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THE POTENTIAL ROLE OF ANDROGRAPHIS PANICULATA AGAINST SEPSIS BY INHIBITING NF-KB P65 AND iNOS IN LIPOPOLYSACCHARIDE-INDUCED RAT MODEL

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Keywords:
NF-B p65, iNOS, sepsis, lipopolysaccharide, Andrographis paniculata.

ABSTRACT

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Andrographis paniculata (AGP) was anti-inflammatory by inhibiting nuclear factor-kappa B (NF-κB), a family of transcription factors that plays a central role in the inducible nitric oxide synthase (iNOS) expression. To prove the effect of AGP on NF-κB p65 and iNOS expression in lipopolysaccharide-induced liver injury in a rat model of sepsis. Immunohistochemistry (IHC) was performed on 30 liver paraffin blocks from 5 groups of male Wistar rats: K1: healthy control; K2: negative control; P1, P2, and P: rats had given AGP doses of 200, 400, and 500 mg/kg BW/day for 14 days. The mean NF-κB p65 and iNOS expression scores in the K1: 3.83±0.408 and 2.83±0.753; K2: 4.00±0.000 and 3.67±0.516; P1: 3.50±0.837 and 3.50±0.837; P2: 3.83±0.408 and 3.83±0.408; P3: 3.83±0.408 and 3.67±0.516. AGP doses of 200, 400, and 500 mg/kg BW/day did not affect NF-κB p65 and iNOS expression in lipopolysaccharide-induced liver injury in a rat model of sepsis. The NF-κB p65 and iNOS expression had a significant correlation ($p<0.05$) with a coefficient value of 0.514 (moderate). AGP extract prevents the accumulation of lipids that lead to liver necrosis. NF-κB p65 expression had a significant correlation with iNOS expression ($p<0.05$).

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1. Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [1], [2]. Excessive production of response immune leads to widespread tissue damage resulting in multi-organ failure and death [3]. The high incidence, mortality, and health costs required to treat a patient with sepsis are a problem in almost every country [1].

Lipopolysaccharides (LPS) are the major outer surface membrane components present in almost all Gram-negative bacteria, [4- 6] trigger the activation of the immune system, [7- 9] activated inducible nitric oxide synthase (iNOS) gene through Nuclear factor-kappa B (NF-κB) pathway, [10] and increased tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, reactive oxygen species (ROS), and nitric oxide (NO) [11]. The over-reactive immune response to an infection could lead to sepsis [12], [13].

The NF-κB consists of a family of transcription factors that play critical roles in immune response and inflammation [14]. In mammals, the NF-κB/Rel family consists of five members: p65 (RelA), RelB, c-Rel (Rel), p50/p105 (NF-κB1), and p52/p100 (NF-κB2) [14- 16]. NF-κB regulates both innate and adaptive host immune responses to infectious pathogens [17]. Many cellular processes are regulated through the NF-κB signaling pathway, dysregulation of the NF-κB pathway results in severe disease [18]. Hypothermia is a significant sign of sepsis, which is associated with a poor prognosis. The iNOS expression is an inflammatory mediator that plays a role in sepsis. Whether there is a decrease in iNOS expression resulting in a decrease in NO production, inflammation can be prevented. Liver dysfunction is common in early sepsis, and is not always accompanied by. The liver is the largest gland in the human body and plays a central role in metabolic homeostasis and immunological [19].

Various attempts have been made to prevent infection leading to sepsis. The potential of AGP in alleviating sepsis complications derived from liver injury has been commonly related to their remedial actions as hepatoprotective, [20- 22] immunomodulator, [23- 27] antioxidant, [28], [29] anti-hyperlipidemia. [30- 33] anti-microbial, [34- 36] and anti-inflammatory [9], [37], [38] agents.

2. Material and methods

2.1 Chemicals and Reagents

AGP was probed into wistar rats in the form of a gel: AGP 2 g dissolved in 100 mL of 1% Na-CMC solution (P1 group); AGP 4 g dissolved in 100 mL of 1% Na-CMC solution (P2 group) and AGP 5 g dissolved in 100 mL of 1% Na-CMC solution (P3 group)

NF-κB p65 expression could be detected using the primary antibody NF-B p65/RelA antibody (A19653) ABclonal with 1:1200 dilution and iNOS expression could be detected using the primary antibody iNOS Rabbit pAb (A0312) ABclonal with 1:1000 dilution in liver tissue. Immunohistochemistry (IHC) staining of both NF-κB and iNOS expression used secondary antibody produced by BioCare Medical (Pacheco, CA, United States).

2.2 Experimental Animals

This research used male; 2-3 months age, 150-200 grams bodyweight of white rats Rattus novergicus strain Wistar. Wistar rats are available and kept with controlled environmental conditions at a temperature of 28±2˚C, relative humidity of 30-70%, and with 12h light/12h dark cycle. The animals were acclimatized to laboratory conditions for 7 days prior to the initiation of the experiment. Approval from the Research Ethics Commission of the Faculty of Medicine, Diponegoro University, and Semarang No. 130/EC/H/FK-UNDIP/XI/2021 was obtained prior to the experiments.

2.3 Experimental Designing and Grouping

After the acclimatization to the housing conditions, the animals were randomized into five groups with 6 animals each: K1= healthy control group administrated with NaCl 0.9% into the peritoneum (i.p); K2 = negative control group without given AGP prior to LPS-induced dose 5 mg/kg BW/i.p; P1, P2, and P3

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groups as treatment groups which were given AGP doses 200 mg/kg BW/day, 400 mg/kg BW/day and 500 mg/kg WB/day prior to LPS-induced dose 5 mg/kg BW/i.p.

2.4 IHC Staining

The thin slices of liver tissue were placed on a glass slide that had been coated (poly-L-lysine incubated at 37ºC overnight), IHC staining of both NF-κB p65 and iNOS expression was performed using the Steptavidin-biotin method: xylol deparaffinization (4x5 mins), absolute ethanol, ethanol 96%, ethanol 70% rehydration (5 mins each), distilled water (5 mins), running water, distilled water (5 mins), phosphate buffered saline (PBS) (2x5 mins), antigen retrieval was performed in a microwave oven with Tris EDTA pH 9 at a temperature of 90ºC (3 mins) then continued at low temperature (10 mins), PBS (2x5 mins), 3% endogenous methanol peroxidase H2O2 (20 mins), running water, blocking serum (10 mins), drained, primary antibody, incubated at 4ºC (18 hours), PBS (2x5 mins), biotin (15 mins), PBS (2x5 mins), streptavidin (10 mins), PBS (2x5 mins), peroxidase enzyme substrate: DAB (3-5 mins), running water (10 mins), hematoxylin (4 mins), running water (10 mins). The final stage of the preparation was then dried at room temperature and then mounted using entellan and covered with a cover glass.

