Antiviral effects of *Curcuma longa* L. against dengue virus in vitro and in vivo

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Abstract. Dengue is the most common infective disease caused by dengue virus (DENV) and endemic diseases in tropical and subtropical areas. Until now, there is no specific antiviral for dengue infection. It is known that viral load is related to disease severity. *Curcuma longa* L. (turmeric) with curcumin as major active compound has been identified for its antiviral effect. This study to determine antiviral effect of *C. longa* extract on DENV-2 in vitro and in vivo along with its toxicity in liver and kidney of ddY mice. Antiviral activity (IC\(_{50}\)) and toxicity (CC\(_{50}\)) in vitro was examined on Huh7it-1 cells by focus assay and a MTT assay, respectively. To determine the selectivity index (SI), we used CC\(_{50}\) and IC\(_{50}\) value. The safe doses obtained were used for toxicity tests of liver and kidney with histopathological and biochemical observations. The *C. longa* extracts was given orally with dose of 0.147 mg/mL for each mice at 2 hours after injected with DENV-2 infected Huh7it-1 cells. Serum was collected from intraorbital at 6 hours and 24 hours after infection and focus assay was used to determine viral load. In this study, the acquired value of IC\(_{50}\) was 17.91 µg/mL whereas the value of CC\(_{50}\) was 85.4 µg/mL. The value of SI of *C. longa* was 4.8. In vivo, we found that *C. longa* remarkably reduced of viral load after 24 hour. Histopathological examination showed no specific abnormalities in liver and kidney. There was no significant increase in levels of SGPT, SGOT, urea, and creatinine. From this study it can be concluded that *C. longa* could potentially be used as antiviral against DENV with low cytotoxicity and effective inhibition.

1. Introduction
In the recent years, it is evident that dengue, which is largely found in the tropical and subtropical areas, is the most common systemic viral disease infecting humans [1]. According to WHO, about 390 million people of the world has infected with DENV where is 500.000 with dengue cases with more than 22.000 deaths every year [2]. Based on the number of dengue cases reported to WHO in 2004-2010, Indonesia ranks second after Brazil [3]. In Indonesia, Bali and DKI Jakarta have the highest number of DHF cases in 2013 up to 168.5/100.000 population [4]. In addition, dengue has always been a major health problem in Indonesia, which became the leading cause of the hospitalization and death of children [4].
DENV is a family of *Flaviviridae* viruses with 4 serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) distinguished by genetic and antigenic properties. The infection of one serotype will not provide immunity to other serotypes [5]. Clinical manifestations of DENV infection range from asymptomatic, Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), up to Dengue Shock Syndrome (DSS) [3]. DHF will be developed into DSS in case of shock due to plasma leakage and may be fatal [6]. Decreased viremia in infected patients was expected to prevent the development of manifestation into DHF/DSS [7-8]. Currently, there are no specific treatments for DENV. Antiviral therapy and vaccines are also unavailable at this moment. Treatment for DENV is only by maintaining the body fluid of the patient, as it is critical in managing the severe form of DENV.

In Asia, *Curcuma longa* L. (Sinonim *C. domestica* Val.) or turmeric has long been used not only as spice but also as remedies [9]. Researchers have discovered that *C. longa* extract and its major active compound curcumin has many benefits in health aspect. Moreover, recent experimental studies also indicate that *C. longa* extract has properties that act as antioxidant, anti-inflammatory, antiviral, antimicrobial, etc [10]. With its component, it is expected that *C. longa* extract has an antiviral effect on the replication of DENV [9-10]. The objective of this study to evaluate the effectiveness of *C. Longa* extract as antiviral to dengue virus infection. The specific objectives were: i) to optimize the antiviral assay for dengue virus, ii) to measure the CC$_{50}$ and IC$_{50}$ of *C. longa* in vitro and iii) to determine the toxicity and antiviral activity in vivo.

2. Materials and Methods

2.1. Ethics statement and experimental animals
Male and female mice strain ddY 8-12 weeks old (30-40 g) and male mice strain ddY 2-4 weeks old (20-30 g), from Institut Pertanian Bogor (IPB) were used in this study. The mice were acclimatized for a week before start of the experiments. The animals were housed under standard laboratory condition at temperature 22 ± 2°C with relative humidity at 65 ± 10%. Standard pellet rodent diet and water were provided to the animals ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee of Universitas Indonesia (no. protocol 16-10-337).

