JAVeST
Journal Of Applied Veterinary Science and Technology

Journal of Applied Veterinary Science and Technology
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Research Reports

Effect of Epigallocatechin Gallate on the Inflammatory Response in Mice *(Mus musculus)* Kidneys

Efek Epigallocatechin Gallate terhadap Respon Inflamasi Ginjal Tikus *(Mus musculus)*

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ABSTRACT

Background: Epigallocatechin gallate is the primary polyphenol constituent of green tea. It has the ability to inhibit the pathological processes caused by oxidants. However, in certain cases, the green tea diet is known to exert pro-oxidant effects. In addition, studies have shown that epigallocatechin gallate negatively affects cells. Several studies on epigallocatechin gallate showed increased oxidative stress and decreased intracellular antioxidants. Furthermore, it can stimulate an inflammatory response from the innate immune system, which may contribute to the elimination of the effects of epigallocatechin gallate. **Purpose:** This study aims to investigate the inflammatory responses (IL-1, IL-6, and TNF-α) in mice kidneys due to epigallocatechin gallate. **Methods:** This study involved the use of experimental animals aged between two and three months with an average body weight of 20 grams. The animals were randomly divided into two groups, namely the control group and the epigallocatechin gallate treatment group, with each group consisting of 16 samples. The dose of epigallocatechin gallate used in this study was 750 mg/kg bw. The treatment was administered for three days, after which the kidneys were collected. Immunohistochemical staining was used to observe the inflammatory response, including IL-1, IL-6 and TNF-α. Subsequently, all the data were collected and statistically analyzed using an independent t-test. **Results:** The results of the data analysis showed a significant difference in the expression of IL-6 ($p = 0.018$) and TNF-α ($p = 0.000$), but no significant difference in the expression of IL-1 ($p = 0.106$). **Conclusion:** In conclusion, epigallocatechin gallate was found to induce an inflammatory response in mice kidneys.

ARTICLE INFO

Received: 2 February 2024
Revised: 13 March 2024
Accepted: 24 April 2024
Online: 30 April 2024

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Keywords: Epigallocatechin Gallate; IL-1; IL-6; Inflammatory Response; TNF-α

ABSTRAK

Latar Belakang: Epigalokatekin galat merupakan penyusun polifenol utama pada teh hijau. Ia memiliki kemampuan untuk menghambat proses patologis yang disebabkan oleh oksidan. Namun, pada kasus tertentu, diet teh hijau diketahui memberikan efek pro-oksidan. Selain itu, penelitian menunjukkan bahwa epigalokatekin galat berdampak negatif pada sel. Beberapa penelitian tentang epigalokatekin galat menunjukkan peningkatan stres oksidatif dan penurunan antioksidan intraseluler. Selain itu, dapat menstimulasi respons inflamasi dari sistem kekebalan bawaan, yang dapat berkontribusi pada penghapusan efek epigalokatekin galat. **Tujuan:** Penelitian ini bertujuan untuk mengetahui respon inflamasi (IL-1, IL-6, dan TNF-α) pada ginjal mencit akibat epigalokatekin galat. **Metode:** Penelitian ini melibatkan penggunaan hewan percobaan berumur antara dua sampai tiga bulan dengan berat badan rata-rata 20 gram. Hewan coba dibagi secara acak menjadi dua kelompok, yaitu kelompok kontrol dan kelompok perlakuan epigalokatekin galat, yang masing-masing kelompok terdiri dari 16 sampel. Dosis epigalokatekin galat yang digunakan pada penelitian ini adalah 750 mg/kg bb. Perlakuan diberikan selama tiga hari, setelah itu ginjal diambil. Pewarnaan imunohistokimia digunakan untuk mengamati respon inflamasi, termasuk IL-1, IL-6 dan TNF-α. Selanjutnya, seluruh data dikumpulkan dan dianalisis secara statistik menggunakan uji t independen. **Hasil:** Hasil analisis data menunjukkan terdapat perbedaan bermakna pada ekspresi IL-6 ($p=0,018$) dan TNF-α ($p=0,000$), namun tidak terdapat perbedaan bermakna pada ekspresi IL-1 ($p=0,106$). **Kesimpulan:** Kesimpulannya, epigalokatekin galat ditemukan dapat menginduksi respon inflamasi pada ginjal tikus.

