

19. Treated Plasmodium berghei infected) decreasing the TLR-4 expression and apoptosis index of placental tissue

by Budi Prasetyo

Submission date: 27-Mar-2023 03:19PM (UTC+0800)

Submission ID: 2047825707

File name: the_TLR-4_expression_and_apoptosis_index_of_placental_tissue.pdf (470.3K)

Word count: 2784

Character count: 15725

Treated *Plasmodium berghei* infected pregnant mice by *Andrographis paniculata* tablet (AS201-01) decreasing the TLR-4 expression and apoptosis index of placental tissue

Budi Prasetyo¹, Diantri Nari Ratih¹, Yustinasari¹, Hilkaatul Ilmi², Lidya Tumewu², Aty Widyawaruyanti^{2,3*}

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Surabaya 60131, Indonesia.

²Institute of Tropical Disease, Universitas Airlangga, Surabaya 60115, Indonesia.

³Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia.

ARTICLE INFO

Article history:

Received on: 02/01/2018

Accepted on: 08/03/2018

Available online: 29/04/2018

Key words:

TLR-4 expression, Apoptosis index, *Plasmodium berghei*, in vivo assay, *Andrographis paniculata* tablet (AS201-01).

ABSTRACT

Objective: This study aimed to evaluate the effect of *Andrographis paniculata* tablet (AS201-01) in decreasing expression of Toll-Like Receptor-4 (TLR-4) and apoptosis index of placental tissue of *Plasmodium berghei* infected pregnant mice. **Material and methods:** A total of 24 pregnant mice were divided into 4 groups, G1 was uninfected pregnant mice group, G2 was untreated *Plasmodium berghei* infected pregnant mice, G3 was *P. berghei* infected pregnant mice treated with AS201-01 tablet and G4 was *P. berghei* infected pregnant mice treated with dihydropiperazine-phosphate (DHP) tablets. All mice were sacrifice at day 15th of pregnancy and the placenta was collected. The TLR-4 expression and apoptosis index were evaluated using immunohistochemistry assay. **Results:** The TLR-4 expression and apoptosis index on G3 was lower than G2 and showed a statistically significant difference ($P = 0.00$). The TLR-4 expression and apoptosis index of placental tissue in *P. berghei* infected pregnant mice which treated with AS201-01 tablet were decreased compared to untreated group and DHP tablet treated group as well. **Conclusion:** AS201-01 tablet containing ethyl acetate fraction of *A. paniculata* could decrease TLR-4 expression and apoptosis index of placental tissue in *P. berghei* infected pregnant mice.

INTRODUCTION

Malaria is a tropical disease caused by *Plasmodium* infection. Pregnant women and fetus are subjects at high risk of malaria infection. *Plasmodium* which infected pregnant women will accumulate in placenta. Pregnancy is a unique condition as there is a changing in antibodies and immune system. These systems causing a special bond between *Plasmodium* proteins with receptors on the surface membrane of syncytiotrophoblast cell that lead to placental malaria (Eriksson *et al.*, 2013).

In the patient's body, *Plasmodium*-infected erythrocytes will stimulate the immune response Th-1 to produce INF- γ in high quantities and increase the number of potential receptors

such as CD36, TLR-4, and TLR-9, on the syncytiotrophoblast cell surface (Rogerson *et al.*, 2007). In addition, infected erythrocytes will express a *Plasmodium* parasite antigen protein known as Glycophosphatidyl Inositol (GPI) *Plasmodium*. This GPI *Plasmodium* protein will act as ligand parasite by identification receptors such as TLR-4, which present on the surface membranes of syncytiotrophoblast cell (Kyes *et al.*, 2001). The binding between GPI *Plasmodium* and TLR-4 stimulates proinflammatory cytokines through the Myeloid Differentiation Factor 88 (MyD88) and NF- κ B signaling pathways and then activates pro-apoptotic proteins (Brabin *et al.*, 2004). *Plasmodium* infections may also induce the formation of Reactive Oxygen Species (ROS) which also lead to increase apoptosis via intrinsic pathways (Wang *et al.*, 2015; Mutabingwa *et al.*, 2005; Sharma *et al.*, 2012).