2.2. *Curcuma longa* L. extract preparation
*Curcuma longa* L. CDS-13 extract contained 19% of curcuminoid was obtained from PT Konimex, Solo. Extraction was performed by PT Konimex, Indonesia with 90% alcohol solvent. The extract is then thickened for further fractionation with methanol. The extract was dissolved in dimethyl sulfoxide (DMSO) (Sigma, Singapore) or in 0.5% Carboxymethyl cellulose (CMC) for in vitro and in vivo assays respectively.

2.3. Preparation of DENV-2 and Huh7it-1 cells.
Dulbecco’s Modified Eagle’s Medium (DMEM) High glucose (GIBCO, UK) with 10% of Fetal Bovine Serum (FBS) (GIBCO) was used to maintain of Huh-7 it-1 cell line in this study. DMEM was added with sodium bicarbonate to maintain of the pH of the medium. Cells were then incubated at 37°C with 5% CO$_2$ for 4-5 days until confluent. We used DENV serotype 2 strain NGC adapted in human cell line of Huh-7 it-1 cell. A monolayer of Huh7it-1 cell in T-75 flasks were infected with DENV-2 NGC at an MOI of 0.5 FFU/cell and incubate at 37°C with 5% CO$_2$ for 7 days. During the time of virus propagation, the FBS concentration of the cell culture medium was reduced to 2%. Supernatant was harvested and centrifuged at 1000 g for 5 minutes. Subsequently, it was filtered using a syringe driven 0.22 mm (Millipore, Co. Bedford MA USA). Supernatant was stored at -80°C and checked for the titer of DENV by Focus assay that was performed previously described by Igarashi et al, 1999 [11].

2.4. Determination of cytotoxicity (CC$_{50}$) in vitro
In vitro cytotoxicity (CC$_{50}$) was determined by MTT assay based on viability of Huh-7 cells after treated with extract. In 96 well flat-bottom plates (Corning, USA), 2 × 10$^4$ cells/well was added and incubated at 37°C with 5% CO$_2$. After 24 hours, the cells were treated with various concentrations of extract from 2.5 to 80 µg/mL and incubated at 37°C with 5% of CO$_2$. After 48 hours, 20 µL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Promega) salt solution was added into each well and incubated for 4 hours according to the manufacturer’s instruction. The absorbance reading of each well was measured using micro-plate reader at 490 nm. First, the theoretical percentage toxicity of the samples were calculated by dividing the mean blanked sample ODs by the mean blanked control ODs for each sample. The resulting percentage toxicity was provided as the “Data In” to curve fit, with the requested interpolation set to 50. The concentrations of the samples were calculated from the curve and the interpolated 50% value was provided. The absorbance was measured at 620 nm. The viability data was used to represent the cytotoxic effect. The viability of the cells was determined by this following equation: The percentage viability cell, we able to determine CC$_{50}$. The CC$_{50}$ was obtained from nonlinear regression analysis of concentration-effect curves by the Graph and represented the means ± standard deviation experiments.

2.5. Determination of antiviral activity (IC$_{50}$) in vitro
A total of 2×10$^4$ cells/well were seeded into 96-well plate and incubated at 37°C with 5% CO$_2$. After 24 hours, the cells were infected with DENV-2 at a MOI of 1 FFU/cell which contained various concentrations of natural extract from 80 µg/mL, 40 µg/mL, 20 µg/mL, 10 µg/mL, 5 µg/mL and 2.5 µg/mL. After 2 hours infection, 100 µL of DMEM+2% FBS contained various concentration of natural extract was added. Plates were further incubated at 37°C for 3 days. Virus was harvested and virus titer was determined by Focus assay [11]. Briefly, a 10-fold serial dilution of supernatant was inoculated onto Huh-7 it-1 cell monolayer in duplicate wells. Absorption was carried out at 37°C in 5% CO$_2$ for 2 hours with agitation at 30 minutes interval. Methylcellulose 0.5% overlay medium was added to the cell and incubated at 35°C in 5% CO$_2$ for 3 days. The infected cells were stained according to previous publication by Payne AF et al, 2006 with slight modification [12]. First, infected cells were fixed with 10% formaldehyde in PBS and incubated at room temperature for 1 hour. Cells were rinsed with PBS 3 times. To permeabilize the cells, 100 µL/well of 1% of Nonidet P40 was added and incubated at room temperature for 30 minutes. Then blocking solution (5% skim milk in PBS) was added and incubated at room temperature for 1 hour. After wash, the cell was added with 1/1000 of human IgG anti-dengue at room temperature for 1 hour. 1/1000 anti-human IgG label HRP was added as a secondary antibody. After wash, substrate was added and observed infected cell with brown colour. The result from Focus assay was used to determine IC$_{50}$ with following equation: Based on percentage inhibition, we able to determine IC$_{50}$. The IC$_{50}$ was obtained from nonlinear regression analysis of concentration-effect curves by the Graph and represented the means ± standard deviation experiments.

