara Kusuma Prasanjaya[2], Asma Bio Kimestri[2], Indah Amalia Amri[3], Desy Cahya Widianingrum[1], Eistifani Fajrin[1], Himmatul Khasanah[1]

[1] Animal Science Study Programme, Faculty of Agriculture, University of Jember, Jember, Indonesia

[2] Faculty of Animal Husbandry, Halu Oleo University, Kendari, Indonesia

[3] Laboratory of Microbiology and Immunology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia

**Corresponding author*: purnaningdhian@unej.ac.id

Abstract. *Andrographis paniculata*, AP, is one of the herbs that are widely utilised as an alternative medicine for its active ingredients, especially andrographolide, which acts as an anti-inflammatory, antibiotic, and immunomodulator. Andrographolide's use as an immunomodulator in many animal models has been recorded, but the effect on the spleen of mice infected by bacteria, particularly *Salmonella sp*. has yet to be done so far. Forty mice were grouped into five different treatments in a completely randomised design: control negative (uninfected, untreated mice), control positive (infected, untreated mice), AP1 (infected, 200mg/kg BW AP), AP2 (infected, 300mg/kg BW AP), and AP3 (infected, 400mg/kg AP). The collected data was analysed to determine the effect of AP extract on mice's spleen. The results showed that AP extract maintain the tissue damage of the spleen in *Salmonella*-infected mice to be minimum, as compared to the control positive. AP3 treatment has not been proved to repair the damaged tissue of spleen infected with *Salmonella sp*.

Keywords: *Andrographis paniculata*, immunomodulator, *Salmonella*, spleen, infection

Abstrak. *Andrographis paniculata*, AP, merupakan salah satu herba yang banyak digunakan sebagai obat alternatif karena kandungan senyawa aktifnya, terutama andrografolid, yang berperan sebagai antiinflamasi, antibiotik, serta imunomodulator. Penggunaan andrografolid sebagai imunomodulator telah banyak diujikan pada berbagai hewan coba, namun efeknya terhadap limpa mencit yang terinfeksi bakteri, khususnya *Salmonella sp*., masih belum banyak diteliti. Sebanyak 40 ekor mencit dikelompokkan menjadi lima kelompok perlakuan dan 4 ulangan menggunakan rancangan acak lengkap. Perlakuan terdiri dari kontrol negatif (tanpa infeksi, tanpa pemberian AP), kontrol positif (diinfeksi, tanpa pemberian AP), AP1 (diinfeksi, pemberian AP 200 mg/kg BB), AP2 (diinfeksi, pemberian AP 300 mg/kg BB), dan AP3 (diinfeksi, pemberian AP 400 mg/kg BB). Data mengenai organ limpa dianalisa untuk menentukan efek pemberian ekstrak AP pada mencit. Hasil penelitian menunjukkan bahwa pemberian ekstrak AP mampu menjaga efek kerusakan jaringan pada organ limpa mencit yang terinfeksi *Salmonella sp* menjadi minimal jika dibandingkan dengan kontrol positif. Perlakuan AP3 belum mamp menunjukkan perbaikan jaringan limpa akibat infeksi *Slamonella sp*.

Kata kunci: *Andrographis paniculata*, imunomodulator, *Salmonella*, limpa, infeksi

1. Introduction

Artificial supplements have been widely used as complementary nutrients to address the deficiency in one's diet. However, its bioavailability is considerably lower than that of the natural supplements, and it poses a risk of toxicity if taken continuously or in large amounts [1]. These supplements include a variety of vitamins, micronutrients, and other compounds assumed to be beneficial to the body. In western countries, the use of natural supplements (nutraceuticals) has increased despite the criticism for the lack of clinical evidence, claims of effects, and issues of self-medication [2]. Various research and efforts have been conducted to prove the effectiveness of naturally sourced compounds, especially for drugs, compared to their synthetic counterparts.

