Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2023 Jan 02; 11(A):111-116.
https://doi.org/10.3889/oamjms.2023.8993
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

The Hepatoprotective Effect of *Curcuma longa* Extract on Dengue Virus Serotype-2 infected BALB/c Mice

Ni Wayan Anantika Riani[1]*, I Made Susila Utama[2], Ida Bagus Oka Winaya[3]

[1]Department of Internal Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia; [2]Tropical and Infectious Disease Division, Department of Internal Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia; [3]Laboratory of Pathology, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia

Edited by: Ksenija Bogoeva-Kostovska
Citation: Riani NWA, Utama IMS, Winaya IBO. The Hepatoprotective Effect of *Curcuma longa* Extract on Dengue Virus Serotype-2 infected BALB/c Mice. Open Access Maced J Med Sci. 2023 Jan 02; 11(A):111-116. https://doi.org/10.3889/oamjms.2023.8993
Keywords: Alanine transaminase; *Curcuma longa*; Dengue; Liver; Pathology
***Correspondence:** Ni Wayan Anantika Riani, Department of Internal Medicine, Udayana University, Udayana University Hospital, Bali, Indonesia.
E-mail: anantika@unud.ac.id
Received: 13-Feb-2022
Revised: 24-Mar-2022
Accepted: 31-Mar-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Dengue infection has a wide clinical spectrum ranging from asymptomatic infection to severe form with organ damage. Liver is among the common organ affected. *Curcuma longa* is a widely used herb that exhibits a hepatoprotective effect.

AIM: This study aimed to investigate effect of *C. longa* on liver necroinflammatory activity and alanine aminotransferase (ALT) levels in dengue virus serotype-2 (DENV-2) infected BALB/c mice.

MATERIALS AND METHODS: BALB/c mice were assigned into Group 1 (healthy mice), Group 2 (without *C. longa* administration), and Group 3 (*C. longa* administered). DENV-2 was inoculated intraperitoneally in Groups 2 and 3. *C. longa* extract was given at dose of 0.147 mg/mL intraorally in the 1st to 7th day. Liver histopathology and ALT level were assessed on the 7th day. Necroinflammatory activity was assessed with a Knodell histology activity index (HAI) score.

RESULTS: The median Knodell HAI score in Group 1, Group 2, and Group 3 were 1.0 (0–5), 7.0 (4–10), and 3.5 (0–8), respectively. The mean difference of Knodell HAI score between Group 2 and Group 3 is 4.1 (95% CI 1.75–6.45, p = 0.002). The mean ALT in Groups 1, 2, and 3 were 58.22 ± 18.31 IU/L, 58.26 ± 18.31 IU/L, and 57.00 ± 10.71 IU/L, respectively. One-way analysis of variance test showed no significant differences in ALT level levels among the three groups (F = 0.26, p = 0.974).

CONCLUSION: This study showed the hepatoprotective effect of *C. longa* that might decrease the liver necroinflammatory activity in dengue infection.

Introduction

Dengue infection is the most common tropical infectious disease caused by dengue virus (DENV) from the genus Flavivirus [1]. DENV was first isolated in 1943 in Japan and to date there are five serotypes have been identified, specifically DENV-1, DENV-2, DENV-3, DENV-4, and most recently DENV-5 that were announced in October 2013 [2]. Dengue infection has become a global health issue with the second highest incidence and mortality among vector-borne diseases after malaria with *Aedes aegypti* and *Aedes albopictus* as the main vectors of DENV [3]. A multicenter study by Utama *et al.* showed that DENV is one of the etiologies of acute febrile illness, it accounted for 32% of acute febrile illness case [4].

Approximately 3.6 billion people living in dengue endemic areas and 50 million people are infected with dengue each year leading to 25.000 deaths [5]. Southeast Asia and West Pacific region have the highest burden of dengue in the world, it is estimated that 75% dengue case in the world has occurred in this region [6]. Dengue infection has a wide clinical spectrum ranging from asymptomatic infection or undifferentiated febrile illness to the severe and life-threatening condition called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [7].