2.5 Assessment Criteria

The staining was assessed in terms of the proportion and intensity of cytoplasmic staining. The proportion (P) of staining in the liver tissue sections is generally assessed as follows: [39], [40] No staining (0); staining <25% (+1); staining 25-50% (+2); staining 50-75% (+3); and staining >75% (+4).

The intensity (I) of staining reflects the intensity of cell chromogen staining which shows cytoplasmic staining in the tissue section with a score of 0 meaning no intensity, +1 weak intensity, +2 moderate intensity, and +3 strong intensity [39], [40].

The immunoreactive score is the value of the proportion (P) multiplied by the intensity (I) and the results are classified as follows: [39], [40]

0 = score 0 (Negative)
1 – 3 = score 1 (weak immunoreactivity)
4 – 6 = score 2 (moderate immunoreactivity)
7 – 9 = score 3 (strong immunoreactivity)
10 – 12 = score 4 (very strong immunoreactivity)

3. FINDINGS AND DISCUSSION

Observation of both NF-κB p65 and iNOS expression in the liver is carried out after the experimental rats are given treatment according to the group, termination and removal of liver organs as well as tissue processing and IHC staining are carried out. Immunoreactive cells are counted under a microscope 400x, the average cells stained at 5 places are counted. The expression of NF-κB p65 and iNOS is observed as a brown color, and the immunoreactive score (proportion x intensity) is an indicator of increased expression observed by 2 expert observers.

3.1 NF-κB p65 Expression

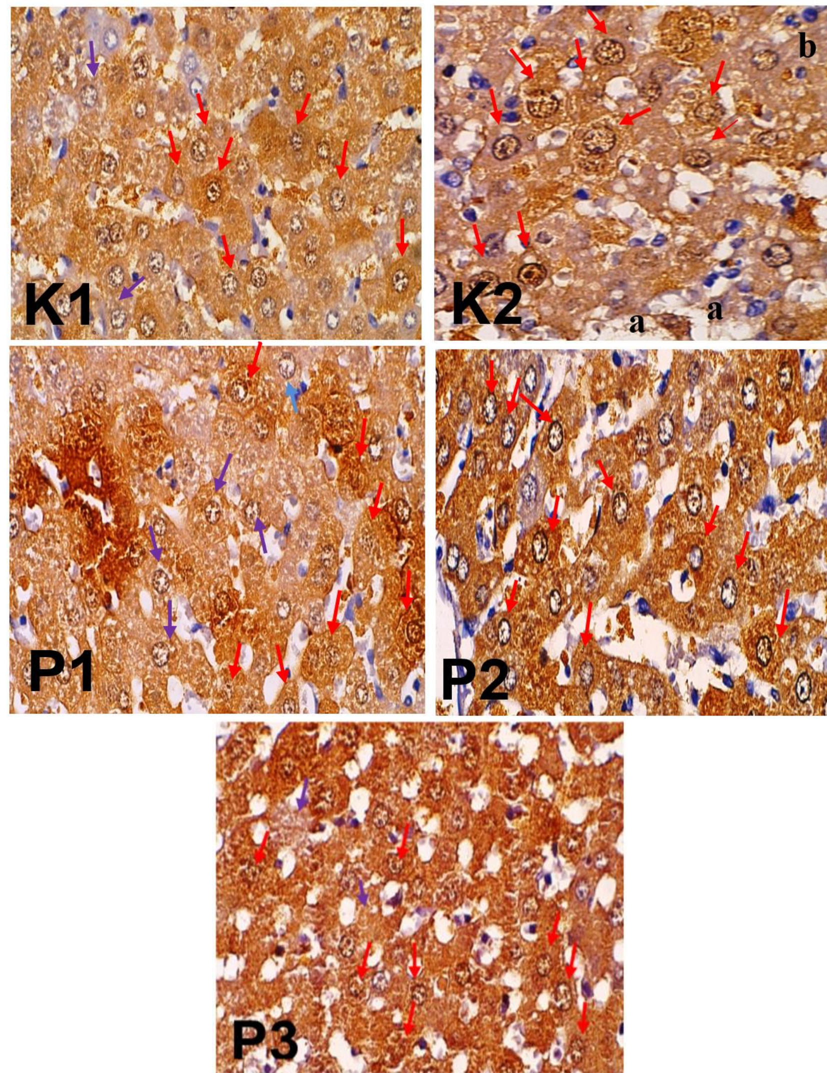

Figure 1. IHC staining results of NF-κB p65 expression in the liver.

The proportion of NF-κB p65 expression (stained in brown) is almost uniform in all groups. LPS causes fatty degeneration and liver necrosis in group K2. AGP treatment groups (P1, P2, P3) do not show fatty degeneration and necrosis cell. Observations view microscopic at 400x by 2 pathologists. The level of the immunoreactivity score is based on the multiplication between the score of the proportion of cells and the intensity of the stained cells. The arrows indicate the cell intensity: blue (weak), purple (moderate), and red (strong); a (fatty degeneration); b (necrosis).

The mean immunoreactive of NF-κB p65 expression shows no statistically significant difference between the healthy control groups (K1) with the negative control group (K2) (sig. 0.317). The significant differences between the K1 and K2 groups are obtained from the microscopic view (figure 1). The healthy control group had a high mean immunoreactivity score, roughly 90% of cells showing moderate/strong intensity of NF-κB p65 expression. Liver tissue in the negative control group shows fatty degeneration and necrosis cell, roughly 85% of the cells show a strong intensity of NF-κB p65 expression. Approximately 88% of cells show weak/moderate/strong intensity of NF-B p65 expression in the P1 group. Approximately 90% of cells show moderate/strong intensity of NF-κB p65 expression in the P2 group. Approximately 91% of cells show moderate/strong intensity of NF-κB p65 expression in the P3 group.