2.6. Determination of toxicity in vivo in mice liver and kidney
Toxicity test was performed on the basis of non-clinical toxicity test guidelines in vivo by BPOM-R1 [13]. The ddY mice was divided into 3 groups with 10 mice (5 male and 5 female mice) each group. Group 1, mice were administered with 500 mg/kgBW of extract in 0.5% CMC. Group 2, mice were administered with 1000 mg/kgBW of extract in 0.5% CMC. Group 3, mice were administered with 0.5% CMC only. Extract was admitted orally admitted orally once day for 14 days. Animals were weighed regularly during the experiment.

At the end of the experiment (i.e. on 14th day), the animals were anesthetized with ketamine intraperitoneally (i.p) injection and sacrificed by cervical decapitation. Blood was collected intraorbitally from all the experimental mice and serum was separated. Serum samples were stored at -80°C and assayed for Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetic Transaminase (SGOT), ureum and creatinine using standard DiaSys kit that were purchased from PT DiaSys Diagnostic System GmbH (Holzheim, Germany). The liver and kidney
were isolated and stored in 10% formalin for Histopathological analysis using Haematoxylin Eosin staining.

2.7. Preparation of Huh7it-1 infected DENV-2 NGC cells for in vivo experiment
A total 5x10⁶ Huh7it-1 cells were infected with 800 µL DENV-2 NGC at an MOI of 0.5 FFU/cell and 200 µL DMEM with 2% FBS was added. The infected cells were incubated for 2 hours at 37°C with 5% CO₂ and homogenized every 20 minutes. After 2 hours of incubation, the infected cells were transferred to the T-75 flasks and 9 mL DMEM with 2% FBS was added. Then, cells were incubated at 37°C with 5% CO₂ for 2 days. After incubation, medium was removed and rinsed with 4 mL PBS 1x. Then, 1 mL of 0.25% trypsin-EDTA was added and incubated for 5 to 10 minutes at 37°C with 5% CO₂. After addition with trypsin, 1 mL of fresh medium with 10% FBS was added and mixed. The mixture was then centrifuged at 4°C for 10 minutes at 1500 rpm. The supernatant was discarded while cells were re-suspended with PBS 1x 2 mL before being injected into mice. The DENV infected Huh7it-1 cells was stained by immunofluoresence assay (IFA) and observed under fluorescence microscope. The infected cell were used when the percentage of infected cells above 80%.

2.8. Determination of antiviral activity in vivo
ddY mice were injected intraperitoneally (i.p) with DENV infected Huh7it-1 to produce DENV viremia in mice. This method was based on the development of Yamanaka A. and Konishi E. method in 2009 in vaccine testing [14].The dose was determined by calculating the conversion value of IC⁵₀ Curcuma longa L. extract [15].

The mice were divided into 2 groups containing 3 male mice each group. Group 1, mice were injected with 1 mL DENV infected cells (5.5 x 10⁶ Huh7it-1 in 1 mL PBS 1x) (i.p) and administered once orally with 0.147 mg/mL C. longa extract in 0.5% CMC at 2 hours after infection. Group 2 (control), mice were injected with 1 mL DENV infected cells (5.5 x 10⁶ Huh7it-1 cells in 1 mL PBS 1x) and administered once orally only with 0.5% CMC at 2 hours after infection.

Blood was collected intraorbitally (i.o) on 6th and 24th hours after infection. The animals were anesthetized with ketamine (i.p) injection. Then, blood was added 10 µl 3.8% sodium citrate and centrifuged at 200 rpm for 5 minutes. Serum samples were collected and stored at -80°C. The titer of DENV in serum was determined by Focus assay [11].

2.9. Statistical Analysis
The data from in vitro assay was analysed with unpaired t-test on GraphPad Prism 6. The homogeneous and normally distributed data were analysed by ANOVA followed by Tukey test as Post Hoc analysis. Data with no normality and homogeneity distribution will be analysed using Wilcoxon test. All the statistical analysis was using SPSS 21 software.