JIPHO (Jurnal Ilmiah Perternakan Halu Oleo): Vol: 8, No 2, April 2026 Halaman: 243-249
eISSN: 2548-1908
DOI: 10.56625/jipho.v8i2.924

Sambiloto (*Andrographis paniculata*/AP) is one of the important plants for natural medication and can be potentially used as phytogenic feed additives/PFA as it contains many active constituents. PFAs are mostly used to increase the digestibility and absorption of nutrients and also to control pathogenic bacteria in the animals' gut [3] [4]. The andrographolide compounds have been associated with antibacterial, anti-viral, and immunomodulatory effects [5]. An immunomodulator is an active compound that helps regulate an individual's immune system.

The spleen is a crucial immune organ responsible for initiating immune reactions and regulating the regeneration of red blood cells [6]. It is suggested that spleen weight correlates with the cellular immune response inside the spleen in some cases, such as skin inflammation [7] and tumours [8]. It can be inferred that the heavier the spleen, the more active is the immune response. However, the spleen weight cannot be a determining factor in measuring the immune response. A histopathological reading of the spleen is needed to measure the mice's immune response to an infection.

Various studies have been done to explore the immunomodulatory effect of AP. Still, its specific impact on the spleen, especially regarding the immune modulatory effect on infected mice, has not been recorded yet. This research aimed to analyse the impact of AP ethanolic extract on the spleen of mice. To examine the effectiveness of AP extract, the mice were infected with *Salmonella sp*.

2. Research Methods

2.1 Ethical clearance

This study was ethically approved by Brawijaya University Animal Care and Use Committee, together with approval number/ID (102-KEP-UB-2024).

2.2 AP extract preparation

The AP leaves were collected from local sources. The leaves were then sun-dried before being put in a 60 °C oven for 24 hours. The dried leaves were ground and made into a fine powder. AP extract was made according to maceration methods by mixing AP leaf flour with 96% ethanol at a 1:5 ratio, and it was continued by evaporating the macerates until a pure extract was obtained [9].

2.3 Research procedure

Forty adult female mice were grouped into five treatment groups consisting of control negative/C- (uninfected and untreated mice), control positive/C+ (*Salmonella*-infected, untreated mice), AP1 (*Salmonella*-infected, treated with 200 mg/kg BW AP), AP2 (*Salmonella*-infected, treated with 200 mg/kg BW AP), and AP3 (*Salmonella*-infected, treated with 400 mg/kg BW AP). The mice were reared for a total of 30 days. For the first 7 days, the mice were put on an adaptation period and were not treated.

The AP was given by oral gavage according to the groups for 21 days after adaptation. On day 22, the C+, AP1, AP2, and AP3 groups were infected with 10^6 CFU (colony-forming unit) of *Salmonella sp* orally [10] [11]. The mice were then sacrificed 48 hours after infection.

The mice were euthanised using chloroform, and the organs were collected, weighed, and put in formaldehyde solutions for storage before being made into tissue histology slides. The slides were made by the standard process and stained with HE (hematoxylin-eosin). This research was done by a completely randomised design with 5 treatment groups and 4 repetitions. The reading of slides was done qualitatively by comparing the tissue with normal spleen tissue, and quantitatively by scoring system [12]. The spleen tissue score was ranged from 0 (no necrosis), 1 (focal/light necrosis), 2 (multifocal/moderate necrosis), and 3 (severe/diffuse necrosis) [12]. The data collected were weight, relative weight, and histopathological features of the spleen. The weight and relative weight of the spleen organ were measured and analysed quantitatively by ANOVA.

3. Results and Discussion

3.1. Spleen macroscopic features

The results (**Table 1**) indicated that AP extract treatment did not affect the spleen weight of mice infected with *Salmonella sp*. The spleen is the organ responsible for the body's immune defence. In this research, the spleen weight was not significantly affected either AP extract treatment. An increase in

eISSN: 2548-1908
DOI: 10.56625/jipho.v8i2.924

spleen weight may lead to splenomegaly, an abnormal enlargement of the spleen. The increase in spleen organ weight might indicate various conditions, including being overweight due to a high-fat, high-sugar diet [13], liver inflammation [14], and tumour growth [8]. Infection by *Plasmodium berghei* also caused splenomegaly in mice [15]. Previous research also concluded that the spleen of immunodeficient mice is relatively smaller than that of normal wild-type mice [16].