Cite This Article:
Arilangga, G.M., Fajriya, B.A., and Hardian, A.B., 2024. ***Effect of Epigallocatechin Gallate on the Inflammatory Response in Mice (Mus musculus) Kidneys.*** Journal of Applied Veterinary Science and Technology. 5(1): 66-71 . https://doi.org/10.20473/javest.V5.I1.2024.66- 71.

Kata kunci: Epigallocatechin Gallate; IL-1; IL-6; Respon Keradangan; TNF-α

INTRODUCTION

Epigallocatechin gallate is one of the polyphenol constituents of green tea, which has antioxidant properties. As evidenced by numerous studies, epigallocatechin gallate can eliminate reactive oxygen species in living cells. However, in certain cases, epigallocatechin gallate has been found to have a toxic side effect. In total, 36 cases of toxicity caused by excessive doses of epigallocatechin gallate have been reported. The patients exhibited a number of parameters indicative of cell damage, such as increased alanine transferase (ALT), bilirubin, and gamma-glutamyl transpeptidase (GGT) (Mazanti *et al.,* 2010).

Several studies have revealed that epigallocatechin gallate can be toxic to the organs of mice. This toxic mechanism begins with epigallocatechin gallate acting as a pro-oxidant. It is known that antioxidant compounds containing hydroxyl groups, such as polyphenols, can bind with reactive oxygen species (ROS). But, when the antioxidant effect reaches a certain level, the pro-oxidant effect begins to take over and damage living cells (Sotler *et al.,* 2019). For instance, Lambert *et al.,* (2010) reported that 750 mg/kg bw of epigallocatechin gallate resulted in increased malondialdehyde levels and the occurrence of apoptosis. Increased malondialdehyde levels indicated that epigallocatechin gallate at a dose of 750 mg/kg bw affected the integrity of cell membranes. Furthermore, lipid peroxidation occurred as a consequence of higher doses of epigallocatechin gallate, which induced the mechanism of apoptosis.

Another study conducted by James *et al.,* (2018) suggested that epigallocatechin gallate had a potential effect on the mitochondria. The results of parameter testing showed that the reduction in mRNA expression of mitochondrial proteins and epigallocatechin gallate exposure affected complex I (Ndufs8) and III (Uqcrc1). It is known that mitochondria are involved in the intrinsic pathway of apoptosis. In addition, epigallocatechin gallate has been shown to participate in the apoptosis of living cells by affecting the mitochondrial complex proteins (Zachary *et al.,* 2017).

During the damaging effect of epigallocatechin gallate, the body initiates a protective mechanism for living cells by stimulating the immune system. The immune system is divided into two categories based on immune responses, namely innate and adaptive immunity. Innate immunity is an early protection mechanism that protects cells, tissues, or organs from microorganisms as a pathogen-associated molecular pattern (PAMP) or cell damage as a damage-associated molecular pattern (DAMP) (Murphy and Casey, 2017). As epigallocatechin gallate has been shown to cause an increase in malondialdehyde levels, it can be assumed that malondialdehyde will induce the release of DAMP. Meng *et al.,* (2016) suggested epigallocatechin gallate induces the release of high mobility group box 1 (HMGB1).

The primary innate immune systems that interact with HMGB1 are macrophages. Macrophages are one of the sentinel cells, along with dendritic cells and mast cells, that reside in any tissue. Macrophages will recognize the HMGB1 induced by epigallocatechin gallate through pattern recognition receptors (PRRs), including toll-like receptors (TLRs). The interaction between TLRs and HMGB1 results in the transmission of a signal through some proteins and transcription factors in the cytoplasm of macrophages, thereby stimulating the inflammatory response (Abbas *et al.,* 2022).