Placental malaria may cause the increase of TLR-4 expressions and apoptosis index beyond normal thus increasing fetus complications such as abortion, low birth

*Corresponding Author

Aty Widyawaruyanti, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia. E-mail: aty_wv@yahoo.com

weight, intrauterine fetal growth restriction, and consequently, intrauterine fetal death (Allaire *et al.*, 2000; Houmsou *et al.*, 2010; Steketee *et al.*, 2001).

One of the antimalarial drugs being developed is *Andrographis paniculata* which traditionally used as an antimalarial drug in Indonesia. Ethyl acetate fraction of *A. paniculata* was found to have antimalarial effect as reported in the previous study (Widyawaruyanti *et al.*, 2014). Further study is to develop ethyl acetate fraction of *A. paniculata* which was formulated in tablet dosage form called AS202-01. The tablet was reported to inhibit the growth of *Plasmodium berghei* in nonpregnant mice (Widyawaruyanti *et al.*, 2017). Our recent study resulted in another formulation of ethyl acetate fraction of *A. paniculata* as a tablet dosage form (called AS201-01 tablet) as well. The tablet was a potential alternative to an antimalarial drug. The effect of the tablet on *P. berghei* infected pregnant mice was unknown, in particular, the effect on TLR-4 expression and apoptosis index. Possibly along with its antimalarial activity, the tablet may also decrease TLR-4 expression and apoptosis index in *P. berghei* infected pregnant mice.

It is hoped, *P. berghei* infected pregnant mice which were treated with an AS201-01 tablet would decrease TLR-4 expression and apoptosis index so that could prevent placental malaria complication and worsening of the fetus. Therefore, this study is aimed to evaluate the effect of the AS201-01 tablet in decreasing of TLR-4 expression and apoptosis index of placental tissue in *Plasmodium*-infected pregnant mice.

MATERIAL AND METHODS

Materials

The AS201-01 tablet contains ethyl acetate fractions of *A. paniculata*, equivalent to 35 mg of andrographolide per tablet. The tablet was produced at Faculty of Pharmacy, University Airlangga, Surabaya, Indonesia. DHP tablet contains dihydroartemisinin 40 mg and piperazine phosphatase/320 mg (D-ARTEPP™) was produced by Guilin Pharmaceutical Co., Ltd., Guangxi, China.

Parasite

P. berghei ANKA strain was obtained from Eijkman Institute for Molecular Biology, Jakarta. On the 9th day post mating, mice were injected with *P. berghei* ANKA strain per 0.2 ml of blood intraperitoneally. This parasite has been maintained at the Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.

Animals

This study used female and male mice Balb/c strain at 16 to 18 weeks of age which were obtained from the Animal Experimental Development Unit - Gajah Mada University (Yogyakarta, Indonesia). They were maintained at Animal Laboratory of Institute of Tropical Disease, Universitas Airlangga. Permission and approval for animal studies were obtained from Faculty of Veterinary Medicine, Universitas Airlangga, with ethical clearance No: 560-KE/2016.

Experimental design

Eighteen of pregnant mice were infected with 1×10^6 of *P. berghei* parasite on day 9th of gestation (GD 9). These infected mice then divided into 3 groups which were G2, G3, and G4 (n = 6). Administered therapy was begun on day 11th of gestation (GD 11). G2 was an untreated group. G3 was treated by AS201-01 tablet, at a dose equal to 25 mg andrographolide/Kg body weight, twice daily for 4 days. Meanwhile, G4 was treated by Dihydroartemisinin Piperquine-Phosphate (DHP) tablet, once a day for 3 days. Additionally, G1 was uninfected pregnant mice (n = 6). All mice were then terminated on the 15th day of gestation (GD 15) and placenta collected in formalin 10% for further analysis.

