PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 15 (6): 515-518, 2016
ISSN 1680-5194

Tomato Extract as an Immunomodulator in Mice (*Mus musculus*) Infected with *Plasmodium berghei*

Retno Sri Iswari[1], Ari Yuniastuti[1] and Muchamad Dafip[2]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences,
Universitas Negeri, Semarang, Indonesia
[2]Department of Master of Biomedical Science, Faculty of Medicine,
Universitas Diponegoro, Semarang, Indonesia

Abstract: To prevent malaria, it is necessary to increase the immune response in the body and reduce disease severity by eradicating the parasites within the body. Tomato (*Lycopersicum esculentum* Mill) is believed to be able to increase the immune response to combat infection. The current study examined 5 different treatment conditions and each treatment was repeated 6 times. The negative control group (K-) was given standard nourishment, while the positive control group (K+) received both standard nourishment and chloroquine treatment. Groups P1, P2 and P3 were given standard nourishment, as well as a dose of 0.1, 1 and 10 mg/kg BW (body weight)/day tomato extract, respectively. Each group of mice was intraperitoneally infected with 10^7 cells/mL *Plasmodium berghei*. On the 8th day post infection, the mice were sacrificed and lymphocytes and macrophages were isolated and subsequently cultured. The expression levels of IL-12 and IFN-γ, as well as the macrophage phagocytosis index of the cells, were then determined. IL-12 levels were significantly different ($p<0.05$) between the groups that were given tomato extract (P2 and P3) and the groups that did not receive tomato extract (K-and K+). However, the mice that received 0.1 mg/kg BW tomato extract were not significantly different from those of the K- and K+ groups. The results of this study suggest that the most effective dose of tomato extract is 10 mg/kg BW.

Key words: Malaria, interleukin-12, tomato extract, lycopene, *Plasmodium berghei*

INTRODUCTION

In Indonesia, there are high numbers of deaths due to malaria, which is concerning to many people in the medical community. In 2014, the prevalence of malaria on the Java-Bali islands was 0.47% per 1000 people and 22.3% per 1000 people on the other Indonesian islands (MH, 2014). Recent research has shown that antimalarial drugs do not effectively reduce the number of *Plasmodium* infections in certain areas, likely due to the adaptation of its parasitic mechanism. Furthermore, low access to health care has also increased the death rate. Therefore, efforts to improve the body's immune response to malaria using local resources are required. In addition, it is important to develop effective medications that can be easily accessed and reduce the rate of *Plasmodium* infection, even killing the parasite within the body. Lycopene is believed to be one such chemical compound comes from the tomato plant and has been shown to increase the immune response against infectious diseases and degenerative diseases (Sergio *et al*., 2013). Lycopene may modulate the immune system to strengthen immunity against malaria (Hardisaputro, 2001; Iribhogbe *et al*., 2012). The structure of lycopene can alternate between a trans-configuration and a cis-configuration. However, the lycopene structure is very delicate and can be affected by processing and heating (Duriancik *et al*., 2010).

Lycopene can stimulate the immune system and has been specifically shown to enhance the proliferation of T lymphocytes (Hall *et al*., 2011). Thus, the consumption of tomato extract may provide beneficial immune effects, such as increasing the level of IFN-γ and the phagocytic ability of macrophages. The aim of this study is to determine the immunomodulatory effects of lycopene in mice infected with *Plasmodium berghei*.

MATERIALS AND METHODS

Ethical clearance for this study was obtained from the Universitas Diponegoro before the research was conducted. Thirty 6 to 8-week-old male mice (*Mus musculus*) of a Swiss strain were obtained from the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada (UGM). The mice were divided into 5 groups. The control group (K-) of mice did not receive tomato lycopene and the positive control group (K+) was given 5 mg/kg body weight (BW) chloroquine. The first treatment group (P1) was given 0.1 mg/kg BW tomato lycopene orally per day, the second treatment group (P2) received 1 mg/kg BW/day and the third treatment group (P3) was given 10 mg/kg BW/day.