Liver injury in dengue characterized by increased levels of alanine transaminase (ALT) and could progress to acute liver failure that increased the mortality rate [8]. Liver injury in dengue infection is presumably cause by hepatocytes apoptosis, hypoxic damage, oxidative stress, and immune system dysregulation induced by viral infection [9]. To date, there is no specific therapy have been determined for dengue treatment.

Curcuma longa is a native plant to Southeast and South Asia that commonly has been used as traditional medicine, including in Indonesia. Curcumin the main active component of *C. longa* showed protective effects on the liver, as well as inhibitory mechanism against viral replication through four mechanisms of ubiquitin-proteasome system, transcription, gene expression, and viral replication [10], [11], [12]. This study aims to investigate effect of *C. longa* extract on liver necroinflammatory activity and ALT levels in DENV serotype-2 (DENV-2) infected BALB/c mice.

Materials and Methods

Experimental animals

Eight weeks old male BALB/c mice weighing 40 g were used in this study. Each cage contained two mice with standard pellet diet and water were provided *ad libitum*. The mice were acclimatized for 1 week, then were assigned into three groups consisting of ten mice in each group, specifically Group 1 (control group, healthy uninfected mice), Group 2 (group of DENV-2 infected mice, but did not receive extract of *C. longa*), and Group 3 (group of DENV-2 infected mice and received *C. longa* extract). The study workflow is shown in Figure 1.

The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines and ethical principles originating in or derived from the Declaration of Helsinki. This study was reviewed and approved by the Research Ethics Commission of Faculty of Medicine, Udayana University/Sanglah General Hospital (193/UN14.2.2.VII.14/LP/2019).

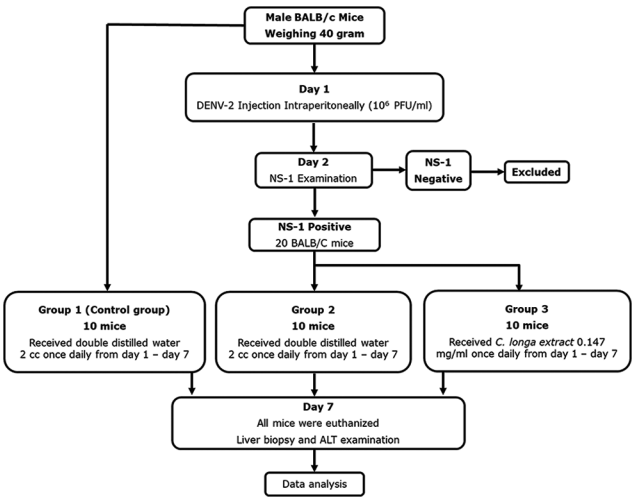

Figure 1: The study workflow

C. longa extract

This study used *C. longa* CDS-13 extract containing 19% curcumin that had been processed into methanol fraction. Mice in Group 3 received 0.147 mg/mL of dissolved *C. longa* extract in 2 cc warm water. The extract was administered orally once daily on the 1st–7th day [13]. Group 1 and Group 2 were given 2 cc/day double distilled water.

DENV

The DENV used in this study was DENV-2 that was obtained from the Health Research and Development Agency, Ministry of Health of Republic of Indonesia. DENV-2 was injected intraperitoneally with a titer of 10^6 PFU/ml in Groups 2 and 3. DENV-2 previous study showed that DENV-2 infected BALB/c mice is an appropriate model of dengue infection and histopathological analysis revealed liver injury with viral antigens detection [14]. Moreover, study Megawati *et al.* in Bali showed that DENV-2 have higher incidence of more severe form of dengue (DHF) [15].

Dengue non-structural protein (NS-1) antigen examination using the enzyme-linked immunosorbent assay was performed on the 2nd day to confirm dengue infection. Mice with negative NS-1 results were excluded from the study.

Examination of alanine aminotransferase

ALT examinations were carried out at a Veterinary Hospital, Faculty of Veterinary Medicine, Udayana University. ALT examination was conducted with International Federation of Clinical Chemistry (IFCC) enzymatic kinetic examination using the IFCC (Biorex Diagnostics, Antrim) ALT liquid reagent and the iChem-535Vet spectrophotometer (iCubio Biomedical Technology, Shenzhen). Peripheral blood was drawn medial to the cantus in the retroorbital venous plexus using a microcapillary pipette. ALT examinations were carried out on the three groups of mice on the 7th day.

