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EFFECTIVENESS OF TENDER COCONUT WATER (*COCOS NUCIFERA* L) AGAINST PARASITEMIA INDEX AND HEMOGLOBIN LEVELS IN MALARIA INFECTION

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ABSTRACT: Red blood cells (RBCs) is the primary target of human Plasmodium. Species. Occurs oxidative stress on uninfected and infected RBC and lead to hemoglobin (Hb) level decreased. The mechanism of decreased hemoglobin level are reduced survival time, diserythropoiesis and increased erythrolysis. Ascorbic acid and phenolics of TCW effectives to prevent RBCs from oxidative damage. **Methods:** The post test only randomized control group desain in 30 Swiss mice inoculated with PbA separated in 2 control groups K(+) and K(+DHP), 4 groups of treatment X1, X2, X3 and X4. TCW treatments of 1mL and 2mL per 100g BW. Statistical analysis uses for parasitemia index and Hb levels was one way ANOVA test. Correlation of both variabel analyzed using Pearson's test. **Results:** There were differences of the parasitaemia index ($p < 0.05$) and mean \pm SD (%) K(+) (20.46 \pm 9.43), K(+DHP) (0.28 \pm 0.33), X1 (14.86 \pm 5.82), X2 (1.26 \pm 1.94), X3 (4.40 \pm 1.45) and X4 (0.32 \pm 0.40). There were significant differences in Hb levels ($p < 0.05$) and mean \pm SD (g%) (8.46 \pm 1.36, 11.92 \pm 1.16, 9.92 \pm 1.77, 11.04 \pm 1.92, 11.06 \pm 0.80 and 12.80 \pm 1.16), multiple range test shown the differences of K(+) against K(+DHP) and X4, $p < 0.05$. Parasitemia index and Hb levels was significantly correlated, ($p < 0.05$) and ($r = -0.724$). **Conclusion:** Tender coconut water is effective to reduce the parasitaemia index and increase hemoglobin levels in mice inoculated Swiss PBA and there is negative correlation among parasitemia index and Hb levels in malaria infection mice.

INTRODUCTION: Malaria still the most problem infectious diseases particularly in the tropical and subtropical countries at the 433 m to 2.666 m highland.¹ The insiden of malaria was estimated 3.3 billion in endemis area from 97 countries and 584 mortilities caused 198 million cases of malaria. Prevalencies of malaria was 52% in Africa, 46% in South East Asia and 14% in America. Most death in 2015 were 88% in African region, 7% in South East Asia Region and 2% in Eastern Mediterania Region.¹

Asia is region with the second of terms of malaria burden after Africa. There are nineteen malaria countries in Asia with 2.31 billion people or 62% of the total population in those countries at risk. In 2011, 2.7 million cases and more than 2000 deaths were reported. India, Indonesia, Myanmar and Pakistan are responsible for more than 85% of the reported cases and deaths in Asia.^{2, 3} Annual Parasites Indeks (API) in Indonesia in 2008–2009 decreased 2.47 per 1000 to 1.85 per 1000. Five provinces of Indonesia with high prevalency of malaria are Papua, Nusa Tenggara Timur, Papua Barat, Sulawesi Tengah dan Maluku.⁴

The important of hematological parameter in malaria infection are RBCs, leukocytes and trombocytes. RBCs is the primary target of Plasmodium infection. The decrease absolute number of RBCs is due to parasite sporulation,

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reduce survival time of RBCs and slow erythropoiesis.^{5,6} All of these result in decrease of Hb levels and subsequently contribute to severe anemia in malaria.⁷⁻¹⁰ Lysis of infected and uninfected RBCs are enhanced by oxidative stress including the generation of reactive oxygen species (ROS), malondialdehyde (MDA) and Thiobarbituric acid reactive substance (TBARS). Oxidative stress is used as marker of increases RBCs membran fragility. This therefore antioxidant plays a significant role as adjunctive therapy in the clinical malaria.¹¹

Besides as an endemic malaria country of malaria, Indonesia also the largest producer of coconut (*Cocos nucifera* L) with growth area 3.701.000 Ha and the average of fruit production is 15.5 billion per year.¹² Total of material obtained was that 3.75 million ton of coconut water, 0.75 million ton of shell and 1.8 million ton of husk. Coconut water is famous as drinking water no exception in Papua. The antioxidant including DPPH free scavenging, phenolic, L-Arginine and ascorbic acids contained in TCW give rise to a hope that coconut water prevent severe anemia in malaria. In this study, we investigated effectiveness of coconut water as adjuvant therapy in malaria.

