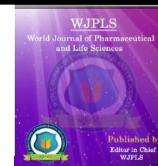
wjpls, 2019, Vol. 5, Issue 11, 12-15. Research Article ISSN 2454-2229

World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129



HEPCIDIN DOWNREGULATION THROUGH INHIBITION INTERLEUKIN-6 RECEPTOR AND JAK/STAT3 SIGNALING WITH EXTRACT OF PHYSALIS MINIMA

Rudy Salam*[1] and Diana Lyrawati[1]

Department of Pharmacy, Faculty of Medicine, University of Brawijaya Jalan Veteran, Malang – 65145.

***Corresponding Author: Rudy Salam**
Department of Pharmacy, Faculty of Medicine, University of Brawijaya Jalan Veteran, Malang – 65145.

Article Received on 28/08/2019 **Article Revised on 18/09/2019** **Article Accepted on 08/10/2019**

ABSTRACT

Inflammation have an impact on iron homeostasis through to activation of hepcidin. Hepcidin is a peptide with 25 amino acids that are expressed mainly in the liver and iron-regulatory molecule. Inflammation stimulates hepcidin expression and causes limitation of iron availability. The inflammatory cytokines, especially IL-6, activate hepcidin through activation of STAT3-mediated signaling to absorb iron in macrophages. The goal of this study was to investigate the inhibition effect of an ethanolic extract from the plant *Physalis minima L.* (Solanacae) on hepcidin expression in zebrafish. In this study, zebrafish were divided into 5 groups: negative control, positive control (exposed with LPS), treatment group 1 (exposed with LPS and 1% extract), treatment group 2 (exposed with LPS and 2,5% extract) and treatment group 3 (exposed with LPS and 5% extract). Results from this experiment indicated that hepcidin expression significantly decrease on treatment group compared with positive groups but there is no significant difference between control groups and treatments groups on IL-6 and STAT3 expression. It could be concluded that an ethanolic extract from the plant *Physalis minima L.* (Solanacae) could decrease the expression of hepcidin but not IL-6 and STAT3.

KEYWORDS: *Physalis minima*, hepcidin, IL-6, zebrafish.

INTRODUCTION

Hepcidin is a peptide with 25 amino acids that are expressed mainly in the liver and iron-regulatory molecule.[1] Inflammation stimulates hepcidin expression and causes limitation of iron availability. The inflammatory cytokines, especially IL-6, activate hepcidin through activation of STAT3-mediated signaling to absorb iron in macrophages.[2] Interaction of IL-6 with IL-6 receptor activate JAK/STAT3 signaling which causes signaling transduction and activation STAT3 transcription factor to attached on hepcidin gene promotor.[3] Inflammation have an impact on iron homeostasis through to activation of hepcidin. Hepcidin is recognized as an acute-phase reactant induced by inflammation in mice and humans.[1]

Natural products are a source of compounds that have important pharmacological activity in humans; scientists work to isolate the active ingredients from herbal medicines that may be developed into effective drugs for the treatment of human disease. In this report, we identify the effect of an ethanolic extract from the plant *Physalis minima L.* (Solanacae). The active components reported from Physalis minima are Physalin B,[4] 5,6 β-epoxyphysalin B (Row *et al*, 1978), withaphysalin A,[5] withaphysalin B (Kirson *et al*, 1976; Glotter *et al*, 1975), Physalin D (Mohana *et al*, 1979) and Physalin L,[6] Prior reports have shown that the whole plant and organic extracts from Physalis minima have anti-inflammatory effects which could inhibit lymphocyte proliferation, cytokine pro-inflammatory production and macrophage activation.[5] The goal of this study was to investigate the inhibition effect of an ethanolic extract from the plant *Physalis minima L.* (Solanacae) on hepcidin expression in zebrafish. The zebrafish, Danio rerio, provides an excellent system for the identification and analysis of genes involved in iron metabolism and erythroid development. The zebrafish hepcidin peptide is 52% identical to human hepcidin and has been shown to mediate degradation of ferroportin and cellular iron retention in vitro.[8]

MATERIALS AND METHODS

Zebrafish Embryo Maintenance

Ethical approval was obtained from Health Research Ethics Committee of Faculty of Medicine University of Brawijaya Malang in accordance with national and international guidelines. *Danio rerio* (Zebrafish) were maintained as described.[9] *Danio rerio* (zebrafish) were maintained at 28.5°C on a 14-hour light/10-hour dark cycle. Wild-type zebrafish were bred and raised according to established procedures approved by the

Laboratory of Pharmacology Faculty of Medicine. Embryos were collected from natural spawning's, cultured, and staged as previously described.