However, AP extract treatment demonstrated an increase in the spleen-to-body weight ratio. An organ-to-body weight ratio indicates the weight proportion of an organ to body weight. The increased value shown in **Table 1** might be due to the reduced body weight of treatment groups compared to control groups, which is related to stress. The treatment utilised oral gavage, which was administered to the mice daily. There was significant weight loss in the treatment groups' mice. A decrease in body weight accompanied by constant spleen weight resulted in an increase in spleen relative weight to body weight. The highest spleen-relative weight was observed in the AP2 treatment compared to other treatments.

Table 1. Spleen weight and spleen to body weight ratio of mice treated with different doses of *Andrographis paniculata* extract

Treatment group	Spleen Weight (gram)	Body weight (gram)	Spleen to body weight ratio (%)
C-	0.28 ± 0.04	27.43 ± 2.07	0.95 ± 0.08^{b}
C+	0.30 ± 0.06	27.50 ± 2.15	1.09 ± 0.09^{ab}
AP1	0.34 ± 0.12	25.83 ± 2.48	1.33 ± 0.28^{ab}
AP2	0.36 ± 0.15	25.00 ± 2.63	1.43 ± 0.40^{a}
AP3	0.25 ± 0.06	23.50 ± 3.82	1.05 ± 0.11^{ab}

Note: different superscripts (a,b) on the same column indicate significant differences between treatments

As mentioned before, the spleen weight is related to the immunomodulatory response of cells in the spleen. Splenomegaly has been mentioned to be positively correlated with the increase of cellular immune response, which usually happens during inflammation or infection. Although the body weight of the treatment group decreased, the spleen weight of all groups was not different statistically. This can be interpreted that even if the treatment group mice were in a constant stressed state, it did not affect their immune response. However, the spleen weight and spleen-to-body weight ratio cannot be the sole indicators of mice's immune response. Further histopathological findings shall be used as consideration for making a diagnosis of spleen function.

3.2 Spleen microscopic features

The spleen is the main organ supplying white blood cells. The decrease or increase of the immune system is typically linked to spleen function. Spleen tissue examination can be used as a pointer to the immune system quality and strength. The microscopic view of the mouse spleen tissue treated with AP extract and infected with *Salmonella sp*. is shown in Figure 1. Observation of spleen tissue in mice in the negative control treatment (C-) showed normal tissue consisting of red pulp dominated by erythrocyte cells, and white pulp dominated by white blood cells. There is a clear boundary between the white pulp and red pulp, with the spleen capsule (KP) still intact. The spleen tissue picture in the control positive treatment (C+) showed an unclear boundary between the red pulp and white pulp areas. This may occur due to increased activity of the white pulp in the spleen, which may be due to *Salmonella typhi* infection [17].

Salmonella sp is one of the bacteria that has the ability to infect a variety of hosts, including humans, rodents, poultry, and ruminants and cause long-term infections that are systemic [18]. *Salmonella sp* bacteria generally attack the digestive system, but the infection can also take place systemically and can be detected through various organs, including the intestines, liver, spleen, kidneys, and even the brain [19] [20] [21] [10]. The observation of spleen tissue in the control positive treatment (C+) also showed visible congestion in the blood vessels caused by spleen tissue damage. Observations also showed localised tissue damage in the form of necrosis, which was suspected to be at the stage of

JIPHO (Jurnal Ilmiah Perternakan Halu Oleo): Vol: 8, No 2, April 2026 Halaman: 243-249
eISSN: 2548-1908
DOI: 10.56625/jipho.v8i2.924

karyopyknotic due to the visible cell shrinkage and an increase in basophilia that occurs because DNA is compressed into a solid basophilic mass.

The results of observations of the spleen tissue of mice in the AP1 treatment (dose of 200 mg/kg BW) showed that the red pulp and white pulp were still clearly visible and intact. Observations also showed congestion in the blood vessels. Yellow arrows indicate localised tissue damage that is thought to be due to spleen tissue necrosis. The condition of the spleen tissue, in general, in the AP1 treatment is not so different when compared to the positive control treatment. The continuous administration of AP extract for 21 days before infection is thought not to have been able to minimise spleen damage caused by *Salmonella sp* infection.