The hypothesis of this study was that epigallocatechin gallate might induce an inflammatory response as well as a damaging effect in cells, while the innate immune system would initiate a process of releasing pro-inflammatory cytokines in the body. This study aims to examine the inflammatory response (IL-1, IL-6, and TNF-α) in mice kidneys caused by epigallocatechin gallate at a dose of 750 mg/kg bw.

It is important to note that the innate immune system uses ana inflammatory response as an initial protective mechanism. This response is effective to eliminate the negative effects of epigallocatechin gallate. However, when epigallocatechin gallate exceeds certain doses, it becomes pro-oxidant. As a consequence, the immune system should be evaluated to elucidate the mechanism of protection for the cells. Moreover, the results of this study will serve as a foundation for further research into the adaptive immunity of epigallocatechin gallate.

MATERIALS and METHODS

Research Site and Design

This study employed a true experiment method with a post-test-only control design. This study was conducted at the Laboratory of Experimental Animal, Laboratory of Anatomic Pathology, Faculty of Veterinary Medicine, and Laboratory of Biochemistry and Biomolecules, Faculty of Medicine, Universitas Brawijaya.

Experimental Materials

This study involved 32 male mice between two to three months old with an average body weight of 20 grams. The mice were randomly divided into two groups, namely the control group and the treatment group receiving epigallocatechin gallate (Catalog No. E4143; Sigma-Aldrich, USA). The epigallocatechin gallate was administered at a dose of 750 mg/kg bw per orally (James *et al.,* 2018). The immunohistochemical staining of inflammatory cytokines was performed using IL-1 (Catalog No. E-AB-65816; Elabscience, USA), IL-6 (Catalog No. sc-28343; Santa Cruz Biotechnology, USA), and TNF-α (Catalog No. sc-52746; Santa Cruz Biotechnology, USA).

Animal Treatment

Mice were placed in the Laboratory of Experimental Animal, Faculty of Veterinary Medicine, Universitas Brawijaya, for seven days to allow for acclimatization. The treatment process commenced on the eighth day and lasted for three days. On the 11th day, a necropsy was carried out at the Laboratory of

Anatomic Pathology, Faculty of Veterinary Medicine, Universitas Brawijaya, to collect the mice kidneys. The kidneys were placed in the organ pots containing 10% formalin. Subsequently, the kidneys were prepared for histopathological examination at the Laboratory of Anatomic Pathology, Faculty of Veterinary Medicine, Universitas Brawijaya and underwent immunohistochemical staining of inflammatory cytokines, including IL-1, IL-6, and TNF-α, at the Laboratory of Biochemistry and Biomoleculed, Faculty of Medicine, Universitas Brawijaya (Khan *et al.,* 2020).

Histopathological Preparation

The histopathological preparation involved a series of steps, including fixation, trimming, dehydration, clearing, paraffinization, embedding, and sectioning. The fixation step involved immersing the kidneys in a 10% formalin solution for 24 hours. The trimming step involved cutting the kidneys to a thickness of approximately 0.5 cm. The next step was dehydration, which involved immersing the kidneys in 70%, 80%, 85%, 90%, and 95% graded ethanol solutions for a minimum of one hour each. Subsequently, the kidneys were immersed in an absolute ethanol solution three times for a minimum of one hour each. The next step was clearing, during which the kidneys were immersed in xylol three times for five minutes, five minutes, and 10 minutes, respectively. The paraffinization step involved immersing the kidneys in paraffin three times for approximately 30 to 60 minutes each inside an incubator at a temperature of 56°C. Following this step was embedding. The kidneys were blocked using liquid paraffin and printed on a metal mold. The final step was sectioning, which involved cutting the paraffin blocks of kidneys using a microtome to a thickness of 5 millimeters. The blocks were placed in a water bath and affixed to a glass object (Khan *et al.,* 2020).