Liver histopathology examination

On the 7th day, all mice in the three groups were euthanized by the cervical dislocation method. The livers fixed with 10% formalin in distilled water. Paraffin block was made using a Leica RM2125 (Leica Microsystem, Germany) microtome with a thickness of 4 μm. The staining was performed with Hematoxylin Harris method. Liver histopathological examinations were carried out in Laboratory of Histopathology, Faculty of Veterinary Medicine, Udayana University. Histopathological abnormalities were assessed by light microscopy with ×400 magnification to observe the presence of hepatic cell necrosis and the results were presented in numerical form based on the Knodell histology activity index (HAI) score [16].

The Knodell HAI score is used to assess necroinflammatory activity and fibrosis in liver biopsy. Knodell HAI consists of three categories of necroinflammation and one category of fibrosis with a total Knodell HAI score ≥4 which is considered a significant necroinflammatory activity. The categories assessed are periportal bridging necrosis, intralobular degeneration and focal necrosis, portal inflammation, and fibrosis. Total Knodell HAI score 0–3 indicate non-significant necroinflammatory activity, score of 4–8 indicate mild necroinflammatory activity, 9–12 indicate moderate necroinflammatory activity, and 13–18 indicate severe necroinflammatory activity [17].

Statistical analysis

This study is an experimental study with a randomized post-test only control group design. Numerical scale that normally distributed is presented in mean and standard deviation. Categorical scale is presented in relative frequency. One-way analysis of variance (ANOVA) analysis was used followed by a *post hoc*-Least significant difference test (LSD) to assess the effect of *C. longa* extract on ALT levels and histopathological features of the livers of mice infected with DENV-2. If the data are not normally distributed, a Kruskal–Wallis test will be performed. The Statistical Package for the Social Sciences (SPSS) statistical software (version 24.0, SPSS Inc., Chicago, IL, USA) was used for all the statistical analysis.

Results

The study subjects were divided into three groups, specifically Group 1 (group of healthy, dengue-uninfected mice), Group 2 (group of mice infected with DENV-2, but did not receive *C. longa* extract), and Group 3 (group of mice infected with DENV-2 and received *C. longa* extract). Dengue infection status was confirmed with NS-1 examination on the 2nd day after virus inoculation. NS-1 examination in all mice in Groups 2 and 3 showed positive results; hence, all mice were included in the study. All mice infected with dengue survived until the end of this study.

The histopathological features in Group 1 (control) showed normal hepatocyte cells with regular hepatocyte architecture and intact cell walls without signs of inflammation (Figure 2). Histopathological examination in Group 2 showed edema of Kupffer cells as well as an increase in the distribution of monocytes and signs of inflammation accompanied by injury to hepatocyte cell membranes. Group 3 histopathological features showed an inflammatory process and distribution of monocytes, but less injury to hepatocyte cell walls than Group 2.

Histopathological changes in subjects were assessed semi-quantitatively using the Knodell HAI score. The median Knodell HAI score in Groups 1, 2, and 3 was 1.0 (interquartile range [IQR] 0–5), 7.0 (IQR 4–10), and 3.5 (IQR 0–8), respectively. The histopathological result in this study is presented in Table 1. The Kruskal–Wallis test showed significant differences in the mean Knodell HAI score in the three groups (p = 0.001).

To determine the difference in the mean Knodell HAI score between groups, the *post hoc* LSD test was used. *Post hoc* LSD (Figure 3) showed a significant difference in the mean Knodell HAI score between Group 1 (control control) and Group 2 (positive dengue that did not receive *C. longa*) (p = 0.001), and between Group 2 (positive dengue who did not receive *C. longa*) and Group 3 (positive dengue that received *C. longa*) (p = 0.002).

This study showed a significant difference in the mean Knodell HAI score between Group 2 compared to Group 1 (7.0 vs. 2.0; mean difference: 5, 95% CI 2.65–7.35, p = 0.001) and Group 2 compared to group 3 (7.0 vs. 2.9; mean difference: 4.1, 95% CI 1.75–6.45, p = 0.002), while Group 3 (2.9) and Group 1 (2.0) did not show a significant difference (mean difference: 0.9, 95% CI –1.45–3.25, p = 0.374).