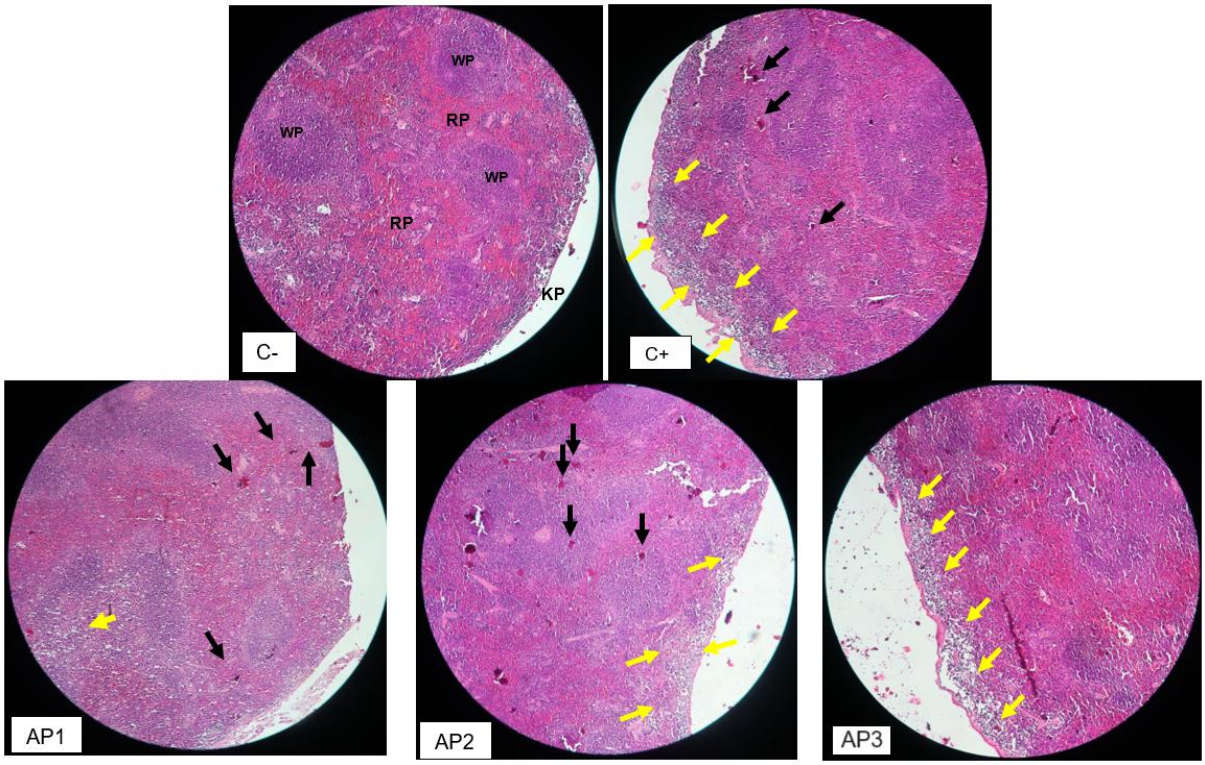

Figure 1. The microscopic view of the spleen of mice treated with AP extract and infected with *Salmonella sp*. RP (Red pulp), WP (white pulp), KP (capsule of spleen), Black arrow (congestion), yellow arrow (tissue degeneration)

The picture of spleen tissue in the AP2 treatment (dose of 300 mg/kg BW) showed congestion in the blood vessels (black arrows). Observations also showed localised tissue damage that was thought to be due to necrosis of the spleen tissue. Interpretation of the results of the examination assumes that spleen tissue damage by *Salmonella sp*. infection can still be observed in mice given AP extract at a dose of 300 mg/kg BW.