Measuring TLR-4 expression and apoptosis index

Placenta samples in paraffin blocks were stained with immunohistochemical KIT using monoclonal anti-rat TLR-4 (NBP 2-45710) Nuvosbio for measuring TLR-4 expressions and TACS-XL in situ apoptosis detection KIT (4828-30-DK) for measuring apoptosis index.

Evaluation of TLR-4 expression and apoptosis index

Observation and counting the number of TLR-4 expression and apoptosis index in placenta tissue under microscope H600L Nikon (10 × 100) were done in five fields of view. TLR-4 expression and apoptosis index was calculated used Immunoreactive Score (IRS) that referred to the result of multiplying the score of percentage immunoreactive cells with a score of color intensity on the cell immunoreactive with Tunnel assay staining. Placenta tissue that consists of syncytiotrophoblast cells undergoing DNA fragmentation will be labeled as brown chromogen.

Statistical analysis

Data was analyzed by statistical software SPSS version 17. The normality data distribution was determined by Kolmogorov-Smirnov test. The significance of the mean difference between independent groups for normal distribution data was determined by using one-way analysis of variance (ANOVA). Meanwhile, for nonnormal distributed data was determined by using nonparametric Kruskal-Wallis test. Significance different of groups was analyzed by using Post Hoc Tukey's HSD test. A p-value <0.05 was considered significant.

RESULTS AND DISCUSSION

Studies to determine the effect of administration of AS201-01 tablets against TLR-4 expression and apoptosis index of placental tissue of plasmodium-infected pregnant mice were performed. Figure 1 showed the placenta TLR-4 expression in all groups. The highest TLR-4 expression was shown in G2 and the lowest in G1. The result of the statistical analysis showed that there was a significant difference (P = 0.00) on TLR-4 expression between group G2 with G1, G3, and G4. Meanwhile, figure 3 showed means of apoptosis index in all groups. The highest apoptosis index was in G2 and the lowest in G1. The statistic analysis result using one way ANOVA showed a significant difference between G2 and G1 (P = 0.00) and also between G2 and G3 (P = 0.00). There was no significant difference in apoptosis index between G1 dan G3 (P = 0.15).

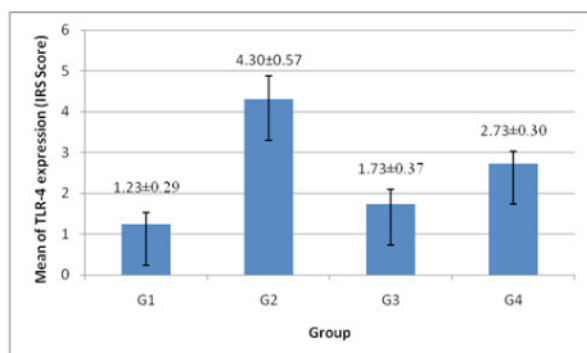


Fig. 1: Mean of placenta TLR-4 expression in all groups (G1: normal group, G2: infected untreated pregnant mice group, G3: infected pregnant mice and treated with AS201-01 tablet group, G4: infected pregnant mice and treated with DHP tablet group).

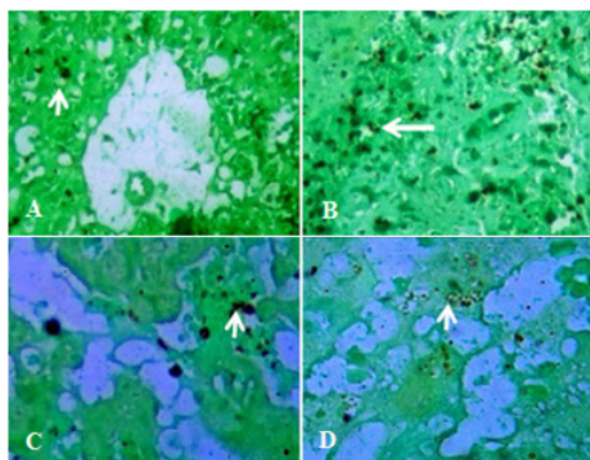


Fig. 2: TLR-4 expression in: A. normal group (G1), B. infected untreated pregnant mice group (G2), C. infected pregnant mice and treated with AS201-01 tablet group (G3), D. infected pregnant mice and treated with DHP tablet group (G4). The arrows show the TLR expression on cell membrane surface. The sample was observed under a light microscope with 100x objective magnification.