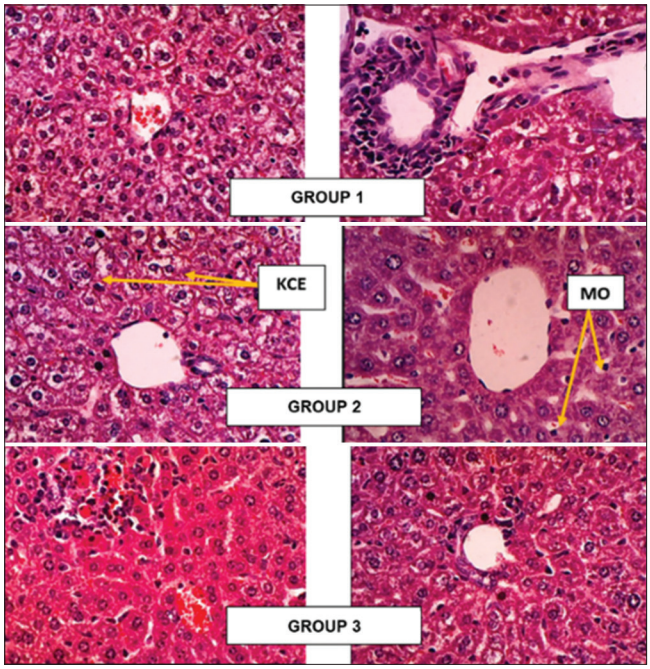

Figure 2: Liver histopathological features. KCE: Kupffer cell edema, MO: Monocytes

Table 1: Histopathological examination of the liver

Group	Mice	Knodell HAI score	Necroinflammatory activity
Group 1	1	4	Mild
	2	4	Mild
	3	0	Non-significant
	4	1	Non-significant
	5	1	Non-significant
	6	3	Non-significant
	7	1	Non-significant
	8	0	Non-significant
	9	5	Mild
	10	1	Non-significant
Group 2	1	10	Moderate
	2	4	Mild
	3	4	Mild
	4	10	Moderate
	5	4	Mild
	6	4	Mild
	7	10	Moderate
	8	4	Mild
	9	10	Moderate
	10	10	Moderate
Group 3	1	4	Mild
	2	4	Mild
	3	8	Mild
	4	4	Mild
	5	2	Non-significant
	6	3	Non-significant
	7	0	Non-significant
	8	0	Non-significant
	9	0	Non-significant
	10	4	Mild

Knodell HAI score: Knodell Histology Activity Index Score. Knodell HAI score interpretation: 1–3 = non-significant necroinflammatory activity, 4–8 = mild necroinflammatory activity, 9–12 = moderate necroinflammatory activity, 13–18 = severe necroinflammatory activity.

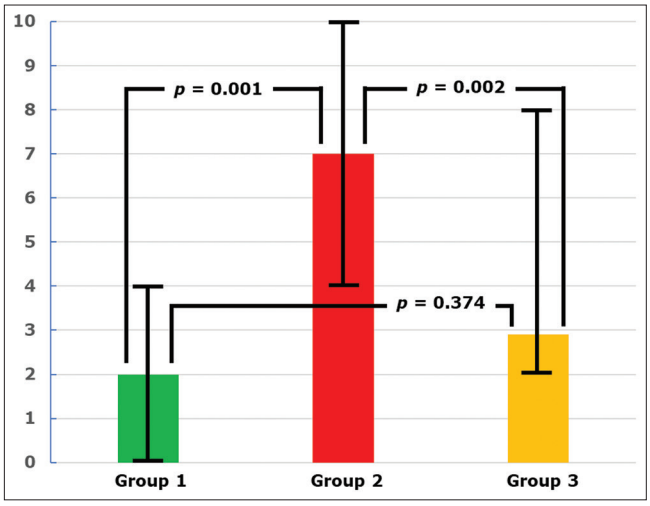

Figure 3: Mean difference of Knodell histology activity index

In this study, the mean ALT levels in Groups 1, 2, and 3 were 58.22 ± 18.31 IU/L, 58.26 ± 18.31 IU/L, and 57.00 ± 10.71 IU/L, respectively. One-way ANOVA test showed no significant differences in ALT levels between the three groups (F = 0.26, p = 0.974).