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Table 1. Shapiro-Wilk Test Normality for NF-κB p65 Ekspression

Groups	NF-κB p65 Mean±SD	Median (min-maks)	Normality	*p*
K1	3.83±0.408	4.00 (3.00-4.00)	0.000*	0.635[K]
K2	4.00±0.000	4.00 (4.00-4.00)	0.006*	
P1	3.50±0.837	4.00 (2.00-4.00)	0.000*	
P2	3.83±0.408	4.00 (3.00-4.00)	0.000*	
P3	3.83±0.408	4.00 (3.00-4.00)	0.000*	

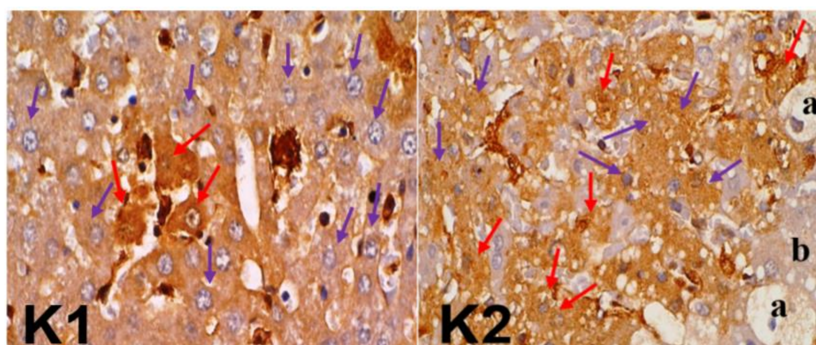
*abnormal distribution (p<0.05); K (Krusskall Wallis)

Table 1 shows that the mean immunoreactive score of NF-κB p65 expression in the healthy control group (K1) was 3.83; the negative control group (K2) is 4.00; the group giving AGP extract dose of 200 mg/kg BW/day (P1) is 3.50; the dose of 400 mg/kg BW/day (P2) is 3.83; and the dose of 500 mg/kg BW/day (P3) is 3.83. The mean immunoreactive score of NF-κB p65 expression in the K2 group shows no significant increase compared to the K1 group. Significant differences between the K1 and K2 groups are obtained from the microscopic images.

The Shapiro-Wilk normality test has obtained a significance value of p<0.05 (not normally distributed). The Mann-Whitney statistical test between the healthy control group (K1) and the negative control group (K2) launch that the difference in liver NF-κB p65 expression is not significant (sig 0.317). Krustal-Walls statistical test launch that the difference in liver NF-κB p65 expression is not significant between two or more groups (sig 0.635), and the Mann-Whitney statistical test between the control group K2 and the treatment groups (P1, P2, and P3) have not performed in this study.

3.2 iNOS Expression

The mean immunoreactive iNOS expression shows no statistically significant difference between the healthy control group (K1) and with negative control group (K2) (sig. 0.057). The significant differences between the K1 and K2 groups are obtained from the microscopic view (figure 2). The healthy control group has a high mean immunoreactivity score, roughly 79% of cells show moderate/strong intensity of iNOS expression. Liver tissue in the negative control group shows fatty degeneration and necrosis cell, roughly 84% of the cells show a strong intensity of iNOS expression. Approximately 90% of cells show weak/moderate/strong intensity of iNOS expression in the P1 group. Approximately 93% of cells show moderate/strong intensity of iNOS expression in the P2 group. Approximately 95% of cells show moderate/strong intensity of iNOS in expression in the P3 group.



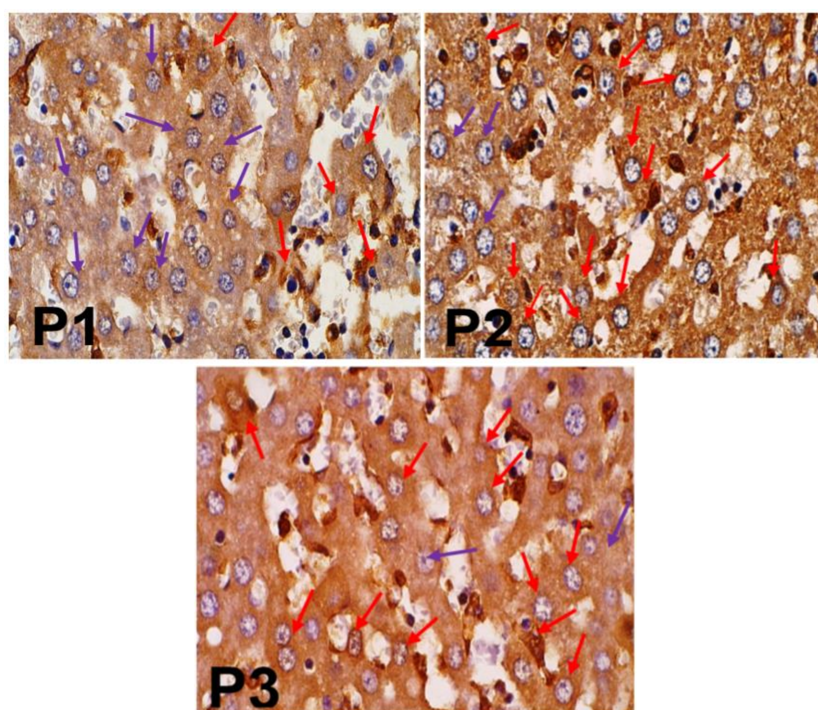


Figure 2. IHC staining results of iNOS expression in the liver.

The proportion of iNOS expression (stained in brown) is almost uniform in all groups. LPS-induced causes fatty degeneration and liver necrosis in the K2 group. AGP treatment groups (P1, P2, P3) do not show fatty degeneration and necrosis cell. Observations view microscopic at 400x by 2 pathologists. The level of the immunoreactivity score is based on the multiplication between the score of the proportion of cells and the intensity of the stained cells. The arrows indicate the cell intensity: blue (weak), purple (moderate), and red (strong); a (fatty degeneration); b (necrosis).

Table 2 shows that the mean immunoreactive score of iNOS expression in the healthy control group (K1) is 2.83; the negative control group (K2) is 3.67; the group giving AGP extract dose of 200 mg/kg BW/day (P1) is 3.50; the dose of 400 mg/kg BW/day (P2) is 3.83; and the dose of 500 mg/kg BW/day (P3) is 3.67. The mean immunoreactive score of iNOS expression in the K2 group showes no significant increase compared to the K1 group. Significant differences between the K1 and K2 groups are obtained from the microscopic images.