Corresponding Author: Muchamad Dafip, Department of Master of Biomedical Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

The mice were infected with 1 x 10^7 cell/mL *Plasmodium berghei*, a Swiss strain from the Laboratory of Parasitology, Faculty of Medicine, UGM. Following infection, blood serum samples were obtained. The media used for the experiments was Roswell Park Memorial Institute (RPMI) 1640 from Thermo Fisher Scientific (Jakarta-Indonesia), containing 10% fetal bovine serum (FBS). The levels of IFN-γ and IL-12 were measured using mouse ELISA kits for IFN and IL-12 from Bender MedSystems (Wien-Austria), following the manufacturer's instructions. The mice were infected with *Plasmodium berghei* intraperitoneally. On the 8th day post infection, the mice were sacrificed and cytokine levels were determined.

Macrophages were obtained from the euthanized mice and counted using a hemocytometer. The macrophages were resuspended at 2.5 x 10^6 cell/ml and incubated at 5% CO_2 for 24 h. The macrophages were then washed twice with RPMI and mixed with 200 µl of latex beads and incubated at 37°C for 60 min. Next, the macrophages were washed with PBS and allowed to dry. The macrophages were stained with 20% Giemsa for 30 min and were then washed with distilled water. Finally, the number of macrophages that phagocytized the latex beads and the number of latex beads in the macrophages were calculated.

RESULTS

We found that the mean IL-12 levels among all of the treatment groups were different (Table 1). The results were analyzed to determine the statistical significance of the data. The data had a normal distribution and ANOVA was conducted.

The expression levels of IL-12 and IFN-γ in the P1 group were 1276 pg/mL and 5359 pg/mL, respectively and were similar to the levels found in the control group ($p = 0.503$). These data suggest that lycopene administration, at 0.1 mg/kg BW, did not affect the secretion of IL-12 and IFN-γ. In contrast, the phagocytic index of the P1 (1.877%) group was significantly enhanced compared to the K-group (0.534%). The one mg/kg BW dose of lycopene also increased the expression of IL-12 and IFN-γ. The higher dose of lycopene, 10 mg/kg BW, increased macrophage phagocytic activity. The optimal dose obtained in this study was able to increase the levels of IL-12 and IFN-γ up to 4.099 pg/mL and 9.657 pg/mL, respectively. The phagocytic index was also 1.4613% higher than the control group (Table 1). In fact, the P2 group had lower levels than the P3 group, although these results did not reach statistical significance.

High levels of IL-12, IFN-γ and an increased phagocytic index were correlated with a decrease of the parasitemia index (Fig. 1). The presence of parasites in the K-group on the first day was 1.083% and increased to 20.450% on the 8th day post infection. Chloroquine treatment in the K+ group was able to inhibit the number of parasites compared to the K-group. Similar to chloroquine, lycopene inhibited the growth of *Plasmodium berghei* for the first 2 days in the P1 and P2 groups and for the first 4 days in the P3 group. The numbers of parasites in the P1 and K+ groups were not significantly different.

Based on the results of ANOVA, we found that there were significant differences in the levels of IL-12 among the treatment groups. The differences were considered to be significant at $p<0.05$ and there was a significant treatment effect of different doses of tomato extract on the secretion of IL-12. Moreover, a different ANOVA test result also suggested a significant difference between the treatment groups. The data were then further analyzed using Fisher's least significant difference (LSD) test and 10 mg/kg BW/day was found to be the optimal dose of lycopene.

DISCUSSION

Based on a study by Sergio et al., lycopene and other carotenoids are found in the human body. The consumption of tomatoes can also increase the amount of lycopene in the body (Sergio *et al*., 2013). Here, we found that the levels of IL-12 and IFN-γ were significantly different between the P1 and P2 groups, whereas there was no significant difference among the K-, K+ and P1 groups. Importantly, chloroquine did not increase the levels of IL-12 and IFN-γ.

Sporozoites entering the bloodstream are immediately confronted by the body's immune system, initially by the non-specific immune system and then by the specific immune system. The non-specific immune response is the first step in conferring resistance to infections and it is carried out by immune cells, cytokines and the spleen (Balnoune *et al*., 2004). Additionally, intracellular parasites can bind to specific receptors and stimulate macrophages to produce IL-12 to reactivate natural killer cells (NK) cells (Tsakonas and Eleanor, 2002).