Discussion

Liver injury in dengue patients may occur directly or indirectly through immune system dysfunction [9]. Injury to the liver may occur directly due to the cytopathic effect of DENV in which DENV antigen is detected in hepatocytes, which cause impaired liver function [18], [19]. A postmortem study in a severe case of dengue conducted by Huerre *et al*. showed that hepatocytes and Kupffer cells were involved in the viral replication process and cell apoptosis occurred in the necrosis area [20].

The pathophysiology of liver injury in dengue may also occur through an indirect mechanism. Avirutnan *et al*. showed that NS-1 may bind to hepatocytes, which is thought to trigger the formation of immune complexes and trigger complement activation [21]. Inflammation and infection of hepatocytes due to DENV infection will lead to apoptosis of hepatocytes [22]. Histopathological features of liver injury due to dengue infection include microvascular steatosis, hepatocyte necrosis, hyperplasia, and injury to Kupffer cells, and mononuclear cell infiltration [9], [14].

In this study, Group 2 which didn't receive *C. longa* had a higher mean Knodell HAI score with a significant mean difference when compared to the group of mice that received *C. longa* extract (group 3). The mean difference in Knodell HAI score in this study showed that the necroinflammatory activity were more severe in Group 2 than in Group 3, whereas when compared to group 1 (healthy mice), Group 3 did not show a significant difference in necroinflammatory activity. These results indicate a protective effect of *C. longa* extract against liver damage in BALB/c mice infected with DENV-2.

To the extent of our understanding, this is the first study to assess the hepatoprotective effect of *C. longa* in dengue infection examining liver histopathological features and this study demonstrates the hepatoprotective effect of *C. longa*. Study by Ichsyani *et al*. showed that *C. longa* administration in dengue infected BALB/c mice did not show liver toxicity on histopathological examination [13]. However, the study did not assess the effect of *C. longa* administration on histopathological improvement.

Curcumin, an active compound from *C. longa*, has long been recognized to inhibit enveloped virus infectivity by inhibiting hemagglutinin activity [23]. The administration of curcumin also plays a role in inhibiting viral replication through the ubiquitin-proteasome system, an important mechanism in the DENV infection cycle [11]. *C. longa* extract administration decreased DENV virus titer in BALB/c mice after 6 and 24 h post infection [13]. Balasubramanian *et al*. also showed that curcumin could inhibit the NS2B/NS3 protease enzyme which plays a role in the cellular pathway that is important for DENV replication [24].

DENV infection increases intracellular reactive oxygen species leading to inflammation and cell death [25]. Oxidative stress in the liver due to dengue infection correlates with the severity of disease, patient with DSS has the highest oxidative stress level, followed by DHF [26]. Chen *et al*. showed antioxidants to be a defense mechanism protecting the cells from apoptosis due to DENV infection [27]. *C. longa* that exhibits antioxidant effects has been shown to protect the liver from acute hepatic stress by preventing the occurrence of oxidative stress [28].

Curcumin will increase intrahepatic antioxidant levels and reduce the production of lipid peroxidase, a free radical [29]. Curcumin also capable of inhibiting proliferation and increase hepatic stellate cell apoptosis by increasing the expression of peroxisome proliferator activated receptor-γ which can prevent hepatic fibrogenesis [30]. Histological analysis by Fu *et al*. demonstrated that curcumin could protect the liver from injury and fibrogenesis due to CCL_4-induced liver injury in mice [31].

Elevated ALT is common in dengue, but the increase is related to the stage of disease. A study by Lee *et al*. showed that the median ALT was higher in patients with severe dengue [32]. Sani *et al*. also showed that patients with severe dengue had a higher median peak ALT level [33]. Oxidative stress in the liver may occur despite normal transaminases enzyme [29]. Therefore, ALT examination is not the ideal test to assess necroinflammatory activity. Approximately 37%

of patients with significant necroinflammatory activity have normal ALT values [16].