Table 2. Shapiro-Wilk Test Normality for iNOS Ekspression

Groups	iNOS Mean±SD	Median (min-maks)	Normality	*p*
K1	2.83±0.753	3.00 (2.00-4.00)	0.212*	0,121^{K}
K2	3.67±0.516	4.00 (3.00-4.00)	0.001*	
P1	3.50±0.837	4.00 (2.00-4.00)	0.006*	
P2	3.83±0.408	4.00 (3.00-4.00)	0.000*	
P3	3.67±0.516	4.00 (3.00-4.00)	0.001*	

*abnormal distribution ($p<0.05$); K (Krusskall Wallis)

The Shapiro-Wilk normality test has obtained a significance value of $p<0.05$ (not normally distributed). The Mann-Whitney statistical test between the healthy control group (K1) and the negative control group (K2) launch that the difference in liver iNOS expression is not significant (sig 0.317). Krustal-Walls statistical test launch that the difference in liver iNOS expression is not significant between two or more groups (sig

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0.635), and the Mann-Whitney statistical test between the control group K2 and the treatment group (P1, P2, and P3) are not performed in this study.

3.3 Correlation between NF-κB p65 and iNOS

The mean of NF-κB p65 expression is to be higher than the mean of iNOS expression in the liver of the K1 group, similarly well as K2 and P3 groups. The mean of NF-κB p65 expression is the same as the mean of iNOS expression in the P1 and P2 groups. The both NF-κB p65 and iNOS expression have almost the same pattern. The decrease of NF-B p65 expression is followed by decreased in iNOS expression in the P1 group and the increase of NF-B p65 expression is followed by increase of iNOS expression in the P2 group.

Table 3. Correlation Kendall-Tou Statistic Test

		iNOS expression
NF-κB p65 expression	r	0,514*
	p	0,004*
	n	30

r = rank correlation coefficient
p = value of significance
* = significant (p<0,05)
n = number of samples

According to the Kendall-Tou correlation test in Table 3, both NF-κB p65 and iNOS expression have a significant correlation ($p<0.05$) with a coefficient value of 0.514 (medium) and a positive correlation direction (in the same direction).

4. Discussion

The cause of the increase in both NF-κB p65 and iNOS expression in healthy control group rats liver in this study is not known with certainty. The difference in the NF-B p65 and iNOS expression in all groups are not significant and the correlation between the NF-κB p65 and iNOS expression do not get the maximum results. Perhaps NF-κB p65 in Wistar rats used in this study is activated by a condition still have not yet known experimentally, furthermore, the healthy control group should be negative or weak but both NF-κB p65 and iNOS expression show a very strong immunoreactive score. The all rats group receive different treatments except in terms of food and the environment in this research. The dietary factors and/or stress are perhaps the main causes of increased NF-κB p65 and iNOS expression in Wistar rats are use in this study, visually is not visible, but molecularly affected. Only two of the six Wistar rats in the healthy control group show moderate immunoreactivity scores.

Wistar rats are available and kept in the PAU animal laboratory (Gajah Mada University Yogyakarta, Indonesia), and they are fed a standard COMFEED AD II diet containing 12% water content, 7% ash, 15% crude protein, 6% crude fiber, 3-7% crude fat. 0.9-1.1% calcium, 0.6-0.9% phosphorus, total aflatoxin 50 g/kg, amino acids (lysine, methionine, cystine, tryptophan, threonine) 1.59%, carbohydrates and other supplements (sodium bicarbonate, vitamins, and minerals) are added in the feed, the percentage is not stated. The impact of the composition and supplementation of ad libitum Wistar rat diet on the expression of NF-κB p65 and liver iNOS is not known with certainty.

The liver plays a central role in produce energy and fat metabolism in the body, [41], [42], and NF-κB NF-B is a family of transcription factors that play a role in cellular responses to stimuli, and are activated by various regulations. NF-κB signaling is critical in a variety of complexly coordination of biological processes [43]. NF-κB activation is triggered not only by infection but also by other signals such as

hypoxia, DNA damage, oxidative stress, endoplasmic reticulum stress, and perhaps the condition represents a threatening situation (including the sunlight) [43], [44]. NF-κB activation can also be influenced by dietary factors. NF-κB is perhaps activated during both overnutrition and undernutrition [45]. Nutrient plays an important role in the maintenance of normal physiological conditions in the body [46]. Food components such as lipids had been reported to increase of NF-B p65 activation and induce lipogenesis in the liver [47], [48]. Previous studies have suggested that a high-cholesterol diet can lead to an overexpression of iNOS [49], [50]. Under high glucose conditions, NF-B is activated and rapidly translocates to the nucleus, leading to increased binding to the iNOS promoter and increased iNOS expression [51]. The High-carbohydrate diet increases NF-κB activation, [52] and the low-carbohydrate diet reduces triglycerides and increases *high-density lipoprotein* (HDL) cholesterol levels [53]. Higher intakes of dietary components such as carbohydrates (fructose, sucrose, and glucose) promote chronic positive energy balance, furthermore promoting liver manifestations such as non-alcoholic fatty liver disease (NAFLD) [54], [55]. Interaction of liver cells and activated immune cells, such as Kupffer cells, T cells, and hepatic stellate cells, with inflammatory factors and TLR, [56], [57] contribute to the development of NAFLD [55].

Initiation of liver disease involve inflammation phase. This is a continuous process of destruction, and regeneration of liver parenchyma leading to fibrosis and cirrhosis. Under many pathological conditions, iNOS produces NO which is an indicator of inflammation in the liver, [58], [59] inhibits collagen and proteoglycan synthesis, and induces chondrocyte apoptosis and pain [60]. Increased production of NO by iNOS and inflammatory cytokines such as TNF-α and IL-1β occurs in the liver injury [61]. Fibrogenesis, inflammation, and necrosis are the major changes in the pathophysiological mechanisms of the liver that cause the change from liver disease to cirrhosis [62]. Failure to prevent infection leading to sepsis can result in several diseases including cancer. One in five cancers is the result of chronic inflammation caused by infection, toxins, and autoimmunity [63]. NO induced by iNOS has been recognized as a mediator and regulator of inflammatory reactions, can trigger harmful effects on host tissues [64].