Plant Extracts

To prepare the extracts, plant used in our study was *Physalis minima* L. Dry material of *Physalis minima* (leafs and stems) were purchased from Balai Tanaman Obat Materia Medika, Batu, Malang. Powdered dry material (2 kg) were extracted with ethanol (1/1 ratio) by maceration at room temperature (x 3). Each extract was evaporated to dryness in vacuum not exceeding 40 °C with, kept at 4 °C and protected from light until use.

Zebrafish Embryo Treatments

Larvae were treated with 10 µg/mL LPS *Salmonella typhimurium* by static immersion for 3 h in a total volume of 3 ml. Extract exposure were conducted for 8 h in 6-well culture plates. Larvae aged 10 *dpf* assigned into 5 group that consist of negative control, positive control (exposed with LPS), treatment group 1 (exposed with LPS and 1% extract), treatment group 2 (exposed with LPS and 2,5% extract) and treatment group 3 (exposed with LPS and 5% extract).

RNA Extraction and real time PCR of Hepcidin, Il-6 and STAT3.

Total RNA was extracted from groups of embryos using *RNA Mini Kit Extraction Geneaid™* according to the manufacturer's instructions. Isolated RNA was checked for quality (A260/280nm 1.8-2.2) and quantity (NanoDrop Spectrophotometer 1000A). Real time PCR was performed by *KAPA SYBR® FAST One-Step qRT-PCR Master Mix* on *Real Time PCR Roche LightCycler* and gene amplification software ABI PRISM 7900 HD SDS. PCR primers (Table I) were used and the thermal profile for real time was 42°C for 5min; 95°C for 3min; followed by 40 cycles of 95°C for 10s; 61°C for 30s; 72 °C for 30s and 72°C for 7min. *ß-actin* was used to normalized the results. Each mRNA level was expressed as a ratio to *ß-actin* mRNA. Three replicates (pools of brain tissues) and three technical replicates of each RNA sample were performed. Relative mRNA expression for each gene was calculated as a fold change compared with the control group.

Statistical significance was analyzed by One Way ANOVA test using SPSS 16 software. The P value represents significance in the pairwise comparison of hepcidin, IL-6 and STAT3 transcript levels between treatment groups, positive control and negative control as determined using *One Way ANOVA*.

Table I: Gene specific primers used for quantitative real time PCR.

Gene	Forward primer	Reverse primer
Hepcidin	CACAGCCGTTCCCTTCATAC	TCAGATGTTGGTTCTCCTGC
IL-6	TCAACTTCTC CAGCGTGATG	TCTTTCCCTCTTTTCCTCCTG
STAT3	CCGCTCGAGATGGCCCAA TGGAATCAGCTAC	ATCGTTAACTCACATGGGGG AGGTAGCGC
B-actin	ATGGATGAGGAAATCGCTG	ATGCCAACCATCACTC CCTG

RESULTS

Effect of leaves and stalks extract of *Physalis minima* on Hepcidin expression

Hepcidin expression increased significantly with LPS exposure on positive control compared to negative control (one-way ANOVA $p<0.05$, Tukey Multiple Comparison of hepcidin expression $p=0.000$). This result showed that inflammation caused by LPS exposure can increase hepcidin expression on zebrafish embryos. Inflammation and infection induce hepcidin synthesis.[10] Releasing cytokine pro-inflammation such as interleukin-1, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) have contribution on elevated hepcidin expression.[11] Following extract of Physalis minima treatment, levels of hepcidin expression decreased significantly compared with positive control.