3. Results

3.1. DENV Inhibition by Curcuma longa L. in vitro
DENV were treated with various concentration of extract before infect to the Huh7it-1 cells. The concentrations were 80 µg/mL, 40 µg/mL, 20 µg/mL, 10 µg/mL, 5 µg/mL and 2.5 µg/mL. CyclosporinA 2 µg/mL was used as positive control whereas DMSO 0.1% was used as negative control. The data distribution of the virus titer from each concentration was normal, with p-value more than 0.05. The Table 1 illustrated the average titer of the virus with its standard deviation.

| Table 1. Titer of DENV-2 upon treatment with various concentration of C. longa extract. |
|--------------------------|-------------|-------|
| Treatment C. longa | Average titer (FFU/mL) | SD |
|--------------------------|-------------|-------|
| 80 µg/mL | 0 | 0 |
| 40 µg/mL | 0.06 x 10² | 3.8 |
| 20 µg/mL | 0.75 x 10² | 16.5 |
The average titer of the virus was then further calculated to find the percentage of the virus that was inhibited upon treatment. The equation from the linear regression curve of the percent inhibition was defined to determine the half inhibitory concentration ($IC_{50}$).

| Treatment                  | Inhibition (%) |
|----------------------------|----------------|
| 10 μg/mL                   | 1.21 x 10^2    |
| 5 μg/mL                    | 1.80 x 10^2    |
| 2.5 μg/mL                  | 1.87 x 10^2    |
| CyA 2 μg/mL (+ control)    | 1.73 x 10^2    |
| DMSO 0.1% (- control)      | 2.04 x 10^2    |

Table 2. Inhibited DENV-2 upon treatment with C. longa extract

The viral inhibition increased as the C. longa extract concentration increased (Table 2., Figure 3.). This means that there was an antiviral activity of C. longa to DENV. From the equation, the $IC_{50}$ of C. longa extract was 17.91 μg/mL (Figure 1.).

3.2. Cytotoxic Effect of C. longa
To ensure that extract was not toxic to the cell, the half cytotoxic concentration ($CC_{50}$) was measured. This was achieved from the result of MTT assay. The cell viability still showed high level after treated with C. longa extract at concentration up to 40 μg/mL (Table 3.).

Table 3. The viability cells upon treatment with various concentration of C. Longa extract.

| Concentration (μg/mL) | Viability (%) | SD   |
|-----------------------|---------------|------|
| 80                    | 51.3          | 2.39 |
| 40                    | 86.5          | 11.94|
| 20                    | 99.1          | 3.14 |
| 10                    | 97.4          | 4.01 |
| 5                     | 101.8         | 2.63 |
| 2.5                   | 111.7         | 4.53 |

The $CC_{50}$ was analyzed from the linear regression equation of the percent viability. It was discovered that the $CC_{50}$ of turmeric was 85.4 μg/mL (Figure 2.)
3.3. Selectivity Index ($CC_{50}/IC_{50}$)
After the $CC_{50}$ was divided by $IC_{50}$, it was found that the selectivity index of Curcuma longa extract in this study was 4.8.

![Figure 3](image)

Figure 3. Focus image of DENV-2 NGC after treated with various concentration of C. Longa extract. (a). 80 μg/mL; (b). 40 μg/mL; (c).20 μg/mL; (d).10 μg/mL, (e).5 μg/mL; (f).2.5 μg/mL and (g).DMSO.

3.4. Toxicity of extract to mice kidney and liver
The results of observation of mice body weight for two weeks both male and female for each dose of C. longa extract showed weight gain during experiment, but statistically the administration with various doses did not affect the body weight of mice and changes in body weight was not significantly different with the group control. The macroscopic images of liver and kidney showed no change occurred compared to control. The liver and kidney were red and shiny, the surface was smooth, the consistency was springy, there was no discoloration.

![Figure 4](image)

Figure 4. Histopathological staining of ddY mice liver (200x). There was not specific abnormality in the liver. (a) dose 500 mg/kgBW, (b) dose 1000 mg/kgBW orally and (c) control. The arrow shows (1) Vena centralis, (2) Tractus portalis. There was no specific abnormality in the liver.
Figure 5. Histopathological staining of ddY mice kidney (200x). There was no specific abnormality in the kidney. (a) dose 500 mg/kgBW, (b) dose 1000 mg/kgBW and (c) control. The arrow shows (1) Glomerulus; (2) Tubulus proximal.

SGPT, SGOT, serum urea and creatinine levels after treatment with 500 mg/kgBW, 1000 mg/kgBW extract and CMC solvent control, both male and female, respectively, were not significantly affected by the administration of the extract. The results of further statistical calculations Tukey test showed results that did not differ significantly with the control group.