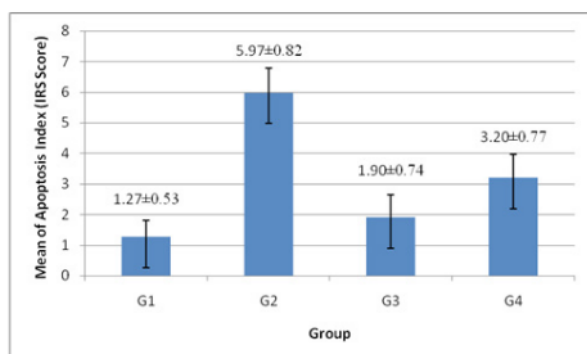


Fig. 3: Mean of placental apoptosis index in all groups (G1: normal group, G2: infected untreated pregnant mice group, G3: infected pregnant mice and treated with AS201-01 tablet group, G4: infected pregnant mice and treated with DHP tablet Group).

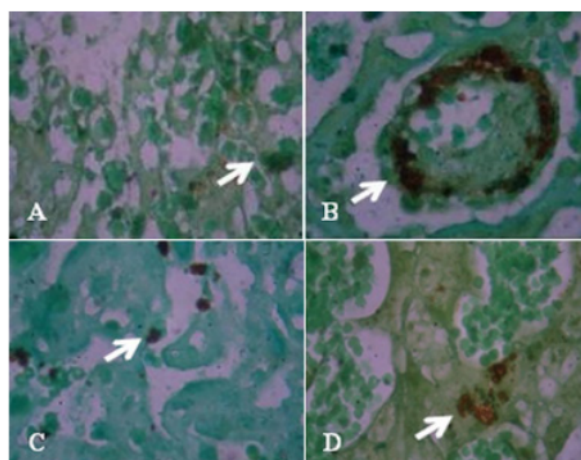


Fig. 4: Apoptosis index in: A. normal group (G1), B. infected untreated pregnant mice group (G2), C. infected pregnant mice and treated with AS201-01 tablet group (G3), D. infected pregnant mice and treated with DHP tablet group (G4). The arrows show the apoptosis on cell membrane surface. The sample was observed under a light microscope with 100x objective magnification.

The study results showed that TLR-4 expression and apoptosis index in uninfected group (G1) were low. This group was used as normal control. Meanwhile, the highest TLR-4 expression and apoptosis index was found in *P. berghei* infected untreated pregnant mice group (G2). This result was in accordance with previous report that found the apoptosis index of *P. berghei* infected pregnant mice was higher than in uninfected pregnant mice (Sharma *et al.*, 2012). *P. berghei* infected pregnant mice which treated with *A. paniculata* tablet AS201-01 (G3) were showed lower TLR-4 expression and apoptosis index compared to G2. This suggests that administration of AS201-01 tablets may decrease TLR-4 expression and apoptosis index. In the placenta, apoptosis normally occurs to maintain a pregnancy. An excessive increase in apoptosis may cause a disruption of blood flow to the placenta resulting in decreased fetal weight (Rogerson *et al.*, 2007; Allaire *et al.*, 2000).

Table 1: Semiquantitative IRS score. The result of multiplication percentage of the immunoreactive cell (A) with color intensity score (B).