Immunohistochemical Staining

The immunohistochemical staining process consisted of deparaffinization, rehydration, antigen retrieval, blocking, administration of primary antibodies, administration of secondary antibodies, enzyme labeling, administration of chromogen, counterstaining, and mounting. Deparaffinization involved immersing the slide preparations in xylol three times, each time for five to 10 minutes. This was followed by rehydration, during which the slide preparations were immersed in an absolute ethanol solution three times and g 90%, 80%, and 70% graded ethanol solutions, each for 15 minutes. In the next stage, the slide preparations were rinsed with phosphate buffered saline (PBS) for nine minutes. The next stage was antigen retrieval, during which the slide preparations were put into a microwave and given a buffer with a pH adjusted to that of the antibody being used. Subsequently, the slide preparations were rinsed again with PBS for nine minutes. Following this stage was blocking, which involved treating the slide preparations with H_2O_2 as an endogenous peroxidase blocker and FBS as a protein blocker for 10 minutes each. Finally, the slide preparations were rinsed with PBS for nine minutes (Khan *et al.,* 2020).

During the administration of primary antibodies, the slide preparations were dripped with 100 to 200 microliths of primary antibodies and incubated for 60 minutes, followed by rinsing them with PBS for nine minutes. During the administration of secondary antibodies, the slide preparations were dripped with 100 to 200 microliths of secondary antibodies and incubated for 60 minutes, followed by rinsing them with PBS for nine minutes. During the enzyme labeling stage, the slide preparations were dripped with strep avidin-horse radish peroxidase (SAHRP) and incubated for 40 minutes. Finally, the slide preparations were rinsed with PBS for nine minutes (Khan *et al.,* 2020).

The following stage was the administration of chromogen or substrate. The slide preparations were dripped with dialminobenzidine (DAB) substrate and left for 10 minutes, followed by rinsing with PBS for nine minutes. Following this stage was counterstaining. The slide preparation were dripped with Mayer's hematoxylin solution and left for 10 minutes, followed by rinsing with PBS for nine minutes. The final stage was mounting. The slide preparations were given Entellan and covered using a glass cover. The slide preparations that had been colored were left to dry for approximately 24 hours (Khan *et al.,* 2020).

Data Analysis

The immunohistochemical preparations were examined under a microscope at 400x magnification. The data were analyzed using ImageJ IHC Profiler, which functions by interpreting digital images of immunohistochemical preparations through color deconvolution. Color deconvolution is obtained by classifying the color of the immunohistochemical preparation images according to the pixel units. The data obtained from the color deconvolution of digital images of immunohistochemical preparation in the ImageJ IHC Profiler software are presented as percentage (Varghese *et al.,* 2014). In addition, the Statistical Package for the Social Science (SPSS) was used to conduct statistical analysis. Pro-inflammatory cytokines in the immunohistochemical preparations were analyzed using the t-test ($\alpha = 0.05$). Meanwhile, the normality test was conducted using the Shapiro-Wilk test for a population of less than 50, while the homogeneity test was conducted using the Levene's test.

RESULTS

The results showed no significant differences in IL-1 expression ($p = 0.106$) between the control and treatment groups (Table 1). This indicated that epigallocatechin gallate did not effectively induce the process of IL-1 secretion in the innate immune system. However, significant differences were observed in IL-6 ($p = 0.018$) and TNF-α ($p = 0.000$) expressions between the control and treatment groups (**Tables 2** and **3**). This indicated that epigallocatechin gallate effectively induces the process of IL-6 and TNF-α secretion in the innate immune system. The results of immunohistochemical examination are presented in **Figures 1, 2,** and **3**.