Necrosis can be defined as cell death characterized by cell swelling and loss of plasma membrane integrity, [65] and fat degeneration occurs due to abnormal fat deposits in diseased cells [66]. LPS induces liver inflammation characterized by fat degeneration, necrosis, fibrosis, [67- 69] furthermore the eventual development of hepatocellular carcinoma [70]. Hepatocyte cell death is an important event in the development of liver disease due to inflammation leading to fibrosis [71] Apoptosis, necrosis, necroptosis, autophagy, pyroptosis and ferroptosis are the pathogenesis of various liver diseases [72]. The pathophysiology of acute liver failure is the occurrence of necrosis which causes cascade activation and the increasing component of oxidative stress so that hepatocyte death increases [73]. Liver dysfunction is not always accompanied by significant histologic changes in the liver, but liver damage usually occurs. Most of the patients died of sepsis, the dominant liver histopathological changes are portal/lobular inflammation, hepatocellular apoptosis, centrilobular necrosis, cholangitis/cholangiolitis, and steatosis at autopsy [74].

Our study is not able to prove differences in both NF-κB p65 and iNOS expression in liver tissue with given AGP doses of 200, 400, and 500 mg/kg BW/day compared to the control group. AGP doses of 200, 400, and 500 mg/kg BW/day have been shown to act as anti-inflammatory agents in the liver of a LPS-induced rat model of sepsis [9], [75- 77]. The treatment groups (P1, P2 and P3) have lipopolysaccharide induced but the liver tissue does not experience fat degeneration and necrosis because the treatment group is given AGP extract. Our study shows that AGP extract prevents the accumulation of liver lipids leading to necrosis in in LPS-induced liver injury in a rat model of sepsis.

Previous research has shown that the compounds contained in AGP, andrographolite and β-sitosterol are

anti-inflammatory, [37], [33] and anti-cholesterol [30], [31], [33]. Andrographolite inhibits the TLR4/NF-κB/MMP-9 signaling pathway, [78- 80]. PI3K-Akt-mTOR [81] dan *Wnt/β-* catenin [82] in colorectal cancer cells, and inhibits inflammatory and oxidative responses in the liver [83]. β-sitosterol inhibits the activation of the ERK/p38 and NF-κB pathways, play a role in inflammation [84- 87]. Flavonoids exhibit anti-inflammatory properties through modulation of NF-κB activation, [88] iNOS regulation, cyclooxygenase-2 (COX-2), [89] and decreases cell-wide production of amyloid protein (Aβ) through NF-κB associated mechanisms [90] Previous studies by [32] showed that AGP prevented cholesterol accumulation, inflammation and reduced liver damage in mice fed a high-fat, high-cholesterol diet and reduced NLRP3 inflammasome activation and oxidative stress in the liver.

5. Conclusion

From our research, NF-κB p65 and iNOS expression no difference in liver tissue between all groups ($p>0.05$). AGP extract doses of 200, 400, and 500 mg/kg BW/day prevents the accumulation of lipids that lead to necrosis. NF-κB p65 expression has a significant correlation with iNOS expression ($p<0.05$).

Limitation

Not doing preliminary research, the effect of the COMFEED II standard and the addition of supplements on NFκB p65 and iNOS expression has not yet known

Data accessibility

The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Statistical Analysis

The collected data are analyzed with IBM SPSS Statistics for Windows, Version 26.0 Armonk, NY: IBM Corp.

Research quality and ethics statement

Approval from the Research Ethics Commission of the Faculty of Medicine, Diponegoro University, Semarang No. 130/EC/H/FK-UNDIP/XI/2021.

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Conflicts of interest

There are no conflicts of interest.

6. References

[1] World Health Organization. Report by the Director-General. Prog reports Seventy-third World Heal Assem. 2020;1–21.

[2] Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. Lancet. 2020;395(10219):200–11.

[3] Ragab D, Eldin HS, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. Front Immunol. 2020;11(June):1–4.

[4] Ni J, Zhao Y, Su J, Liu Z, Fang S, Li L. Toddalolactone Protects Lipopolysaccharide-Induced Sepsis and Attenuates Lipopolysaccharide-Induced Inflammatory Response by Modulating HMGB1-NF-κB Translocation. Front Pharmacol. 2020;11(February):1–12.

[5] Bossu P, Cutuli D, Palladino I, Caporali P, Angelucci F, Laricchiuta D. A single intraperitoneal injection of endotoxin in rats induces long-lasting modifications in behavior and brain protein levels of TNF-α and IL-18. J Neuroinflammation. 2012;9(1):1.

[6] Batista CRA, Gomes GF, Candelario JE, Fiebich BL, Oliveira ACP. Lipopolysaccharide-induced neuroinflammation as a bridge to understand neurodegeneration. Int J Mol Sci. 2019;20(9).

[7] Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. Biomol Concepts. 2018;9(1):64–79.

[8] Nie X, Chen SR, Wang K, Peng Y, Wang YT, Wang D. Attenuation of Innate Immunity by Andrographolide Derivatives Through NF-κB Signaling Pathway. Sci Rep. 2017;7(1):1–10.

[9] Li Y, He S, Tang J, Ding N, Chu X, Cheng L. Andrographolide Inhibits Inflammatory Cytokines Secretion in LPS-Stimulated RAW264.7 Cells through Suppression of NF-κB/MAPK Signaling Pathway. Evidence-Based Complement Altern Med. 2017;1–9.

[10] Choudhury MG, Kumari S, Das KB, Saha N. Lipopolysaccharide causes NFκB-mediated induction of inducible nitric oxide synthase gene and more production of nitric oxide in air-breathing catfish, Clarias magur (Hamilton). Gene. 2018;658(November 2017):18–27.

[11] Woznica EA, Inglot M, Woznica RK, Lysenko L. Liver dysfunction in sepsis. Adv Clin Exp Med. 2018;27(4):547–51.

[12] Purwanto DS, Astrawinata DAW. Mekanisme Kompleks Sepsis dan Syok Septik. J Biomedik. 2018;10(3):143.

[13] Strassheim D, Dempsey EC, Gerasimovskaya E, Stenmark K, Karoor V. Role of inflammatory cell subtypes in heart failure. J Immunol Res. 2019;1–9.