A	B
Score 0: no positive cell	Score 0: no color reaction
Score 1: positive cell less than 10%	Score 1: low color Intensity
Score 2: positive cell between 11–50%	Score 2: medium color Intensity
Score 3: positive cell between 51–80%	Score 3: high color Intensity
Score 4: positive cell more than 80%	

The ability of AS20-01 tablets in decreasing the TLR-4 expression and apoptosis index because of the *A. paniculata* effect as an immunomodulator and antioxidant, which inhibit T lymphocyte cells in activating TLR-4 (Lin *et al.*, 2009; Qin *et al.*, 2006). This TLR-4 activation inhibition leads to activation inhibition of cytokine and pro-apoptosis proteins which may decrease apoptosis index of the placenta through extrinsic pathways (Rogerson *et al.*, 2007). It was also inhibited ROS formation thus

decreasing apoptosis via intrinsic pathway (Rogerson *et al.*, 2007; Brabin *et al.*, 2004; Lin *et al.*, 2009).

A. paniculata tablet has an antimalarial effect. The antimalarial effect is due to the andrographolide which is the major compound of this plant. The period of activity of andrographolide was found evidently on the ring stage of the parasite (Luo *et al.*, 2013). *A. paniculata* extract and andrographolide effects include inhibition of the nuclear transcription factor-kappa B (NF- κ B). *Plasmodium*-infected erythrocytes have shown to induce NF- κ B regulated inflammatory pathways in human cerebral endothelium. NF- κ B is activated and translocated into the nucleus, where it binds to the DNA regulatory site to regulate specific gene expression, especially cell signaling for parasite growth and development. Therefore, the inhibition of transcription factor of *A. paniculata* extract against *P. berghei* ANKA might be a critical process to inhibit blood stage propagation of parasites in vivo (Luo *et al.*, 2013; Mishra *et al.*, 2009).

Tablets AS201-01 can be used as an alternative to treat malaria in pregnancy. Therapy with this tablet is expected to prevent the side effects of malaria in pregnancy, such as low birth weight, abortion, and fetal growth inhibited.

CONCLUSION

In conclusion, *A. paniculata* tablet (AS201-01) which contain ethyl acetate fraction of *A. paniculata* could decrease the TLR-4 expression and apoptosis index of placental tissue in *P. berghei* infected pregnant mice.

ACKNOWLEDGMENT

The authors acknowledge Universitas Airlangga Mandat Research Grant 2016 with contract no. 564/UN3.14/LT/2016 for funding this research.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

- Allaire AD, Ballenger KA, Wells SR, McMahon MJ, Lessey BA. Placental apoptosis in preeclampsia. *Obstet and Gynecol.* 2000; 96(2):271-6.
- Brabin BJ, Romagosa C, Abdelgalil S, Menéndez C, Verhooff FH, McGready R, *et al.* The sick placenta-the role of malaria. *Placenta.* 2004; 25(5):359-78.
- Eriksson EM, Sampaio NG, Schofield L. Toll-Like Receptors and malaria – sensing and susceptibility. *J Trop Dis.* 2013; 2:126.

Houmsou RS, Amuta EU, Sar TT, Adie AA. Malaria infection in pregnant women attending antenatal clinics in Gboko, Benue State-Nigeria. *International Journal of Academic Research.* 2010; 2(1):33-36.

Kyes S, Horrocks P, Newbold C. Antigenic variation at the infected red cell surface in malaria. *Annu Rev Microbiol.* 2001; 55:673-707.

Lin FI, Wu SJ, Lee SC, Ng IT. Antioxidant, antioedema and analgesic activities of *Andrographis paniculata* extract and their active compound andrographolide. *Phytother Res.* 2009; 23(7):958-64.

Luo W, Liu Y, Zhang J, Luo X, Lin C, Guo J. Andrographolide inhibits the activation of NF-kappaB and MMP-9 activity in H3255 lung cancer cells. *Exp Ther Med.* 2013; 6(3):743-6.

Mishra K, Dash AP, Swain BK, Dey N. Antimalarial activities of *Andrographis paniculata* and *Hedyotis corymbosa* extracts and their combination with curcumin. *Malar J.* 2009; 8(26):1-9.

Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, Fried M, *et al.* Maternal malaria and gravidity interact to modify infant susceptibility to malaria. *Plos Med.* 2005; 2(12):e407.

Qin LH, Kong L, Shi GJ, Wang ZT, Ge BX. Andrographolide inhibits the production of TNF-alpha and IL-12 in lipopolysaccharide-stimulated macrophages: role of mitogen-activated protein kinase. *Biol Pharm Bull.* 2006; 29(2):220-4.

Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in pregnancy: pathogenesis and immunity. *Lancet Infect Dis.* 2007; 7(2):105-17.

Sharma L, Kaur J, Shukla G. Role of oxidative stress and apoptosis in the placental pathology of *Plasmodium berghei* infected mice. *PLoS One.* 2012; 7(3):e32694.

Steketee RW, Nahlen BL, Parise NE, Menendez C. The burden of malaria in pregnancy in malaria-endemic areas. *Am J Trop Med Hyg.* 2001; 64(2):28-35.

Wang D, Shi J, Lv S, Xu W, Li J, Ge W, *et al.* Artesunate attenuates lipopolysaccharide - stimulated proinflammatory responses by suppressing TLR4, MyD88 expression, and NF- κ B activation in microglial cells. *Inflammation.* 2015; 38(5):1925-32.

Widyawaruyanti A, Asrory M, Ekasari W, Setiawan D, Radjaram A, Tumewu L, *et al.* In vivo antimalarial activity of *Andrographis Paniculata* tablet. *Procedia Chemistry.* 2014; 13:101-104.

Widyawaruyanti A, Astrianto D, Ilmi H, Tumewu L, Widyastuti E, Dachliati L, *et al.* Antimalarial activity and survival time of *Andrographis paniculata* fraction (AS202-01) on *Plasmodium berghei* infected mice. *Res J Pharm Biol Chem Sci.* 2017; 8(1S):49-54.

How to cite this article:

Prasetyo B, Ratih DN, Yustinasari, Ilmi H, Tumewu L, Widyawaruyanti A. Treated *Plasmodium berghei* infected pregnant mice by *Andrographis paniculata* tablet (AS201-01) decreasing the TLR-4 expression and apoptosis index of placental tissue. *J App Pharm Sci*, 2018; 8(04): 105-108.

19. Treated Plasmodium berghei infected) decreasing the TLR-4 expression and apoptosis index of placental tissue

ORIGINALITY REPORT

17%

SIMILARITY INDEX

13%

INTERNET SOURCES

11%

PUBLICATIONS

1%

STUDENT PAPERS

PRIMARY SOURCES

1	Yuyun Ika Christina, Meyla Restia Diana, Eka Noviya Fuzianingsih, Nurhayati et al. "Hormone-balancing and protective effect of combined extract of <i>Sauropus androgynus</i> and <i>Elephantopus scaber</i> against <i>Escherichiacoli</i> -induced renal and hepatic necrosis in pregnant mice", <i>Journal of Ayurveda and Integrative Medicine</i> , 2021 Publication	1%
2	phcogj.com Internet Source	1%
3	dergi.fabad.org.tr Internet Source	1%
4	www.dovepress.com Internet Source	1%
5	escholarship.org Internet Source	1%
6	medicopublication.com Internet Source	1%

7	repository.stikim.ac.id Internet Source	1 %
8	Punsawad, Chuchard, Srivicha Krudsood, Yaowapa Maneerat, Urai Chaisri, Noppadon Tangpukdee, Emsri Pongponratn, Kwannan Nantavisai, Rachanee Udomsangpetch, and Parnpen Viriyavejakul. "Activation of nuclear factor kappa B in peripheral blood mononuclear cells from malaria patients", <i>Malaria Journal</i> , 2012. Publication	1 %
9	www.thefreelibrary.com Internet Source	1 %
10	ftp.academicjournals.org Internet Source	1 %
11	Submitted to Universitas Airlangga Student Paper	1 %
12	chimie-biologie.ubm.ro Internet Source	1 %
13	downloads.hindawi.com Internet Source	1 %
14	Izzatul Laili, Sri Gunarso, Nashrul Wathan, Noor Erma Sugijanto, Gunawan Indrayanto. "Identification of Massoia Lactone and its Hydroxy-derivative from <i>Kabatiella caulivora</i> ,	1 %

an Endophyte of the Terrestrial Plant *Alyxia reinwardtii*", Makara Journal of Science, 2017