Observation of spleen tissue images in the AP3 treatment (dose of 400 mg/kg BW) showed unclear boundaries (diffuse) between the red pulp and white pulp areas. The spleen capsule is still visible, but in the capsule area, there is tissue necrosis. This spleen tissue damage is thought to be due to *Salmonella sp*. infection. The administration of AP extract at a dose of 400 mg/kg BW has not been able to repair the damage that occurs in the spleen of mice by *Salmonella sp*. The administration of water and ethanol extracts of AP is known to protect liver, kidney, heart, lung, and spleen tissue damage in rats given a nicotine challenge [22], however in this research, a 400 mg/kg BW of AP ethanolic extract did not present the same result. Research by [22] mainly focusing in the function of the spleen by analyzing the protein oxidation activity, meanwhile this research focuses on the histopathological effect

of AP extract. Even given the highest dose of 400 mg/kg BW, the damage caused by *Salmonella* infection could not be repaired instantly. Considering that the organ collection was done 48 hours after infection, the damage inflicted by *Salmonella* on spleen tissue was not fully recovered yet. Further research is needed in order to examine the spleen tissue condition of *Salmonella*-infected mice treated with AP extract in longer period.

Overall, spleen tissue damage seen in mice treated with AP extract (AP1, AP2, and AP3) has not shown improvement when compared to the positive control treatment. This study's results differ from the study of in vivo administration of andrographolide to spleen cells of mice immunised with hepatitis B antigen, which showed a decrease in cells producing interleukin 4 [23]. A longer study time is needed to see how far the effect of administration of AP extract on the improvement of the spleen tissue picture of mice infected with *Salmonella sp*. Studies show that infection by *Salmonella sp* can last up to 6 months [24], so it is suspected that the administration of AP extracts up to a dose of 400 mg/kg BW for 21 days before infection has not been able to prevent spleen tissue damage.

3.3 Spleen tissue score

Aside from the qualitative examination, the spleen tissues were also examined quantitatively by scoring. The scoring of spleen tissues is displayed in **Table 2**. The scores consisted of four levels: score 1 for normal tissue; score 2 for focal or light bleeding or necrosis; score 3 for multifocal or moderate bleeding or necrosis; and score 4 for diffused or heavy bleeding or necrosis [12].

Table 2. Spleen histopathological scoring of mice treated with different doses of *Andrographis paniculata* extract.

Treatment group	Spleen tissue score
C-	1.42 ± 0.23^{a}
C+	3.00 ± 0.42^{c}
AP1	2.35 ± 0.09^{b}
AP2	2.00 ± 0.20^{b}
AP3	1.85 ± 0.38^{ab}

Note: different superscripts (a,b,c) on the same column indicate significant differences between treatments

The control negative and AP3 treatment groups exhibited similar results of spleen tissue score between 1.42 and 1.85. Those scores are higher than normal (score 1), but still lower than score 2, which indicates light bleeding and/or necrosis of spleen tissue. This indicated that AP extract at a 400 mg/kg BW dose improved the spleen tissue of Salmonella-infected mice to a level similar to that of uninfected mice (control negative). The highest tissue score was demonstrated by the control positive treatment. This indicated that the spleen tissue of Salmonella-infected mice underwent moderate or multifocal bleeding and/or necrosis.

Those damages caused by *Salmonella sp* infection in this study were not considered heavy necrosis, which might be due to the duration of the infection. The acute infection model demonstrated in this study has not yet damaged the spleen tissue much. *Salmonella sp* is a multi-species infectious agent, including humans, rodents, poultry, and ruminants, and it causes chronic and systemic infection [18]. The *Salmonella* infection in this study was categorised as an acute infection, in which the samples were taken two days after the bacterial inoculum was given to the mice. In an acute infection, a drastic increase in the spleen's activity is indicated by bleeding, swelling, and tissue inflammation. The spleen acts in regulating the white blood cells, thus increasing activity during infection.