[14] Berger T, Szentmary N, Latta L, Seitz B, Stachon T. NF-κB, iNOS, IL-6, and collagen 1 and 5 expression in healthy and keratoconus corneal fibroblasts after 0.1% riboflavin UV-A illumination. Graefe's Arch Clin Exp Ophthalmol. 2021;259(5):1225–34.

[15] Zinatizadeh MR, Schock B, Chalbatani GM, Zarandi PK, Jalali SA, Miri SR. The Nuclear Factor Kappa B (NF-kB) signaling in cancer development and immune diseases. Genes Dis. 2021;8(3):287–97.

[16] Liu AR, Ramakrishnan P. Regulation of Nuclear Factor-kappaB Function by O-GlcNAcylation in Inflammation and Cancer. Front Cell Dev Biol. 2021;9:1–9.

[17] Peng C, Ouyang Y, Lu N, Li N. The NF-κB Signaling Pathway, the Microbiota, and Gastrointestinal Tumorigenesis: Recent Advances. Front Immunol. 2020;11:1–13.

ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

[18] Vu D, Huang D, Vemu A, Ghosh G. A structural basis for selective dimerization by NF-κB RelB. J Mol Biol. 2013;425(11):1934–45.

[19] Wilkinson AL, Qurashi M, Shetty S. The Role of Sinusoidal Endothelial Cells in the Axis of Inflammation and Cancer Within the Liver. Front Physiol. 2020;11:1–25.

[20] Mahardika GG, Dewi NWS, Aman I. Ekstrak etanol daun Sambiloto (Andrographis paniculata) menurunkan HAI (Histology Acctivity Indeks)-Knodell Scor pada hepar mencit (Mus musculus) jantan yang diinduksi CCL. J Med udayana. 2020;9(4):1–6.

[21] Rachman F, Yanti SN, Andriani. Uji Efek Hepatoprotektor Ekstrak Metanol Daun Sambiloto (A. Paniculata) terhadap Kadar Malondialdehid Plasma Tikus Jantan Galur Wistar yang Diinduksi Parasetamol. Vol. 13, Naskah Publikasi. 2015.

[22] Salunkhe AJ, Patil RN. Hepatoprotective Effect of Ethanolic Extract of Andrographis Paniculata Against Thioacetamide Induced. Res J Life Sci Bioinformatics, Pharm Chem Sci. 2018;4:549–59.

[23] Palanikani R, Chanthini KMP, Soranam R, Thanigaivel A, Karthi S, Nathan S. Efficacy of Andrographis paniculata supplements induce a non-specific immune system against the pathogenicity of Aeromonas hydrophila infection in Indian major carp (Labeo rohita). Environ Sci Pollut Res. 2020;27(19):23420–36.

[24] Churiyah, Pongtuluran OB, Rofaani E, Tarwadi. Antiviral and Immunostimulant Activities of Andrographis paniculata. Hayati J Biosci. 2015;22(2):67–72.

[25] Radhika P, Annapurna A, Rao SN. Immunostimulant, cerebroprotective & nootropic activities of andrographis paniculata leaves extract in normal & type 2 diabetic rats. Indian J Med Res. 2012;135(5):636–41.

[26] Alkandahri MY, Subarnas A, Berbudi A. Aktivitas Immunomodulator Tanaman Sambiloto (Andrographis paniculata Nees). Farmaka. 2018;16(3):16–20.

[27] Wurlina, Meles DK, Mustofa I, Zakaria S, Adnyana DPA. Alkaloid Immunomodulatory Effects of Sambiloto (Andrographis paniculate l .) On The Response of Gamma Interferon and T Helper Cell. Adv Nat Appl Sci. 2017;11(9):154–8.

[28] Aulya ZV, Arthamin MZ, Chilmi S, Widodo MA, Sujuti H. Kombinasi elektroporasi dan aspirin menghambat aktivasi nuclear factor kappa B (NFkB) pada kultur sel mononuklear darah tepi pasien leukemia akut. Maj Kesehat Fak Kedokt Univ Brawijaya. 2014;1(1):10–6.

[29] Rachmani EPN, Pramono S, Nugroho AE. Aktivitas Antioksidan Fraksi Flavonoid Bebas Andrografolit dari Herbal Sambiloto (Andrographis paniculata). J Farm Medica/Pharmacy Med J. 2018;1(2):42–9.

[30] Krestianto DP, Jatmiko SW, Bestari RS. The Effect of Total Cholesterol Decrease In Blood At Galur Wistar White Rat From The Extract. I S S N 2 7 2 1 - 2882. 2019;99–107.

[31] Susanti N, Wardiatini N, Dewi K, Oka M. Aktivitas Antihiperlipidemia Andrografolid dari Sambiloto (Andrographis paniculata (Burm. f.) Ness) secara In Silico. J Farm Udayana. 2016;5(2):58–62.

[32] Liu YT, Chen HW, Lii CK, Jhuang JH, Huang CS, Li ML. A diterpenoid, 14-deoxy-11, 12-didehydroandrographolide, in andrographis paniculata reduces steatohepatitis and liver injury in mice fed a high-fat and high-cholesterol diet. Nutrients. 2020;12(2):523.

[33] Lahare RP, Kumar A. HPTLC Based Screening of β-Sitosterol from Andrographis paniculata. Nat Environ Pollut Technol. 2019;18(3):949–54.

[34] Radji M, Kurniati M, Kiranasari A. Comparative antimycobacterial activity of some Indonesian medicinal plants against multi-drug resistant Mycobacterium tuberculosis. J Appl Pharm Sci. 2015;5(1):019–22.

[35] Gopinath R, Sneha S, Manickan E. Anti-Mycobacterial Activity of Acalypha indica and Andrographis paniculata, the Indian Medicinal Plants against Mycobacterium tuberculosis (MTB). Ijppr. 2016;7(4):33–43.

[36] Prabu A, Hassan S, Prabuseenivasan, Shainaba AS, Hanna LE, Kumar V. Andrographolide: A potent antituberculosis compound that targets Aminoglycoside 2′-N-acetyltransferase in Mycobacterium tuberculosis. J Mol Graph Model. 2015;61:133–40.

[37] Zou W, Xiao Z, Wen X, Luo J, Chen S, Cheng Z. The anti-inflammatory effect of Andrographis paniculata (Burm. f.) Nees on pelvic inflammatory disease in rats through down-regulation of the NF-KB pathway. BMC Complement Altern Med. 2016;16(1):1–7.