Objective:

The aim of this study was to determine the effectiveness of coconut water on parasitemia index and Hb level in female Swiss mice inoculated Pba and treated ACT.

MATERIALS AND METHODS:

Preparation of Tender Coconut Water:

Fresh coconut water of green dwarf variety in range of 6 to 9 months were obtained from the local market, Kebumeh Central of Java. They were identified by Laboratorium Penelitian dan Pengujian Terpadu (LPPT) of Universitas Gajah Mada. It was punctured at the holes using sterilized spuit and then placed in sterilized bowl. The coconut water were collected and used for each day experiment.

Animals handling

Thirty adult female of Swiss mice, weighing 25-35 g and 8-10 weeks old were bought from LPPT Universitas Gajah Mada. They were housed in six

plastic cages in a room maintained at $25 \pm 4^\circ \text{C}$ with alternate exposure to light and dark for 12 hours. The animals were acclimatized for 7 days under hygienic conditions. All procedures were done in accordance with ethical guidelines for care & use of laboratory animals and were approved by ethical committee of health researches, Medical Faculty of Diponegoro University and Karyadi Hospital Semarang. The mice divided in 4 treated and 2 control groups. Treated groups were X1, X2, X3 and X4 received orally 1 ml TCW, 1 mL TCW+DHP, 2 mL TCW and 2 mL TCW+DHP for 14 days. Two control groups were K(+) and K(+DHP).

Sample collection:

At the end of the experiment, the animals were sacrificed by decapitation and blood samples were collected for biochemical determinations. Blood samples were collected from sinus orbitalis under mild anaesthesia using diethylether. The parasitemia index was expressed as percentage (%) of RBCs infected in 1000 of total RBCs stained with Giemsa and examined using light microscope. Hematology analyzer was used to measure Hb level.

Statistical Analysis:

The statistical analysis was done by one-way anova. The results are presented as the mean value \pm SD for the controls and treated groups. Differences among the means for the groups were assayed using the Post Hoc Bonferoni Multiple Range Test to determine. The significantly different was $p \leq 0.05$.

RESULTS AND DISCUSSION:

Effectiveness of coconut water against level of parasitemia:

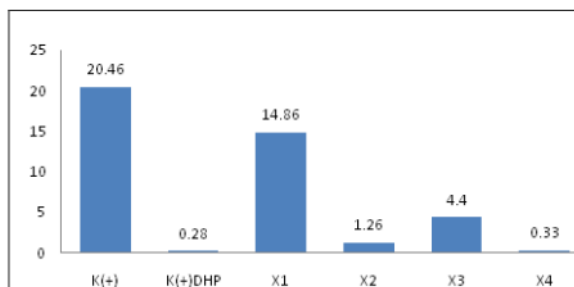


FIG.1: MEAN OF PARASITEMIA INDEX BETWEEN GROUPS

Parasitemia index of these treated with TCW including X1, X2, X3, X4 were significantly lower, $p < 0.05$. Different of groups mean describe in **Table 1**.