The tissue scores of the spleen recorded in this study improved gradually from the control positive, AP1, AP2, AP3 and control negative treatments. This can be interpreted that on infected mice, the spleen tissue showed the worst damage compared to other treatments (C+ versus C-, AP1, AP2, and AP3). However, administration of AP extract at 200 mg, 300 mg, and 400 mg/kg BW doses showed improving score of spleen tissue to a similar score on non-infected/control negative, especially in AP3 (1.42 and 1.85, respectively). However, even at the highest dose of 400 mg/kg BW, *Andrographis paniculata* extract did not manage to repair all the damage caused by *Salmonella* infection in the mice's spleen.

4. Conclusions

Treatment of *Andrographis paniculata* extract for mice as an immunomodulator up to 400 mg/kg bodyweight still did not manage to repair the tissue damage of the spleen caused by *Salmonella sp*. infection. However, the spleen tissue damage was not observed macroscopically, and the spleen weights were normal.

5. References

[1] Nangare SD, Kandalkar PS and Kawade RM. 2024. Artificial Vitamins and Supplements: A comprehensive review of their development, benefits, risks, and emerging trends. International Journal of Novel Research and Development, 9(12). 541-550.

[2] Williamson EM, Liu X and Izzo AA. 2020. Trends in use, pharmacology, and clinical applications of emerging herbal nutraceuticals. Br J Pharmacol. 2020 Feb 6;177(6):1227–1240. doi: 10.1111/bph.14943

[3] Athanasiadou S, Githiori J and Kyriazakis I. 2007. Medicinal plants for helminth parasite control: Facts and fiction. *Animal*, *1*(9), 1392–1400. https://doi.org/10.1017/S1751731107000730

[4] Balunas MJ and Kinghorn AD. 2005. Drug discovery from medicinal plants. *Life Sciences*, *78*(5), 431–441. https://doi.org/10.1016/j.lfs.2005.09.012

[5] Vetvicka V and Vannucci L. 2021. Biological properties of andrographolide, an active ingredient of Andrographis Paniculata: a narrative review. *Annals of Translational Medicine*, *9*(14), 1186–1186. https://doi.org/10.21037/atm-20-7830

[6] Cesta MF. 2006. Normal Structure, Function, and Histology of the Spleen. *Toxicologic Pathology*, 34(5):455-465. doi:10.1080/01926230600867743

[7] Shinno-Hashimoto H, Eguchi A, Sakamoto A, Wan X, Hashimoto Y, Fujita Y, Mori C, Hatano M, Matsue H and Hashimoto K. 2022. Effects of splenectomy on skin inflammation and psoriasis-like phenotype of imiquimod-treated mice. *Sci Rep* 12, 14738. https://doi.org/10.1038/s41598-022-18900-7

[8] Jiang W, Li Y, Zhang S, Kong G and Li Z. 2021. Association between cellular immune response and spleen weight in mice with hepatocellular carcinoma. *Oncol Lett.* 22(2): 625. DOI: 10.3892/ol.2021.12886.

[9] Susanti NMP, Warditiani NK, Laksmiani NPL, Widjaja INK, Rismayanti AAMI and Wirasuta I. MAG. 2014. Perbandingan Metode Ekstraksi Maserasi dan Refluks Terhadap Rendemen Andrografolid dari Herba Sambiloto (*Andrographis paniculata* (Burm.f.) Nees). *Universitas Udayana*, 29–32.

[10] Nilsson OR, Kari L and Steele-Mortimer O. 2019. Foodborne infection of mice with Salmonella Typhimurium. *PLoS ONE*, 14(8), 4–15. https://doi.org/10.1371/journal.pone.0215190

[11] Wickham ME, Brown NF, Provias J, Brett BB and Coombes BK. 2007. Oral infection of mice with *Salmonella enterica* serovar Typhimurium causes meningitis and infection of the brain. *BMC Infectious Diseases*, *7*: 1–6. https://doi.org/10.1186/1471-2334-7-65

[12] Hidayati E, Berata IK, Samsuri S, Sudimartini LM and Merdana IM. 2018. Gambaran Histopatologi Limpa Tikus Putih yang Diberi Deksametason dan Vitamin E. *Buletin Veteriner Udayana*, 10(1), 18. https://doi.org/10.24843/bulvet.2018.v10.i01.p03

