Influence of Process Methods on the Hepatoprotective Effect of Curcumin Analogs Synthesized from Culilawan Oil in Mice (*Mus musculus* L.) with CCl_4 Induced Liver Damage

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ABSTRACT. One of the downstream products which can be synthesized from culilawan oil is an analog curcumin compound (AKS) with a dioxolane ring. AKS products can be synthesized using conventional and microwave methods. The method of synthesis can influence physical properties, compound geometry, and pharmacological effects. The purpose of this study was to determine the effect of the processing method on the hepatoprotective ability of AKS, and to determine a protective dose. AKS was synthesized using insulated safrole compounds from Lawang oil and involved isomerization, oxidation, and aldol condensation of curcumin analogues. At the final stage of the analog curcumin synthesis process, 2 different methods were employed: the conventional method heated the chemical in a water bath at 30 °C for 3 hours, the microwave method heated the chemical using 140 watts of power for 2 minutes. Analogs were tested in vivo in mice (*Mus musculus* L.) with CCl_4 induced liver damage. Hepatoprotective efficacy of AKS products processed by the conventional method and the microwave method were compared using histology and liver enzyme (AST and ALT) assessment. Animals treated with conventionally produced AKS products had lower AST and lower ALT levels—and fewer histological signs of liver damage at a lower dose of AKS—than seen in either untreated animals or those treated with microwave produced AKS. Thus, products that are processed by conventional methods are more hepatoprotective.

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INTRODUCTION

Indonesia is well known for its wide variety of essential oil producing plants. One plant, *Curcuma longa* L., the source of turmeric, which contains the active compound curcumin, has been studied extensively. It has anti-diabetic (Kanitkar et al. 2008), anti-cancer, anti-inflammatory (Rao et al. 2013; Allegra et al. 2016), and hepatoprotective (Maiti et al. 2006; Mehta et al. 2012) activity. Curcumin appears to be a chemopreventative compound, slowing the process of carcinogenesis (Johnson and Mukhtar 2007), presumably by acting as an antioxidant.

Hepatoprotectors protect and repair damage to liver cells (Khan et al. 2012) and have been widely used to treat liver damage via their antioxidant actions. Known natural hepatoprotectors include gotu kola (Tang et al. 2012), curcumin, and curcumin analog compounds (AKS) (Kapelle and Manalu 2018). Analog compounds are those that have similar structure and the possibility of similar or improved pharmacological properties when compared to the parent compound (Yang et al. 2013).

Culilawan (*Cinnamomum culilawan* Blume) is an example of a plant with potential chemoprotective and hepatoprotective properties using the same mechanisms as curcumin. It grows in eastern Indonesia (especially Maluku and Papua) and is the source of culilawan oil, which contains a secondary metabolite with great pharmacological potential and the ability to be converted into derivative products. Culilawan oil, obtained from distillation of the bark with a yield of 0.94% (Kapelle et al. 2016), has 2 main components, eugenol and safrole. Safrole has an active dioxolane ring that can be used as a precursor for synthetic drugs. Compounds that have a dioxolane ring have a pair of free electrons that can resonate in the ring and capture free radicals.

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One of the downstream products that can be synthesized from culilawan oil is the curcumin analog compound (AKS) {symmetrical curcumin analog (1,5-bis-benzo [1,3] dioxol-5-yl-penta-1,4-dien-3- one)} (Kapelle et al. 2015a). This particular curcumin analog compound expresses cytotoxic activity in breast cancer cell cultures T47D (Kapelle et al. 2015b) and has demonstrated hepatoprotective effects (Kapelle and Manalu 2018). It can be produced both by conventional distillation and microwave methodology.

Carbon tetrachloride (CCl_4) is a hepatotoxic compound that is widely used in liver damage models (Khan et al. 2012). Liver damage can be measured in vivo by analysis of blood levels of liver enzymes (Khan et al. 2012). Common indicators of liver injury include a rise in serum alanine transaminase (ALT) and aspartate transaminase (AST). Liver injury may also be associated with a lipid peroxidation reaction that causes malondialdehyde (MDA) levels to increase and glutathione (GSH) levels to decrease with subsequent hepatocyte degradation (Mansour et al. 2006). In the early stages of liver damage, hydropic degeneration is evident histologically, followed by fatty degeneration and cell death (or necrosis) (Weber et al. 2003).

In this study, liver damage was induced in male mice* (Mus musculus *L.*) *using carbon tetrachloride (CCl_4). Subsequently, curcumin analog compounds from culilawan oil (AKS-k from the conventional method and AKS-m from the microwave method), were administered to assess hepatoprotective ability in this mouse model.