Publication

15

backend.orbit.dtu.dk

Internet Source

<1 %

16

heanoti.com

Internet Source

<1 %

17

jhu.pure.elsevier.com

Internet Source

<1 %

18

Augustine Ankomah, Samson B Adebayo, Ekundayo D Arogundade, Jennifer Anyanti et al. "Determinants of insecticide-treated net ownership and utilization among pregnant women in Nigeria", BMC Public Health, 2012

Publication

<1 %

19

apjtb.org

Internet Source

<1 %

20

www.ssbfn.net

Internet Source

<1 %

21

Voravuth Somsak, Preeyanuch Borkaew, Chokdee Klubsri, Kittiyaporn Dondee, Panatda Bootprom, Butsarat Saiphet. " Antimalarial Properties of Aqueous Crude Extracts of and Leaves in Combination with Artesunate in - Infected Mice ", Journal of Tropical Medicine, 2016

Publication

<1 %

22	dergipark.org.tr Internet Source	<1 %
23	doczz.net Internet Source	<1 %
24	journal-inflammation.biomedcentral.com Internet Source	<1 %
25	link.springer.com Internet Source	<1 %
26	nozdr.ru Internet Source	<1 %
27	"Nanomaterials and Plant Potential", Springer Science and Business Media LLC, 2019 Publication	<1 %
28	Christopher J.M. Whitty. "Malaria in pregnancy", BJOG An International Journal of Obstetrics and Gynaecology, 9/2005 Publication	<1 %
29	Mingli Liu, Audu S. Amodu, Sidney Pitts, John Patrickson, Jacqueline M. Hibbert, Monica Battle, Solomon F. Ofori-Acquah, Jonathan K. Stiles. "Heme Mediated STAT3 Activation in Severe Malaria", PLoS ONE, 2012 Publication	<1 %
30	Wang, Yifei, Juanjuan Liu, Bei Lin, Changzhi Wang, Quanrong Li, Shuice Liu, Limei Yan, Shulan Zhang, and Masao Iwamori. "Study on	<1 %

the Expression and Clinical Significances of Lewis y Antigen and Integrin α_v, β_3 in Epithelial Ovarian Tumors", International Journal of Molecular Sciences, 2011.

Publication

31

Xinlei Wang, Jie Chen, Jingtong Zheng. "The roles of COX-2 in protozoan infection", Frontiers in Immunology, 2023

Publication

<1 %

32

www.frontiersin.org

Internet Source

<1 %

33

www.scielo.br

Internet Source

<1 %

34

Su Jing Chan. "Neuroprotective effects of andrographolide in a rat model of permanent cerebral ischaemia : Effect of andrographolide on cerebral ischaemic damage", British Journal of Pharmacology, 05/2010

Publication

<1 %

35

Mertaniasih, Ni Made, Didik Handijatno, Agnes Dwi Sis Perwitasari, Desak Nyoman Surya Suameitria Dewi, Much Zaenal Fanani, and Ika Qurrotul Afifah. "Sequence Analysis of the Gene Region Encoding ESAT-6, Ag85B, and Ag85C Proteins from Clinical Isolates of Mycobacterium tuberculosis", Procedia Chemistry, 2016.

Publication

<1 %

Exclude quotes On

Exclude matches Off

Exclude bibliography On

19. Treated Plasmodium berghei infected) decreasing the TLR-4 expression and apoptosis index of placental tissue

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4