[13] Buchan L, St Aubin CR, Fisher AL, Hellings A, Castro M, Al-Nakkash L, Broderick TL and Plochocki JH. 2018. High-fat, high-sugar diet induces splenomegaly that is ameliorated with exercise and genistein treatment. *BMC Research Notes*, 11(1), 4–9. https://doi.org/10.1186/s13104-018-3862-z

[14] Wang C-B, Wang Y, Yao Y, Wang J-J, Tsuneyama K, Yang Q, Liu B, Selmi C, Gershwin ME, Yang S-H and Lian Z-X. 2022. The gut microbiome contributes to splenomegaly and tissue inflammation in a murine model of primary biliary cholangitis. *Annals of Translational Medicine*, 10(9), 507–507. https://doi.org/10.21037/atm-21-5448

[15] Wang H, Li S, Cui Z, Qin T, Shi H, Ma J, Li L, Yu G, Jiang T and Li C. 2021. Analysis of spleen histopathology, splenocyte composition and haematological parameters in four strains of mice

infected with Plasmodium berghei K173. *Malar J*. 20(1):249. doi: 10.1186/s12936-021-03786-z.

[16] Lee JH, Park J., Nam TW, Seo SM, Kim JY, Lee HK, Han JH, Park SY, Choi YK and Lee HW. 2018. Differences between immunodeficient mice generated by classical gene targeting and CRISPR/Cas9-mediated gene knockout. *Transgenic Res* 27, 241–251. https://doi.org/10.1007/s11248-018-0069-y

[17] Rajab NA and Turki AM. 2021. Assessment of histopathological changes in the liver and spleen of mice infected with salmonella typhi following treatment with ciprofloxacin. *Iraqi Journal of Science*, *62*(3), 761–768. https://doi.org/10.24996/ijs.2021.62.3.6

[18] Lawley TD, Chan K, Thompson LJ, Kim CC, Govoni GR and Monack DM. 2006. Genome-wide screen for Salmonella genes required for long-term systemic infection of the mouse. *PLoS Pathogens*, *2*(2), 0087–0100. https://doi.org/10.1371/journal.ppat.0020011

[19] Umezawa K, Ohnishi N, Tanaka K, Kamiya S, Koga Y, Nakazawa H and Ozawa A. 1995. Granulation in livers of mice infected with Salmonella typhimurium is caused by superoxide released from host phagocytes. *Infection and Immunity*, *63*(11), 4402–4408. https://doi.org/10.1128/iai.63.11.4402-4408.1995

[20] Mathur R, Oh H, Zhang D, Park S-G, Seo J, Koblansky A, Hayden MS and Gosh S. 2012. A mouse model of Salmonella typhi infection. *Cell*, *151*(3), 590–602. https://doi.org/10.1016/j.cell.2012.08.042.A

[21] Chaudhuri D, Chowdhury AR, Biswas B and Chakravortty D. 2018. Salmonella Typhimurium infection leads to colonization of the mouse brain and is not completely cured with antibiotics. *Frontiers in Microbiology*, *9*(JUL), 1–12. https://doi.org/10.3389/fmicb.2018.01632

[22] Neogy S, Das S, Mahapatra SK, Mandal N and Roy S. 2008. Amelioratory effect of Andrographis paniculata Nees on liver, kidney, heart, lung and spleen during nicotine-induced oxidative stress. *Environmental Toxicology and Pharmacology*, *25*(3), 321–328. https://doi.org/10.1016/j.etap.2007.10.034

[23] Wang W, Wang J, Dong SF, Liu CH, Italiani P, Sun SH, Xu J, Boraschi D, Ma SP and Qu D. 2010. Immunomodulatory activity of andrographolide on macrophage activation and specific antibody response. *Acta Pharmacologica Sinica*, *31*(2), 191–201. https://doi.org/10.1038/aps.2009.205

[24] Wu S, Lu R, Zhang, Y Guo and Sun J. 2010. Chronic salmonella infected mouse model. *Journal of Visualized Experiments*, *39*, 1–4. https://doi.org/10.3791/1947