[38] Patala R, Levita J, Milanda T. Aktivitas Penghambatan in Vitro Dan Penambatan Molekular Senyawa Flavonoid Pada Inducible Nitric Oxide Synthase : Review. Farmaka. 2018;16(3):22–30.

[39] Gezmis A, Balkan B, Yektas A. Effect of Pyrrolidine Dithiocarbamate to Liver Injury in a Sepsis Model with Cecum Ligation and Perforation-An Animal Study. iMedPub Journals Prot. 2019;7:1–11.

[40] Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. Diagn Pathol. 2014;9:221.

[41] Lemberger UJ, Fuchs CD, Schofer C, Bileck A, Gerner C, Stojakovic T. Hepatocyte specific expression of an oncogenic variant of β-catenin results in lethal metabolic dysfunction in mice. Oncotarget. 2018;9(13):11243–57.

[42] Hyatt HW, Zhang Y, Hood WR, Kavazis AN. Physiological, Mitochondrial, and Oxidative Stress Differences in the Presence or Absence of Lactation in Rats. Reprod Biol Endocrinol. 2018;16(1):1–14.

[43] Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer. 2013;12:86.

[44] Schmitz ML, Shaban MS, Albert BV, Gokcen A, Kracht M. The crosstalk of Endoplasmic Reticulum (ER) stress pathways with NF-κB: Complex mechanisms relevant for cancer, inflammation and

ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

infection. Biomedicines. 2018;6(2):1–18.

[45] Kracht M, Ladner UM, Schmitz ML. Mutual regulation of metabolic processes and proinflammatory NF-κB signaling. J Allergy Clin Immunol. 2020;146(4):694–705.

[46] Sangi SMA. Potential therapeutic agents for the treatment of fatty degeneration of liver and atheromatous plaques: An experimental study in rats. Pharmacogn Mag. 2016;12(47):S414–23.

[47] Lu H, Lei X, Zhang Q. Moderate activation of IKK2-NF-kB in unstressed adult mouse liver induces cytoprotective genes and lipogenesis without apparent signs of inflammation or fibrosis. BMC Gastroenterol. 2015;15(1):1–14.

[48] Zeng T, Zhou J, He L, Zheng J, Chen L, Wu C. Blocking Nuclear Factor-Kappa B Protects against Diet-Induced Hepatic Steatosis and Insulin Resistance in Mice. PLoS One. 2016;11(3):1–15.

[49] Miron N, Tirosh O. Cholesterol Prevents Hypoxia-Induced Hypoglycemia by Regulation of a Metabolic Ketogenic Shift. Oxid Med Cell Longev. 2019;1–11.

[50] Anavi S, Obercyger HM, Madar Z, Tirosh O. Mechanism for HIF-1 activation by cholesterol under normoxia: A redox signaling pathway for liver damage. Free Radic Biol Med. 2014;71:61–9.

[51] Jia J, Liu Y, Zhang X, Liu X, Qi J. Regulation of iNOS expression by NF-κB in human lens epithelial cells treated with high levels of glucose. Invest Ophthalmol Vis Sci. 2013;54(7):5070–7.

[52] Daniel PV, Dogra S, Rawat P, Choubey A, Khan AS, Rajak S. NF-κB p65 regulates hepatic lipogenesis by promoting nuclear entry of ChREBP in response to a high carbohydrate diet. J Biol Chem. 2021;296(22):100714.

[53] Hu T, Mills KT, Yao L, Demanelis K, Eloustaz M, Yancy WS. Effects of low-carbohydrate diets versus low-fat diets on metabolic risk factors: A meta-analysis of randomized controlled clinical trials. Am J Epidemiol. 2012;176(7):S44–54.

[54] Wahed AA, Guilmeau S, Postic C. Sweet Sixteenth for ChREBP: Established Roles and Future Goals. Cell Metab. 2017;26(2):324–41.

[55] Ullah R, Rauf N, Nabi G, Ullah H, Shen Y, Zhou YD. Role of nutrition in the pathogenesis and prevention of non-alcoholic fatty liver disease: Recent updates. Int J Biol Sci. 2019;15(2):265–76.

[56] Chen W, Wang X, Huang L, Liu B. Hepcidin in non-alcoholic fatty liver disease regulated by the TLR4/NF-κB signaling pathway. Exp Ther Med. 2016;11(1):73–6.

[57] Dattaroy D, Seth RK, Das S, Alhasson F, Chandrashekaran V, Michelotti G. Sparstolonin B attenuates early liver inflammation in experimental NASH by modulating TLR4 trafficking in lipid rafts via NADPH oxidase activation. Am J Physiol - Gastrointest Liver Physiol. 2016;310(7):G510–25.

[58] Nakamura Y, Iida H, Nakatake R, Sakaguchi T, Kaibori M, Okumura T. L-Carnitine has a liver-protective effect through inhibition of inducible nitric oxide synthase induction in primary cultured rat

hepatocytes. Funct Foods Heal Dis. 2018;8(3):212–27.

[59] Iwakiri Y, Kim MY. Nitric Oxide in Liver Diseases. Physiol Behav. 2015;36(8):524–36.

[60] Suantawee T, Tantavisut S, Adisakwattana S, Tanpowpong T, Tanavalee A, Yuktanandana P. Upregulation of inducible nitric oxide synthase and nitrotyrosine expression in primary knee osteoarthritis. J Med Assoc Thai. 2015;98(1):S91–7.

[61] Ostojic M, Zevrnja A, Vukojevic K, Soljic V. Immunofluorescence analysis of nf-kb and inos expression in different cell populations during early and advanced knee osteoarthritis. Int J Mol Sci. 2021;22(12):1–15.

[62] Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet. 2014;383(9930):1749–61.

[63] Barnabei L, Laplantine E, Mbongo W, Laucat RF, Weil R. NF-κB: At the Borders of Autoimmunity and Inflammation. Front Immunol. 2021;12:1–27.

[64] Jeong YE, Lee MY. Anti-inflammatory activity of populus deltoides leaf extract via modulating NF-κB and p38/JNK pathways. Int J Mol Sci. 2018;19(12).

[65] Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P. Molecular mechanisms of cell death: Recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 2018;25(3):486–541.