Table 4. Result of biochemical analysis in mice serum

|          | Male    |          |          | Female   |          |          |
|----------|---------|----------|----------|----------|----------|----------|
|          | 500 mg/kgBW | 1000 mg/kgBW | Control CMC | 500 mg/kgBW | 1000 mg/kgBW | Control CMC |
| SGPT (IU/L) | 74,4±22,8 | 84,1±9,5 | 72,9±11,4 | 64,7±00,0 | 88,9±3,4 | 83,0±1,8 |
| SGOT (IU/L) | 141,5±38,2 | 141,6±39,7 | 153,3±59,5 | 152,8±36,4 | 79,2±20,5 | 102,4±16,6 |
| Ureum (mg/dL) | 57,1±12,7 | 62,5±5,5 | 59,7±6,5 | 57,8±9,3 | 64,2±9,6 | 63,4±21,2 |
| Creatinine (mg/dL) | 0,59±0,17 | 0,53±0,37 | 0,89±0,24 | 0,79±0,35 | 1,00±0,43 | 0,92±0,15 |

3.5. Effect of C. longa extract as DENV-2 antiviral in vivo

In the preliminary study, the titer of DENV in ddY mice injected with DENV infected Huh7it-1 cells was detected up to 24 hours. Virus titer in mice serum was 5.8x10^3 FFU/mL at 6 hours after infection and decreased at the level of 3.6x10^2 FFU/mL at 24 hours after infection.

To determine effect of C. longa extract to the DENV titer in mice, we infect mice with DENV infected Huh7it-1 cells and serum was collected at 6 and 24 hours after infection. Mice serum from each group were pooled for testing because of the limitation of serum’s amount.

Figure 6. Level of DENV titer in DENV infected mice after treated with C. longa extract.

The results showed that the titer of DENV decreased in treated group compared to control (Figure 6). The titer of DENV from the group with of C. Longa treatment showed decreased to the level of 1.11x10^2 FFU/mL at 6 hours of infection and was not detected at 24 hours after infection. While on the control of solvent, the virus titer was detected up to 4.0x10^2 FFU/mL and up to 0.95x10^1 FFU/mL after 6 and 24 hours after infection. Treatment with C. Longa reduced viremia period in mice.
4. Discussion

Dengue has been a significant problem in most tropical and sub-tropical countries. There is no specific treatment yet for dengue. Treatment is only supportive based on the maintenance of the patient’s body fluid since it is crucial in managing the severe form of dengue. As of today, approved antiviral agents for dengue are not available yet although studies regarding this have becoming more popular. Most of the studies directly or indirectly used natural product as sources for their antiviral study since the active substances for most therapies derived conventionally from natural sources [16]. Furthermore, the active substances for most therapies derived conventionally from natural sources [16].

The method, however, were a bit different. They used Vero cell instead of Huh7it-1 cell [20]. Based on the same journal, it stated that the inhibited virus was probably not directly from curcumin [20].

One of the obstacles in the development of DENV antiviral is the difficulty of getting experimental models other than the natural hosts of humans and mosquitoes.

The purpose of this study is to find antiviral to DENV when it infect to human. Thus, Huh7it-1 cell, which is a human cell line derived from the liver, was used in this study. In addition, Huh7 could be infected by dengue virus since the characteristic of the cell is similar to hepatocyte in which basically the place of dengue virus to replicate itself [18].

It was found in this study that the $CC_{50}$ was 85.4 $\mu$g/mL and the percentage of cell viability decreased in higher concentration. Thus, it can be said that the *C. longa* extract, in fact, killed the cells although not significantly. Half cytotoxic concentration is a concentration of a substance in which it may reduce the viability of a cell by 50%. Thus, theoretically, a good substance should have high $CC_{50}$. According to a review of studies about potential plants that can be used as anti-dengue, *Alternanthera philoxeroides* or also called as alligator weed had the lowest cytotoxic effect on cells (CC$_{50}$ = 535.91) [19].