Table 1. Data Analysis of the IL-1 Expression

Group	Mean ± SD	p-Value
Control	36.67[a] ± 6.5	0.106
Epigallocatechin gallate treatment	40.53[a] ± 6.2	

Table 2. Data Analysis of the IL-6 Expression

Group	Mean ± SD	p-Value
Control	36.20[a] ± 6.5	0.018
Epigallocatechin gallate treatment	42.60[b] ± 7.5	

Table 3. Data Analysis of the TNF-α Expression

Group	Mean ± SD	p-Value
Control	40.60[a] ± 9.2	0.000
Epigallocatechin gallate treatment	51.93[b] ± 6.3	

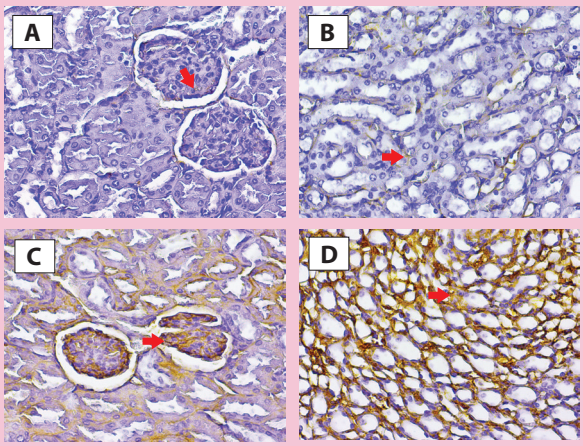

Figure 2. The immunohistochemical staining of IL-6. **(A).** Cortex area of the control group, **(B).** Medulla area of the treatment group, **(C).** Cortex area of the control group, **(D).** Medulla area of the treatment group. The red arrows indicate the expression of IL-6.

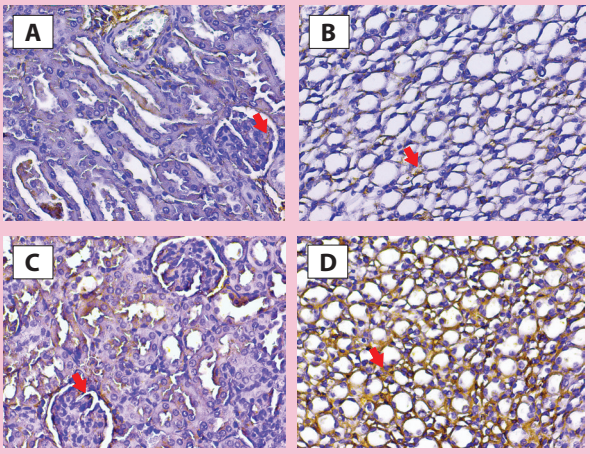

Figure 1. The immunohistochemical staining of IL-1. **(A).** Cortex area of the control group, **(B).** Medulla area of the treatment group, **(C).** Cortex area of the control group, **(D).** Medulla area of the treatment group. The red arrows indicate the expression of IL-1.

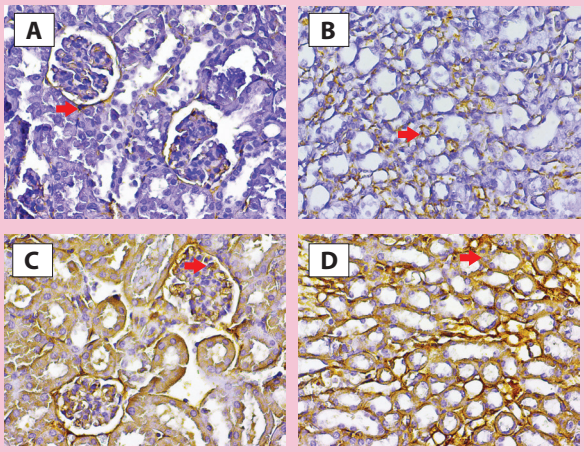

Figure 3. The immunohistochemical staining of TNF-α. **(A).** Cortex area of the control group, **(B).** Medulla area of the treatment group, **(C).** Cortex area of the control group, **(D).** Medulla area of the treatment group. The red arrows indicate the expression of TNFα.