[66] Suzuki T, Shinjo S, Arai T, Kanai M, Goda N. Hypoxia and fatty liver. World J Gastroenterol. 2014;20(41):15087–97.

[67] Huang XY, Ansari AR, Huang HB, Zhao X, Li NY, Sun ZJ. Lipopolysaccharide mediates immuno-pathological alterations in young chicken liver through TLR4 signaling. BMC Immunol. 2017;18(1):1–9.

[68] Gao LN, Yan K, Cui YL, Fan GW, Wang YF. Protective effect of Salvia miltiorrhiza and Carthamus tinctorius extract against lipopolysaccharide-induced liver injury. World J Gastroenterol. 2015;21(30):9079–92.

[69] Guo X, Li W, An R, Huang M, Yu Z. Composite ammonium glycyrrhizin has hepatoprotective effects in chicken hepatocytes with lipopolysaccharide/enrofloxacin-induced injury. Exp Ther Med. 2020;20(5):1–1.

[70] Liu WT, Jing YY, Gao L, Li R, Yang X, Pan XR. Lipopolysaccharide induces the differentiation of hepatic progenitor cells into myofibroblasts constitutes the hepatocarcinogenesis-associated microenvironment. Cell Death Differ. 2020;27(1):85–101.

[71] Koyama Y, Brenner DA. Liver inflammation and fibrosis. J Clin Invest. 2017;127(1):55–64.

[72] Fang H, Liu A, Chen X, Cheng W, Dirsch O, Dahmen U. The severity of LPS induced inflammatory injury is negatively associated with the functional liver mass after LPS injection in rat model 11 Medical and Health Sciences 1103 Clinical Sciences. J Inflamm (United Kingdom). 2018;15(1):1–9.

[73] Trautwein C, Koch A. Mechanisms of Acute Liver Failure. Liver Immunol Princ Pract. 2014;373–88.

[74] Garofalo AM, Ros LM, Goncalvez G, Carriedo D, Barragan BA, Fernandez AV. Histopathological changes of organ dysfunction in sepsis. Intensive Care Med Exp. 2019;7:1–15.

[75] Kim N, Lertnimitphun P, Jiang Y, Tan H, Zhou H, Lu Y. Andrographolide inhibits inflammatory responses in LPS-stimulated macrophages and murine acute colitis through activating AMPK. Biochem Pharmacol. 2019;170(August):113646.

[76] Putra BPW, Murwani S, Oktavianie DA. Potensi Ekstrak Daun Sambiloto (Andrographis paniculata Ness) Sebagai Terapi Preventif Pada Tikus (Rattus norvegicus) Model Sepsis Yang Diinduksi Lipopolisakarida Terhadap Ekspresi TNF-α (Tumor Necrosis Factor-Alfa) Dan Histopatologi Ginjal. Brawijaya Knowl Gard. 2017;

[77] Wong SY, Tan MGK, Banks WA, Wong WSF, Wong PTH, Lai MKP. Andrographolide attenuates LPS-stimulated up-regulation of C-C and C-X-C motif chemokines in rodent cortex and primary astrocytes. J Neuroinflammation. 2016;13(1):1–11.

[78] Zhang R, Zhao J, Xu J, Jiao DX, Wang J, Gong ZQ. Andrographolide suppresses proliferation of human colon cancer SW620 cells through the TLR4/NF-κB/MMP-9 signaling pathway. Oncol Lett. 2017;14(4):4305–10.

[79] Farooqi AA, Attar R, Uteuliyev YS, Alaaeddine N, Sousa DP, Xu B. The prowess of andrographolide as a natural weapon in the war against cancer. Cancers (Basel). 2020;12(8):1–15.

[80] Lin HH, Shi M, Tseng HC, Chen JH. Andrographolide sensitizes the cytotoxicity of human colorectal carcinoma cells toward cisplatin via enhancing apoptosis pathways in vitro and in vivo. Toxicol Sci. 2014;139(1):108–20.

[81] Li X, Tian R, Liu L, Wang L, He D, Cao K. Andrographolide enhanced radiosensitivity by downregulating glycolysis via the inhibition of the PI3K-Akt-mTOR signaling pathway in HCT116 colorectal cancer cells. J Int Med Res. 2020;48(8).

[82] Reabroi S, Saeeng R, Boonmuen N, Kasemsuk T, Saengsawang W, Suksen K. The anti-cancer activity of an andrographolide analogue functions through a GSK-3β-independent Wnt/β-catenin signaling pathway in colorectal cancer cells. Sci Rep. 2018;8(1):1–14.

[83] Pan CW, Yang SX, Pan ZZ, Zheng B, Wang JZ, Lu GR. Andrographolide ameliorates d-galactosamine/lipopolysaccharideinduced acute liver injury by activating Nrf2 signaling pathway. Oncotarget. 2017;8(25):41202–10.

[84] Sun Y, Gao L, Hou W, Wu J. β-Sitosterol Alleviates Inflammatory Response via Inhibiting the Activation of ERK/p38 and NF-κ B Pathways in LPS-Exposed BV2 Cells. Biomed Res Int. 2020;2020.

[85] Kasirzadeh S, Ghahremani MH, Setayesh N, Jeivad F, Shadboorestan A, Taheri A. β -Sitosterol Alters the Inflammatory Response in CLP Rat Model of Sepsis by Modulation of NF κ B Signaling.

Biomed Res Int. 2021;2021.

[86] Chakraborty K, Joy M, Raola VK. Anti-inflammatory β-sitosterols from the Asiatic loop-root mangrove Rhizophora mucronata attenuate 5-lipoxygenase and cyclooxygenase-2 enzymes. Steroids. 2021;172(1603):108860.

[87] Arivarasu L, Gunam V. Antinociceptive and Anti-Inflammatory Activity of Beta-Sitosterol. Asian Pacific J Heal Sci. 2022;9(2):26–8.

[88] Badshah SL, Faisal S, Muhammad A, Poulson BG, Emwas AH, Jaremko M. Antiviral activities of flavonoids. Biomed Pharmacother. 2021;140(March):111596.

[89] Chen W, Zhong Y, Feng N, Guo Z, Wang S, Xing D. New horizons in the roles and associations of COX-2 and novel natural inhibitors in cardiovascular diseases. Mol Med. 2021;27(1).

[90] Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci. 2016;5.