The $IC_{50}$ of *C. longa* extract was 17.91$\mu$g/mL and there was a viral inhibition even though at lower concentration, it showed dengue virus enhancement. This enhancement may be useful for diagnosing patient, which it can be used to enhance and culture the virus of the patient. Half cytotoxic concentration is a concentration of a substance that can be used to inhibit the viral activity by 50%. Alligator weed and extracts from several plants also was shown to have high inhibitory activity towards dengue virus [19]. The $IC_{50}$ of them was less than 5 $\mu$g/mL [19]. The data in this study was analyzed by using unpaired t-test on GraphPad Prism. It showed that the virus titer of the cells that were treated with *C. longa* extract at concentrations 80 $\mu$g/mL, 40$\mu$g/mL, and 20 $\mu$g/mL was significant (p value < 0.05) while the others were not.

One of the major active constituents of *C. longa* called curcumin. It was reported that curcumin has many pharmacological effects such as its antiviral properties. In 2013, Padilla S et al studied the antiviral effect of curcumin against dengue virus [20]. The selectivity index found was 2.56, which differs from this study (SI = 4.8). The method, however, were a bit different. They used Vero cell instead of Huh7it-1 cell [20]. Based on the same journal, it stated that the inhibited virus was probably due to cellular alterations from apoptosis, which induced by curcumin. Therefore, the inhibition was not directly from curcumin [20].

On the other hand, other study reported that the extract of *Curcuma longa* or turmeric is a potent inhibitor of dengue virus by targeting the NS2B/NS3 protein that coded protease enzyme DENV [21]. IMDPH (Inosine Monophosphate Dehydrogenase) is an enzyme that catalyzes the oxidation of several purine. Thus, it plays a significant role in synthesizing DNA and RNA and also signal transduction. Through inhibiting this enzyme, curcumin is suggested as a potential antiviral compound [22].

One of the obstacles in the development of DENV antiviral is the difficulty of getting experimental animals that exhibit clinical manifestations, DENV pathogenesis as in humans including viral protein identification and potential host to target treatment of DENV infection. The development of model animals is constrained because viruses cannot replicate well on models other than the natural hosts of humans and mosquitoes.

The effect of *C. longa* extract on DENV-2 in vivo was done by inducing Huh7it-1 infected DENV-
2 NGC cells in ddY mice intraperitoneally (i.p) to see the level of viremia in mice. This method is based on the development of Yamanaka S and Konishi E method in 2009 in vaccine testing [14]. Researchers used K562 cells infected with dengue and inoculated into ICR, Balb/c, and ddY mice. The results explain that viremia levels last up to 34 hours after infection in mice ddY and ICR, whereas in Balb/c viremia occurs only lasts for 24 hours [14]. Laboratory of microbiology has tested the ddY mice inoculated K562 infected DENV-2 (i.p). From the results of this test, virus still detected until 24 hours in mice serum. Therefore ddY mice was selected as animal models of this study, in addition ddY mice are cheap and easy to develop.

In this study, virus titer detected up to 24 hours in mice serum that was inoculated with DENV infected Huh7it-1 cells. The titer of DENV in mice treated with *C. longa* was decreased at 6 and 24 hours after infection. In the 24 hours after infection, mice treated with *C. longa* extract orally, no DENV-2 detected while in controls were present. In other words that treatment with *C. Longa* reduced viremia period in mice.

In the development of antivirus, the materials should be safely consumed every day orally. The literature review confirmed on safety aspects of curcumin and essential oil fractions of *C. longa* [23-26]. Since the source of plant may give different toxicity effect, therefore toxicity study of *C. Longa* from Indonesia was needed. In this study we evaluated cytotoxic effect of *C. longa* extract by determined of LD50 and its effect in liver and kidney of mice. Several studies have been conducted to determine LD50 *C. longa* extract. It was found that LD50 of *C. longa* extract orally was 10 g/kgBW in the mice, and in that dose not cause toxic symptoms and deaths were observed for 72 hours [27]. In addition, acute toxicity of *C. longa* extract has also been studied in mice and monkeys by Bhavani Shangkar et al [28]. From previous study LD50 of *C. longa* extract was 5 g/kgBW and no toxicity effects on histopathological examination and cytology of the liver, heart and kidneys. In this study, histopathological examination showed no specific abnormalities in liver and kidney organ after treated orally with 500 mg/kgBW and 1000 mg/kgBW of *C. longa* extract for 14 days. There was no significant increase in levels of SGPT, SGOT, urea, and creatinine.

5. Conclusion
CC50 of *C. longa* extract was 85.4 μg/mL, IC50 was 17.91 μg/mL, and the SI of *C. longa* extract in this study was 4.8. *C. longa* extract was not provide toxic symptoms in mice liver and kidney. In vivo study showed that *C. longa* extract at doses of 0.147 mg/mL has antiviral effect against DENV-2 and reduce viremia period.

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