To date, no study has assessed the correlation between ALT levels and Knodell HAI scores in dengue patients. However, studies assessing the correlation between the Knodell HAI score and elevated ALT in other viral infections, such as hepatitis have been reported. A study conducted by Lee *et al*. showed that there was no correlation between ALT levels and HAI scores in patients with hepatitis C [34]. The same results were also shown in patients with hepatitis B infection, where there were histopathological changes in the liver despite persistently normal ALT levels [35]. A study conducted by Paes *et al*. showed no correlation between liver injury and ALT levels in infected mice compared to controls [36]. The study conducted by Ichsyani *et al*. also showed no difference in ALT levels in dengue-infected mice receiving *C. longa* compared to healthy controls [13].

Although not statistically significant, the mean ALT level in Group 3 that received C. longa was lower when compared to Group 2 and Group 1. Moreover, the histopathological examination in Group 3 showed non-significant necroinflammatory activity when compared to control. The insignificant difference in ALT between groups and the discrepancy with histopathological results is thought to be caused by interindividual variation. In addition, the stage of dengue infection itself also affects transaminase levels in dengue infection that cannot be assessed in this study.

Conclusion

This study showed necroinflammatory activity in DENV-2 infected mice. In the study group that received *C. longa* extract has a lower necroinflammatory activity compared to other groups. The hepatoprotective effect of *C. longa* might decrease the necroinflammatory activity of the liver in dengue infection. Further studies are needed to investigate the optimal dosage of *C. longa* extract administration for hepatoprotection in dengue infection.

Acknowledgment

The authors would like to thank the Animal Laboratory Unit of Pharmacology and Therapy Department, Faculty of Medicine, Udayana University and Veterinary Hospital of Faculty of Veterinary, Udayana University for the supports until the completion of this study.

References

1. Hasan S, Jamdar SF, Alalowi M, Al Beaiji SM. Dengue virus: A global human threat: Review of literature. J Int Soc Prev Community Dent. 2016;6(1):1-6. https://doi.org/10.4103/2231-0762.175416
   PMid:27011925
2. Mustafa MS, Rasotgi V, Jain S, Gupta V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Med J Armed Forces India. 2015;71(1):67-70. https://doi.org/10.1016/j.mjafi.2014.09.011
   PMid:25609867
3. Jing Q, Wang M. Dengue epidemiology. Glob Health J. 2019;3(2):37-45. https://doi.org/10.1016/j.glohj.2019.06.002
4. Utama IM, Lukman N, Sukmawati DD, Alisjahbana B, Alam A, Murniati D, *et al*. Dengue viral infection in Indonesia: Epidemiology, diagnostic challenges, and mutations from an observational cohort study. PLoS Negl Trop Dis. 2019;13(10):e0007785. https://doi.org/0.1371/journal.pntd.0007785
   PMid:31634352
5. Harrington J, Kroeger A, Runge-Ranzinger S, O'Dempsey T. Detecting and responding to a dengue outbreak: Evaluation of existing strategies in country outbreak response planning. J Trop Med. 2013;2013:756832. https://doi.org/10.1155/2013/756832
   PMid:24222774
6. Shepard DS, Undurraga EA, Halasa YA. Economic and disease burden of dengue in Southeast Asia. PLoS Negl Trop Dis. 2013;7(2):e2055. https://doi.org/10.1371/journal.pntd.0002055
   PMid:23437406
7. Harapan H, Michie A, Mudatsir M, Sasmono RT, Imrie A. Epidemiology of dengue hemorrhagic fever in Indonesia: Analysis of five decades data from the national disease surveillance. BMC Res Notes. 2019;12(1):350. https://doi.org/10.1186/s13104-019-4379-9
   PMid:31221186
8. De Souza L, Nogueira R, Soares L, Ribas B, Alves F, Vieira F, *et al*. The impact of dengue on liver function as evaluated by aminotransferase levels. Braz J Infect Dis. 2007;11(4):407-10. https://doi.org/10.1590/s1413-86702007000400007
   PMid:17873994
9. Samanta J, Sharma V. Dengue and its effects on liver. World J Clin Cases. 2015;3(2):125-31. https://doi.org/10.12998/wjcc.v3.i2.125
   PMid:25685758
10. Farzaei MH, Zobeiri M, Parvizi F, El-Senduny FF, Marmouzi I, Coy-Barrera E, *et al*. Curcumin in liver diseases: A systematic review of the cellular mechanisms of oxidative stress and clinical perspective. Nutrients. 2018;10(7):855. https://doi.org/10.3390/nu10070855
    PMid:29966389
11. Padilla-S L, Rodríguez A, Gonzales MM, Gallego-G JC, Castaño-O JC. Inhibitory effects of curcumin on dengue virus Type 2-infected cells *in vitro*. Arch Virol. 2014;159(3):573-9. https://doi.org/10.1007/s00705-013-1849-6
    PMid:24081825
12. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": From kitchen to clinic. Biochem Pharmacol. 2008;75(4):787-809. https://doi.org/10.1016/j.bcp.2007.08.016
    PMid:17900536
13. Ichsyani M, Ridhanya A, Risanti M, Desti H, Ceria R, Putri DH, *et al*. Antiviral effects of *Curcuma longa* L. against dengue virus *in vitro* and *in vivo*. IOP Conf Ser Earth Environ Sci. 2017;101(1):012005. https://doi.org/10.1088/1755-1315/101/1/012005