TABLE 1: DIFFERENCE BETWEEN GROUP OF PARASITEMIA INDEX

Group	Homogeneity	p	Difference between groups					
			K(+)	K(+)/DHP	X1	X2	X3	X4
K(+)	0.288 ^a	0.000*	-	0.009*	0.465	0.009*	0.009*	0.009*
K(+)/DHP			-	-	0.009*	0.248	0.009*	0.916
X1			-	-	-	0.009*	0.009*	0.009*
X2			-	-	-	-	0.047*	0.465
X3			-	-	-	-	-	0.009*
X4			-	-	-	-	-	-

* One Way Anova test, significant value $p < 0.05$, ^aHomogeneity test, $p > 0.05$

Thus this suggested that TCW has effect in reducing parasitemia index. Further analysis showed that parasitemia index of X3 group was lower than X1 group ($p = 0.009$). This indicate that the dose of 2 ml TCW was the most effective dose in controlling malaria infection. Parasitemia index of K(+) receiving DHP group significantly lower than X1 and X3 groups. Additionally parasitemia index of K(+) receiving DHP group was not different than X2 and X4 groups. All of these emphasized that TCW remain need standard malaria therapy to complete in controlling Plasmodium infection. Ascorbic acids in coconut water increase antimalaria activities, in line with the previous study which observed the effectiveness of ascorbic in combination with arthemeter to reduce paraitemia index ¹³.

The most amino acid contains in coconut water is L-arginine. L-arginine is involved in many metabolic pathways, including the synthesis of NO. NO production is influenced by several cytokines, including interferon gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α) which up

regulate iNOS expression involve in an increasing NO production. NO has

Effectiveness of coconut water againts Hb level:

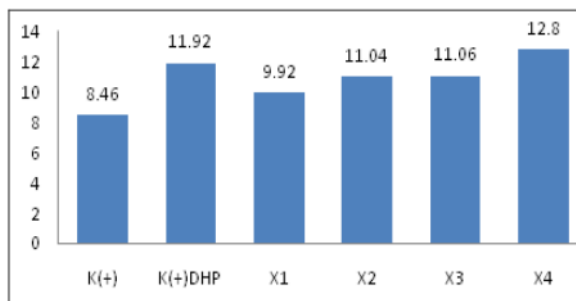


FIG.2: MEAN OF Hb LEVELS

Effectiveness of coconut water on Hb level showed that no differences mean of Hb levels between groups X3 (2 ml/100 g BW TCW) and control K(+)/DHP. The mean hemoglobin level of TCW treatment were lowest than control K(+). Describe in **Table 2**.

TABLE 2: DIFFERENCES OF Hb LEVELS BETWEEN GROUP

Group	Homogeneity	p	K(+)	K(+)/DHP	X2	X3	X4
K(+)	0.453 ^a	0.001*	-	0.022*	0.253	0.063	0.006*
K(+)/DHP			0.022*	-	0.941	0.748	0.829
X1			0.696	0.379	0.919	0.772	0.127
X2			0.253	0.941	-	1.000	0.546
X3			0.063	0.748	1.000	-	0.17
X4			0.006*	0.829	0.546	0.177	-

* One Way ANOVA Test, significant value $p < 0.05$, ^aHomogeneity test, $p > 0.05$

Content 0.106 mg/mL of Vitamin C in TCW enhance iron absorbs ion and prevent the RBCs damage. In malaria infection, Vitamin C serum was lower than normal. The previous study

showed that Vitamin C in dose of 0.3 mL/20 g BW can increase Hb levels in Balb C mice infected by *Plasmodium vivax*. ¹⁶ The antioxidants effectivities are usually associated with their ability to inhibit

oxidative damage by DPPH free radicals scavenging. Most herb study showed that phenolic compound has antioxidant activity. Phenolic compound in TCW are probably play a role in improving Hb levels This however, has never been studied. Than K(+), $p < 0.05$. Post hoc bonferoni test showing the different between groups was described in Table 1. Activity to parasites killing.¹⁴
¹⁵ Effectiveness of TCW againts parasitemia index is the novelty study.

CONCLUSION: This study indicates that effectiveness tender coconut water to reduce of parasitemia index and increase Hb level in Swiss mice, but effect in human malaria is not confirmed and need further investigation.

CONFLIC OF INTEREST: This study is original research carried out by the cited authors. The authors declare that there is no conflict of interests regarding the publication of this paper.

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