DISCUSSION

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Epigallocatechin gallate is one of the most abundant polyphenol components of green tea. It is known as an antioxidant that can interact with the reactive oxygen species (ROS) to become more stable. Epigallocatechin gallate has been investigated in the field of pathology, and it has been demonstrated that this compound can effectively mitigate the adverse effects of pathological events. However, at certain doses, epigallocatechin gallate may act as a pro-oxidant. A study by Lambert *et al.*, (2010) revealed that epigallocatechin gallate had a toxic effect and elevated the levels of certain damaged cell markers, such as malondialdehyde. The formation of malondialdehyde is attributed to the activity of epigallocatechin gallate as a pro-oxidant. Meng *et al.*,(2016) reported that damaged cells released HMGB1 as a DAMP in response to epigallocatechin gallate. DAMP is one of the antigens that is recognized by the immune system to initiate innate and adaptive immunity. The innate immune response involves two mechanisms to eliminate the antigen, namely inflammatory and antiviral responses. Subsequently, the activation of adaptive immune response induced by the innate immune system involves two mechanisms to eliminate the antigen, namely cell-mediated immunity which is mediated by T lymphocytes and humoral immunity which is mediated by B lymphocytes (Puledran, 2024).

The initial mechanism to eliminate HMGB1 is innate immunity. The sentinel cells that inhabit the organ will recognize the HMGB1, which will be recognized by one of the sentinel cells, known as macrophages. Macrophages are phagocyte cells that will eliminate pathogens or damaged cells through certain mechanisms. In addition, toll-like receptors (TLRs) will recognize the HMGB1 (He *et al.*, 2018). TLR is one of the

pattern recognition receptors in the innate immune system that transmits signals from ligands (PAMP or DAMP) and initiates an inflammatory response. In this case, HMGB1 will interact with TLR4 and transmit the signal through adaptor proteins, namely MyD88 and TRIF, which activate the transcription factor NF-κB. Furthermore, the NF-κB stimulates the inflammatory cytokine encoding genes, including IL-1, IL-6, and TNF-α (Yang *et al.,* 2020).

The inflammatory cytokines facilitate the elimination of HMGB1 through various innate immune systems, including macrophages, neutrophils, dendritic cells, and mast cells. The initial step in the elimination of HMGB1 is the recruitment of leukocytes to the tissues. Mast cells release histamine to endothelial cells to promote vasodilation and increase the capillary permeability in blood vessels. This allows leukocytes, such as neutrophils and monocytes, to enter the tissue. Here the inflammatory cytokine stimulates the endothelial cells to express E- and P-selectin to facilitate the rolling of leukocytes from central flow to the vessel lining. The adhesion of leukocytes to the vessel lining is of low affinity. Therefore, the endothelial cells express ligands that bind with integrin receptors on the leukocytes, such as ICAM-1 binding with LFA-1, and VCAM-1 binding with VLA-4. These adhesion events will result in the arrest of the leukocytes on the endothelium, subsequently facilitating their transmigration to the tissues (Mitroulis *et al.,* 2015).

The recruitment of leukocytes to the tissues will help neutrophils and macrophages eliminate the HMGB1. This elimination process is mediated by molecules in the neutrophils and macrophages, such as reactive oxygen species, nitric oxide, and proteolytic enzymes (Virag *et al.,* 2019). Following this elimination process, neutrophils and macrophages will release other innate soluble molecules, namely complements. These molecules bind to the HMGB1 to assist phagocyte cells in the process of phagocytosis of HMGB1 (Merle *et al.,* 2015). Furthermore, the inflammatory response mediated by the innate immune system will be followed by the subsequent stage of the immune response, namely adaptive immunity.

The results of this study indicated no significant difference in IL-1 levels between the groups. However, IL-6 and TNF-α showed significant differences. The formation of IL-1 on the macrophages was possibly disrupted by the presence of epigallocatechin gallate. IL-1 has two forms, namely IL-1α and IL-1β. It is known that the release of IL-1α is caused by necrosis events, while the release of IL-1β is caused by apoptosis events (England *et al.,* 2014). James *et al.,* (2018) reported that epigallocatechin gallate caused a damaging effect on the mitochondrial complex protein, thereby contributing to the induction of apoptosis in the cells. As a result, it can be concluded that the apoptosis event induced by epigallocatechin gallate through the intrinsic pathway resulted in a low level of IL-1α formation. Conversely, the formation of IL-1β was dependent on the inflammasome, and mitochondria played a role in the activation of the inflammasome (Billingham *et al.,* 2022).