NK cells and macrophages play an important role in the body's defense against *Plasmodium berghei* in mice (Rosales *et al*., 2000). In the initial phase, the rapid growth of microorganisms in the liver and spleen is suppressed by activated macrophages. IL-12 directly affects the phagocytic function of macrophages, which can be activated by IFN-γ and TNF-α (Raverdeau and Mills, 2014). Previous studies have shown that the consumption of tomatoes, a source of lycopene, can increase the production of IL-12 and IL-4. IL-12 and IL-4 can increase the activity of macrophages and T_h cells, respectively (Nugroho *et al*., 2000).

Lycopene can also play a role in regulatory T cell differentiation (Agarwal and Rao, 2003). Lycopene is absorbed into cells through an active transport system and is then converted into retinoid (Burria and Clifford, 2004). In the cytoplasm, retinoid can bind to the active site of the Retinoid A Receptor (RAR) and Retinoid X

Table 1: Mean levels of IL-12, INF-γ and the phagocyte index*

Treatment group	IL-12 (pg/mL)	INF-γ (pg/mL)	Phagocyte index (%)
K⁻	1.065[a]	5.002[a]	0.534[a]
K⁺	1.003[a]	5.052[a]	5.556[b]
P1	1.276[a]	5.359[a]	1.877[b]
P2	3.226[b]	7.600[b]	4.081[c]
P3	4.099[c]	9.657[c]	5.186[c]

*Values indicate statistical significance at or above a 95% confidence level using the LSD test

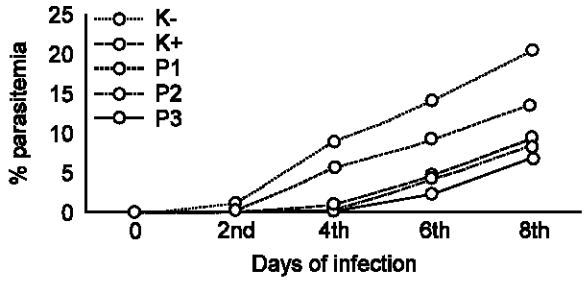

Fig. 1: Parasitemia index

Receptor (RXR). The RAR and RXR heterodimer can then trigger the DNA transcription of acute-phase response proteins, such as IL-12 and TNF-α (Takeuchi, *et al*., 2010).

High levels of IL-12 can stimulate T cell proliferation and T_h cell differentiation and influence NK cells. T_{h1} and T_{h2} cells can also secrete IFN-γ (Gonzales *et al*., 2007). In addition, previous work has suggested that mice treated with 1 and 10 mg of lycopene have significantly increased IFN-γ levels. IFN-γ can activate macrophages and dendritic cells, boosting their phagocytosis activity against antigens (Tsakonas and Eleanor, 2002; Frevert and Nardin, 2008).

Macrophages are able to reduce the number of *Plasmodium* parasites in several different ways. They can directly phagocytose the parasites, secrete cytokines to activate other macrophages, secrete NO and IL-12 to stimulate natural killer cells (NK cells) and also function as antigen presenting cells to T lymphocytes (Ross, 2012).

Based on the present data, the macrophage phagocytic indices of the groups of mice receiving lycopene were similar to the group treated with chloroquine. This suggests that lycopene can reduce the *Plasmodium* burden in mice. Lycopene intake, in appropriate amounts, can also increase the expression of IL-12 (Duriancik *et al*., 2010). In addition, routine consumption of lycopene can trigger protein transcription in macrophages and T_{h1} cells, leading to acute-phase responses, such as secretion of IL-12 and TNF-α (Lobo *et al*., 2010).

A previous study showed that consumption of tomatoes, which are rich sources of lycopene, is associated with lower mortality in malaria patients (Metzger *et al*., 2011). A high dose of tomato lycopene was also able to delay the *Plasmodium* growth phase in mice. These results suggest that lycopene may be required for the immune system to function properly. Thus, a tomato extract supplementation program may improve the health of the Indonesian people in malaria case management efforts, particularly in endemic areas.

Conclusion: The use of lycopene tomato extract at a concentration of 10 mg/kg BW increased the expression of INF-γ and IL-12, as well as the phagocytic index of immune cells in a Swiss strain of mice infected with *Plasmodium berghei*. Interleukin-12 acts as marker of macrophage functional ability within the humoral branch of the immune system. Further pre-clinical research is needed to characterize the toxicity of tomato extract and its ability to reduce the number of parasites in malaria patients.

REFERENCES

Agarwal, S. and A.V. Rao, 2003. Role of antioxidant lycopene in cancer and heart disease. J. Am. Coll. Nutr., 19: 563-572.