14. Paes MV, Lenzi HL, Nogueira AC, Nuovo GJ, Pinhão ÂT, Mota EM, *et al*. Hepatic damage associated with dengue-2 virus replication in liver cells of BALB/c mice. Lab Invest. 2009;89(10):1140-51. https://doi.org/10.1038/labinvest.2009.83
PMid:19721415
15. Megawati D, Masyeni S, Yohan B, Lestarini A, Hayati RF, Meutiawati F, *et al*. Dengue in Bali: Clinical characteristics and genetic diversity of circulating dengue viruses. PLoS Negl Trop Dis. 2017;11(5)e0005483. htpps://doi.org/10.1371/journal.pntd.0005483
PMid:28531223
16. Shen FF, Wang Y, Wang YF, Zheng RD, Xian JC, Shi JP, *et al*. Prediction of hepatic necroinflammatory activity in patients with chronic hepatitis B by a simple noninvasive model. J Transl Med. 2018;16(1):166. https://doi.org/10.1186/s12967-018-1538-z
PMid:29914513
17. Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: The knodell histology activity index and beyond. Hepatology. 2000;31(1):241-6. https://doi.org/10.1002/hep.510310136
PMid:10613753
18. Alcon-LePoder S, Drouet MT, Roux P, Frenkiel MP, Arborio M, Durand-Schneider AM, *et al*. The secreted form of dengue virus nonstructural protein NS1 Is endocytosed by hepatocytes and accumulates in late endosomes: Implications for viral infectivity. J Virol. 2005;79(17):11403-11. https://doi.org/10.1128/jvi.79.17.11403-11411.2005
PMid:16103191
19. Barth OM, Barreto DF, Paes MV, Takiya CM, Pinhão AT, Schatzmayr HG. Morphological studies in a model for dengue-2 virus infection in mice. Mem Inst Oswaldo Cruz. 2006;101(8):905-15. https://doi.org/10.1590/S0074-02762006000800014
PMid:17293987
20. Huerre MR, Lan N, Marianneau P, Hue N, Khun H, Hung NT, *et al*. Liver histopathology and biological correlates in five cases of fatal dengue fever in vietnamese children. Virchows Arch. 2001;438(2):107-15. https://doi.org/10.1007/s004280000329
PMid:11253111
21. Avirutnan P, Zhang L, Punyadee N, Manuyakorn A, Puttikhunt C, Kasinrerk W, *et al*. Secreted NS1 of dengue virus attaches to the surface of cells via interactions with heparan sulfate and chondroitin sulfate E. PLoS Pathog. 2007;3(11):e183. https://doi.org/10.1371/journal.ppat.0030183
PMid:18052531
22. Ageep K. Degree of liver injury in Dengue virus infection. J Gen Mol Virol. 2012;4(1):1-5. https://doi.org/10.5897/JGMV11.023
23. Chen TY, Chen DY, Wen HW, Ou JL, Chiou SS, Chen JM, *et al*. Inhibition of enveloped viruses Infectivity by Curcumin. PLoS One. 2013;8(5):e62482. https://doi.org/10.1371/journal.pone.0062482
PMid:23658730
24. Balasubramanian A, Pilankatta R, Teramoto T, Sajith A, Nwulia E, Kulkarni A, *et al*. Inhibition of dengue virus by curcuminoids. Antiviral Res. 2019;162:71-8. https://doi.org/10.1016/j.antiviral.2018.12.002
PMid:30529358
25. Olagnier D, Peri S, Steel C, Van Montfoort N, Chiang C, Beljanski V, *et al*. Cellular oxidative stress response controls the antiviral and apoptotic programs in dengue virus-infected dendritic cells. PLoS Pathog. 2014;10(12):e1004566. https://doi.org/10.1371/journal.ppat.1004566