Inflammasome is one of the pattern recognition receptors in the cytosol that is responsible for the production of IL-1β and the occurrence of pyroptosis events. During the signalling events of HMGB1 through TLR4 and the adaptor protein, HMGB1 induces the formation of inflammasome through NLRP3 (Chi *et al.,* 2015). Inflammasome consists of the NOD-like receptor (NLR) subfamily, namely NLRP3, which binds an adaptor protein called apoptosis-associated speck-like protein containing CARD (ASC) to form an oligomer. The formation of NLRP3 with ASC causes the alteration of conformational molecules and initiated the recruitment of an inactive pro-caspase-1 to become an active caspase-1 (Evavold and Jonathan, 2019). Caspase-1 cleaves the precursor form of IL-1β to become active IL-1β, and the secretion of IL-1β promotes an inflammatory response that is mediated by Gasdermin D, which forms pores in the cell membrane of macrophages to release IL-1β to the extracellular environment (Zheng *et al.,* 2020).

As previously stated, epigallocatechin gallate has been demonstrated to disrupte complex I (Ndufs8) and III (Uqcrc1) as part of the electron transport chain (Billingham *et al.,* 2022). The dysfunction of the electron transport chain will prevent the activation of the inflammasome. Therefore, it was assumed that the disturbance of the electron transport chain by epigallocatechin gallate affected the formation of IL-β through the inhibition of inflammasome activation.

CONCLUSION

The results of this study indicated that epigallocatechin gallate is capable of eliciting an inflammatory response in the mice kidneys. This study also indicated that the expression of IL-1 was not found to be significantly different due to the effect of epigallocatechin gallate. Furthermore, further research is needed to investigate the molecular process of inflammasome inhibition by epigallocatechin gallate in the innate and adaptive immune systems.

ACKNOWLEDGEMENT

The authors wish to thank the laboratory technicians of the Laboratory of Veterinary Pathologic Anatomy, Faculty of Veterinary Medicine, Universitas Brawijaya and the Laboratory of Biochemistry and Biomolecules, Faculty of Medicine, Universitas Brawijaya for their assistance throughout this study.

CONFLICT of INTEREST

The authors have no conflicts of interest to declare.

RESEARCH FUNDING

This study was funded by the Faculty of Veterinary Medicine, Universitas Brawijaya through the 2022 DPP/SPP research grant scheme with a certificate number 2049/UN10.F13/TU/2022.

ETHICAL APPROVAL

This study received ethical approval by the Animal Care and Use Committee of Universitas Brawijaya with a certificate number 088-KEP-UB-2022.

AUTHORS' CONTRIBUTIONS

Conception and design of the study: GWA; data collection: BAF; data analysis and/or interpretation: GWA, BAF, ABH; drafting the manuscript: GWA, ABH; critical review/revision: GWA

REFERENCES

Abbas, A.K., Andrew, H.L., and Shiv, P., 2022. Cellular and Molecular Immunology. 10th Edition. Pennsylvania: Elsevier.

Billingham, L.K., Stoolman, J.S., and Vasan, K., 2022. Mitochondrial Electron Transport Chain is Necessary for NLRP3 Inflammasome Activation. *Nature Immunology,* 23, 692–704.

Chi, W., Hongrui, C., Fei, L., Yingting, Z., Wei, Y., and Yehong, Z., 2015. HMGB1 Promotes the Activation of NLRP3 and Caspase-8 Inflammasomes via NF-kB Pathway in Acute Glaucoma. *Journal of Neuroinflammation,* 12, 1-12.