Belnoue, E., F.T. Costa, T. Frankenberg, A.M. Vigário, T. Voza, N. Leroy, M.M. Rodrigues, I. Landau, G. Snounou and L. Rénia, 2004. Protective T cell immunity against malaria liver stage after vaccination with live sporozoites under chloroquine treatment. The J. Immunol., 172: 2487-2495.

Burria, B.J. and A.J. Clifford, 2004. Carotenoid and retinoid metabolism: insights from isotope studies. Arch. Biochem. and Biophysics, 430: 110-119.

Duriancik, D.M., D.E. Lackey and K.A. Hoag, 2010. Vitamin A as a regulator of antigen presenting cells. J. Nutr., 140: 1395-1399.

Frevert, U. and E. Nardin, 2008. Cellular effector mechanism against Plasmodium liver stages. Cellular Microbiol., 10: 1956-1967.

Zapata-Gonzalez, F., F. Rueda, J. Petriz, P. Domingo, F. Villarroya, A. de Madariaga, and J.C. Domingo, 2007. 9-cis-Retinoic acid (9cRA), a retinoid X receptor (RXR) ligand, exerts immunosuppressive effects on dendriticcells by RXR-dependent activation: inhibition of peroxisome proliferator-activated receptor gamma blocks some of the 9cRA Activities and Preclude. J. Immunol., 178: 6130-6139.

Hall, J.A., J.R. Grainger, S.P. Spencer and Y. Belkaid, 2011. The role of retinoic acid in tolerance and immunity. Immunity, 35: 13-22.

Hardisaputro, S., 2001. Managemen penyakit infeksi: perkembangan baru antibiotika dan imunostimulan. Semarang, Universitas Diponegoro.

Iribhogbe, O.I., E.O. Agbaje, I.A. Oreagba, O.O. Aina and A.D. Ota, 2012. Oxidant versus antioxidant activity in malaria rolee of nutritional therapy. Res. Paper, 12: 229-233.

Lobo, G.P., S. Hessel, A. Eichinger, N. Noy, A.R. Moise, A. Wyss, K. Palczewski and J. von Lintig, 2010. ISX is a retinoic acid-sensitive gatekeeper that controls intestinal ß, ß-carotene absorption and vitamin A production. The FASEB J., 24: 1656-1666.

Metzger, A., G. Mukasa, A.H. Shankar and G. Ndeezi, 2011. Antioxidant Status and Acute Malaria in Children in Kampala, Uganda. Am. J. Med. Hyg., 65: 115-119.

MH (Ministry of Health), 2014. Anual Report of Indonesian Health Profile, Jakarta: Kemenkes.

Nugroho, A., P.N. Harijanto and E.A. Datau, 2000. Immunology of Malaria. In: P. N. Harijanto, ed. Malaria: Epidemiology, Pathogenicity, Clinical Manifestation and Cases Handling. Jakarta: EGC.

Raverdeau, M. and K.H. Mills, 2014. Modulation of T cell and innate immune responses by retinoic acid. J. Immunol., 192: 2953-2958.

Rosales, F.J., James D. Topping, John E. Smith, Anu H. Shankar and A. Catharine Ross, 2000. Relation of serum retinol to acute phase proteins and malarial morbidity in Papua New Guinea children. Am. J. Clin. Nutr., 71: 1582-1588.

Ross, A.C., 2012. Vitamin A and retinoic acid in T cell-related immunity. Am. J. Clin. Nutr., 96: 1166S-1172S.

Sergio, A.R., M.D. Paiva, M. Robert and M.D. Russell, 2013. β-Carotene and Other Carotenoids as Antioxidants. J. Am. Coll. Nutr., 18: 426-433.

Takeuchi, H., A. Yokota, Y. Ohoka, H. Kagechika, C. Kato, S.Y. Song and M. Iwata, 2010. Efficient induction of CCR9 on T cells requires coactivation of retinoic acid receptor and retinoid X receptors (RXRs): exaggerated T cell homing to the intestine by RXR activation with organotins. J. Immunol., 185: 5289-5299.

Tsakonas, K.A. and M.R. Eleanor, 2002. Innate immune response to malaria: rapidinduction of IFN-g from human NKcells by live plasmodium falciparum-infected erythrocytes. J. Immunol., 169: 2956-2963.