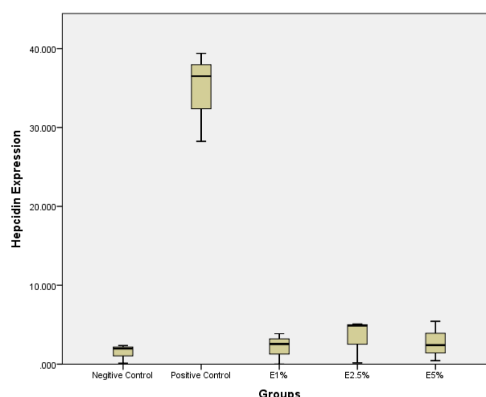


Figure 1: Hepcidin expression on control groups and treatment groups.

Figure 1 showed that hepcidin expression on positive control increase 34.716 times compared with negative control. One Way ANOVA test indicated that treatment using leaves and stalks extract of *Physalis minima* could inhibit hepcidin expression significantly. Post-Hoc test indicated decreasing of hepcidin expression significantly on treatment group compared with positive groups. Physalins are secosteroid that's found in *Physalis*. Physalins B, F or G, but not D, decreased nitric oxide production by macrophages stimulated with lipopolysaccharide and interferon-gamma. Physalin B inhibits macrophages activation and produced lower levels of TNF-α, IL-6 and IL-12.[12] Inhibition of hepcidin expression on treatment groups due to anti-inflammation effect of extract of Physalis minima.

Effect of leaves and stalks extract of *Physalis minima* on IL-6/STAT3 expression

Hepcidin expression influenced by interleukin-6 under inflammatory condition.[13] Hepcidin is directly influenced by IL-6 signaling through STAT3 activation and following with hepcidin promoter binding.[14] IL-6 induces signal transducer and activator of transcription (STAT)-3 to bind to the hepcidin promoter then increasing hepcidin's activity.[13]

Based on One Way ANOVA and Post-Hoc test that showed on figure 2, there is no significant difference between control groups and treatments groups on IL-6 expression. This results were inversely with hepcidin expression which is significantly difference among control groups and treatment groups. Figure 3 shows that there is no significant difference in the expression of STAT3 between control groups and treatments groups. The observed STAT3 expression is the total number of STAT3 either phosphorylated or not.

Activation hepcidin transcription through 2 mechanism, BMP6-HJV-SMAD and IL-6-STAT3 signaling pathways. Activation of hepcidin transcription through the IL-6-STAT3 signaling pathway occurs under inflammatory conditions. Inflammation triggers the release of pro-inflammatory cytokines, IL-6, that will interact with its receptors. Interaction of IL-6 with IL-6R results in the activation of the associated Janus kinases (JAKs) signalling pathway which following by phosphorylates STAT3 which will induce hepcidin transcription.

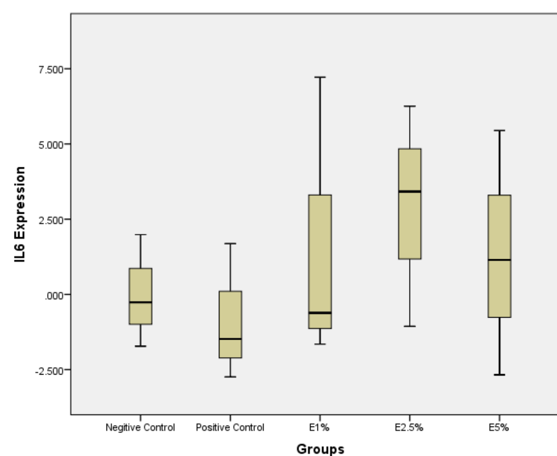


Figure 2: IL-6 expression on control groups and treatment groups.

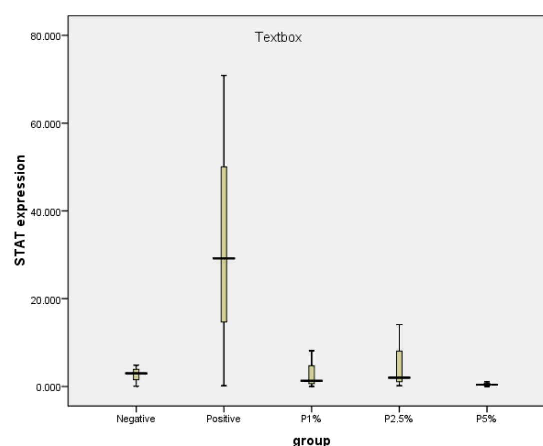


Figure 3: STAT3 expression on control groups and treatment groups.