PMid:25521078
26. Soundravally R, Sankar P, Bobby Z, Hoti SL. Oxidative stress in severe dengue viral infection : Association of thrombocytopenia with lipid peroxidation. Platelets. 2008;19(6):447-54. https://doi.org/10.1080/09537100802155284
PMid:18925513
27. Chen TH, Tang P, Yang CF, Kao LH, Lo YP, Chuang CK, *et al*. Antioxidant defense is one of the mechanisms by which mosquito cells survive dengue 2 viral infection. Virology. 2011;410(2):410-7. https://doi.org/10.1016/j.virol.2010.12.013
PMid:21216424
28. Lee GH, Lee HY, Choi MK, Chung HW, Kim SW, Chae HJ. Protective effect of *Curcuma longa* L. Extract on CCl 4induced acute hepatic stress. BMC Res Notes. 2017;10(1):77. https://doi.org/10.1186/s13104-017-2409-z
PMid:28143589
29. An S, Jang E, Lee JH. Preclinical evidence of *Curcuma longa* and its noncurcuminoid constituents against hepatobiliary diseases: A review. Evid Based Complement Alternat Med. 2020;2020:8761435. https://doi.org/10.1155/2020/8761435
PMid:32802138
30. Xu J, Fu Y, Chen A. Activation of peroxisome proliferator-activated receptor-gamma contributes to the inhibitory effects of curcumin on rat hepatic stellate cell growth. Am J Physiol Gastrointest Liver Physiol. 2003;285(1):G20-30. https://doi.org/10.1152/ajpgi.00474.2002
PMid:12660143
31. Fu Y, Zheng S, Lin J, Ryerse J, Chen A. Curcumin protects the rat liver from CCl4-Caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. Mol Pharmacol. 2008;73(2):399-409. https://doi.org/10.1124/mol.107.039818.genesis
PMid:18006644
32. Lee LK, Gan VC, Lee VJ, Tan AS, Leo YS, Lye DC. Clinical relevance and discriminatory value of elevated liver aminotransferase levels for dengue severity. PLoS Negl Trop Dis. 2012;6(6):e1676. https://doi.org/10.1371/journal.pntd.0001676
PMid:22679523
33. Sani SS, Han WH, Bujang MA, Ding HJ, Ng KL, Shariffuddin MA. Evaluation of creatine kinase and liver enzymes in identification of severe dengue. BMC Infect Dis. 2017;17(1):505. https://doi.org/10.1186/s12879-017-2601-8
PMid:28732476
34. Lee YS, Yoon SK, Chung ES, Bae SH, Choi JY, Han JY, *et al*. The relationship of histologic activity to serum ALT, HCV genotype and HCV RNA titers in chronic hepatitis C. J Korean Med Sci. 2001;16(5):585-91. https://doi.org/10.3346/jkms.2001.16.5.585
PMid:11641527
35. Xing YF, Zhou DQ, He JS, Wei CS, Zhong WC, Han ZY, *et al*. Clinical and histopathological features of chronic hepatitis B virus infected patients with high HBV-DNA viral load and normal alanine aminotransferase level: A multicentre-based study in China. PLoS One. 2018;13(9):e0203220. https://doi.org/10.1371/journal.pone.0203220
PMid:30180183
36. Paes MV, Pinhão AT, Barreto DF, Costa SM, Oliveira MP, Nogueira AC, *et al*. Liver injury and viremia in mice infected with dengue-2 virus. Virology. 2005;338(2):236-46. https://doi.org/10.1016/j.virol.2005.04.042
PMid:15961136