England, H., Hazel, R.S., Michelle, E.E., Nancy, J.R., and David, B., 2014. Release of Interleukin-IL-1α and Interleukin-1β Depends on Mechanism of Cell Death. *Journal of Biological Chemistry,* 289, 15942-15950.

Evavold, C. L., and Jonathan, C. K., 2019. Inflammasomes: Threat-Assessment Organelles of the Innate Immune System. *Immunity Review,* 51, 677-687.

He, M., Marco, E.B., Tom, R.C., Kevin, J.T., and Yousef, A.A., 2018. Exploring the Biological Functional Mechanism of the HMGB1/TLR4/MD-2 Complex by Surface Plasmon Resonance. *Molecular Medicine,* 24, 1-9.

James, K.D., Mary, J.K., and Joshua, D.L., 2018. Potential Role of the Mitochondria as a Target for the Hepatotoxic Effects of (-)-epigallocatechin-3-gallate in Mice. *Food and Chemical Toxicology,* 111, 302-309.

Khan, H. A., Khalid E. I., Sara T. A., Salman A., Salman H. A., Najla A., Mohsen G. A., Samia H. S., and Isra K., 2020. Immunohistochemistry of IL-1β, IL-6 and TNF-α in Spleens of Mice Treated with Gold Nanoparticles. *Saudi Journal of Biological Sciences,* 27(4), 1163-1168.

Lambert, J.D., Mary, J.K., Shengmin, S., Kenneth, R.R., Jihyeung, J., and Chung, S.Y., 2010. Hepatotoxicity of High Oral Dose(-)-epigallocatechin-3-gallate in Mice. *Food and Chemical Toxicology Journal,* 48, 409-416.

Meng, X.Y., Li, B., Liu, S., Kang, H., Zhao, L., and Zhou, R., 2016. EGCG in Green Tea Induces Aggregation of HMGB1 Protein through Large Conformational Changes with Polarized Charge Redistribution. *Scientific Reports,* 6, 1-10.

Merle, N.S., Remi, N., Lise, H.M., Veronique, F.B., and Lubka, T.R., 2015. Complement System Part II: Role in Immunity. *Frontiers Immunology,* 6, 1-26.

Mitroulis, I., Vasileia, I.A., Ioannis, K., Athanassios, Z., George, H., and Triantafyllos, C., 2015. Leukocyte Integrins: Role in Leukocyte Recruitment and as Therapeutic Targets in Inflammatory Disease. *Pharmacology and Therapeutics,* 147, 123-135.

Murphy, K., and Casey, W., 2017. Janeway's Immunobiology. 9th Edition. New York: Garland Science.

Puledran, B., 2024. Integrated Organ Immunity: a Path to a Universal Vaccine. *Nature Reviews Immunology,* 24, 81-82.

Sotler, R., Borut, P., Raja, D., Tomislav, J., Doroteja, P.J., Cecilija, R., Polonca, T., and Andrej, S., 2019. Prooxidant Activities of Antioxidants and Their Impact on Health. *Acta Clina Croatica,* 58(4), 726-736.

Varghese, F., Amirali, B.B., Renu, M., and Abhijit, D., 2014. IHC-Profiler: An Open-Source Plugin for the Quantitative Evaluation and Automated Scoring of Immunohistochemistry Images of Human Tissue Samples. *PLOS One,* 9(5), 1-11.

Virag, L., Rafael, I.J., Zsolt, R., Lisardo, B., and Patricia, P., 2019. Self-Defense of Macrophages Against Oxidative Injury: Fighting for Their Own Survival. *Redox Biology,* 26, 1-9.

Yang, H., Haichao, W., and Ulf, A., 2020. Targeting Inflammation Driven by HMGB1. Frontiers Immunology, 11, 1-9.

Zachary, J.F., 2017. Pathology Basis of Veterinary Disease 6th Edition. China: Elsevier.

Zheng, D., Liwinski, T., and Elinav, E., 2020. Inflammasome Activation and Regulation: Toward a Better Understanding of Complex Mechanisms. *Cell Discovery,* 6, 1-22.