As a transcription factor, STAT3 mediates the expression of various genes because its translocated into the nucleus. Administration of lipopolysaccharide in the positive control group has been shown increasing of hepcidin levels and this was consistent with Kemna *et al* (2005) where healthy subjects injected with lipopolysaccharide have been shown increasing blood levels of hepcidin, contrastly no increase in IL-6 levels.[15] Although there was no significant difference in IL-6 and STAT3 levels, the significant difference in hepcidin levels in the treatment group compared with positive control showed the potential of ethanolic extract of *Physalis minima* as hepcidin inhibitor.

REFERENCES

1. Sasu BJ, Cooke KS, Arvedson TL, Plewa C, Ellison AR, Sheng J, et al. Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammation-induced anemia. Blood, 2010; 115(17): 3616-24.
2. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, Camaschella C. The Serine Protease Matriptase-2 (TMPRSS6) Inhibits Hepcidin

Activation by Cleaving Membrane Hemojuvelin. Cell Metabolism, 2008; 8(6): 502-11.
3. Tsuchiya K, Nitta K. Hepcidin is a potential regulator of iron status in chronic kidney disease. Therapeutic apheresis and dialysis : official peer-reviewed journal of the International Society for Apheresis, the Japanese Society for Apheresis, the Japanese Society for Dialysis Therapy, 2013; 17(1): 1-8.
4. Matsuura T, Kawai M, Nakashima R, Butsugan Y. Structures of physalin A and physalin B, 13, 14-seco-16, 24-cyclo-steroids from Physalis alkekengi var. Francheti. Journal of the Chemical Society C: Organic, 1970; (5): 664-70.
5. Mulchandani N, Iyer S, Badheka L. Physalin D, a New 13, 14–Seco–16, 24–Cyclo Steroid from Physalis minima. Planta Medica, 1979; 37(11): 268-73.
6. Sen G, Pathak HD. Physalin L, a 13,14-seco-16,24 cyclosteroid from Physalis minima. Phytochemistry, 1995; 39(5): 1245-6.
7. Wu S-J, Ng L-T, Chen C-H, Lin D-L, Wang S-S, Lin C-C. Antihepatoma activity of Physalis angulata and P. peruviana extracts and their effects on apoptosis in human Hep G2 cells. Life Sciences, 2004; 74(16): 2061-73.
8. Fraenkel PG, Gibert Y, Holzheimer JL, Lattanzi VJ, Burnett SF, Dooley KA, et al. Transferrin-a modulates hepcidin expression in zebrafish embryos. Blood, 2009; 113(12): 2843-50.
9. De Domenico I, Vaughn MB, Yoon D, Kushner JP, Ward DM, Kaplan J. Zebrafish as a model for defining the functional impact of mammalian ferroportin mutations. Blood, 2007; 110(10): 3780-3.
10. D'Angelo G. Role of hepcidin in the pathophysiology and diagnosis of anemia. Blood research, 2013; 48(1): 10-5.
11. Wang J, Song N, Jiang H, Wang J, Xie J. Pro-inflammatory cytokines modulate iron regulatory protein 1 expression and iron transportation through reactive oxygen/nitrogen species production in ventral mesencephalic neurons. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2013; 1832(5): 618-25.
12. Soares MBP, Bellintani MC, Ribeiro IM, Tomassini TCB, Ribeiro dos Santos R. Inhibition of macrophage activation and lipopolysaccaride-induced death by seco-steroids purified from Physalis angulata L. European journal of pharmacology, 2003; 459(1): 107-12.
13. Tsuchiya K, Nitta K. Hepcidin is a potential regulator of iron status in chronic kidney disease. Therapeutic Apheresis and Dialysis, 2013; 17(1): 1-8.
14. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood, 2006; 108(9): 3204-9.
15. Kemna E, Pickkers P, Nemeth E, van der Hoeven H, Swinkels D. Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. Blood, 2005; 106(5): 1864-6.