METHODS AND MATERIALS

Sample Preparation and Characterization

The treatments in this study were turmeric from Maluku and curcumin analog compounds synthesized from culilawan oil (distillation products of culilawan bark from Maluku). Curcumin was isolated from turmeric by ethanol extraction (Yadav et al. 2017). For curcumin analog compounds (AKS-k and AKS-m) the protocol from Kapelle et al. (2015a) was followed using Lawang oil base material. The process begins with isolating safrole from Lawang oil, isomerization of isosafrole, oxidation of isosafrole, and finally an aldol condensation process. The final stages of the synthesis of curcumin analogs used 2 different methods: a conventional method with process conditions at 30 °C for 3 hours to produce AKS-k, and a microwave method with 140 watts of power for 2 minutes to produce AKS-m. The analog curcumin products (AKS-k and AKS-m) produced were purified and crystallized using methanol solvents and analyzed using liquid chromatography–mass spectrometry (LCMS). Turmeric extracts (TM) were obtained using a maceration method with ethanol.

In-vivo Procedures

Three-week-old mice (*Mus musculus* L.), balb/c strain, (n = 80) were divided into 10 equal groups (n = 8) according to Table 1. Mice were acclimatized for 7 days in a room with a 12-hour cycle (light/dark), humidity at 70% ± 2%, and temperature of 22 °C ± 2 °C. On day 7 (immediately following the acclimatization period), mice were weighed and 0.02 ml of CCl_4/200 g body weight was dissolved in coconut oil and administered orally to all treatment groups except the normal control (MN) group. On day 14, treatments were administered to all groups according to Table 1.

Histopathology

Experimental mice were sacrificed by cervical dislocation on days 7, 14, 17, 21, and 29. Livers were photographed in situ and collected at necropsy. Livers were fixed in 10% neutral-buffered formalin (NBF) solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Histopathology parameters observed were liver pathology which included cell ballooning, steatosis, and presence of inflammatory cells.

Serum Enzyme Measurement

Blood was collected from mice through the orbital vein using a capillary tube (Superior Marienfeld®) on days 7, 14, 17, 21, and 29, and stored in Eppendorf Tubes™. Blood was kept refrigerated for a few hours (4 °C) before being processed, then centrifuged (Hettich® MIKRO 22 R centrifuge) at a speed of 10,000 rpm for 10 minutes at 4 °C to obtain serum. Serum AST (serum glutamic oxaloacetic transaminase) and ALT (glutamic pyruvate transaminase serum) were measured using DiaSys® reagents and a UV-Vis spectrophotometer (GENESYS™ 10uv).

Table 1
Group division and treatment of mice

Group	CCl_4 (day 7)	Treatment (day 14)	Treatment dose mg/200 g body weight	Notes
MA1	Yes	AKS-k	13	---
MA2	Yes	AKS-k	26	---
MA3	Yes	AKS-k	52	---
MB1	Yes	AKS-m	13	---
MB2	Yes	AKS-m	26	---
MB3	Yes	AKS-m	52	---
M+1	Yes	Turmeric extract	130	Positive control
M+2	Yes	Hepa-Q*	60	Positive control
M-1	Yes	No treatment	---	Negative control
MN	No	No treatment	---	Normal control

* Herbal liver-support capsules.

RESULTS AND DISCUSSION

Chemical Analysis of Analogs

LCMS analysis of curcumin analog samples—obtained via both conventional and microwave methods—produced 1 product at $t_R = 3.38$ minutes with a molecular mass of 322 g/mol. Further analysis of these products using a Fourier-transform infrared spectroscopy (FTIR) spectrophotometer (Fig. 1) showed that the compounds AKS-k and AKS-m are almost identical (Table 2).

The shape of the fingerprints of the AKS-k and AKS-m products had sufficient similarity that the infrared spectrum peaks could be considered virtually identical. Thus, the species produced by both microwave and conventional methods were analogous to curcumin with molecular weights of 322 g/mol and had chemical structures similar or identical to that of other curcumin analogs (Fig. 2). Based on the chemical structure in Fig. 2, several isomers could be obtained as products. These isomers would appear identical by FTIR or LCMS, but have potentially different hepatoprotective activity. For example, Kapelle et al. (2015b) showed—in a study of breast anticancer activity in vitro in T47D breast cancer cells—that the AKS-m product provided a better IC_{50} value than did AKS-k.

Body Weight

The effect of treatment on body weight, which was measured on days 17, 21, and 29, was calculated as a percentage of post-treatment body weight compared to the initial pre-treatment body weight (day 14). While weight gain differed significantly between groups (ANOVA $F_{(9,20)} = 18.292$; $p = 0.001$), an increase in body weight was seen in all groups (as expected for mice growing from juveniles to adults). This indicated that administration of curcumin analog compounds to mice with CCl_4-induced liver damage improved their ability to gain weight (Fig. 3a).

The greatest percentage of weight gain was observed in high-dose AKS-m (MB3) treated animals, which was significantly more effective than that seen in the Hepa-Q positive control group (M+2) ($p = 0.001$). Furthermore, by day 29 the AKS-m (MB) analogs were significantly ($p = 0.001$) more effective than AKS-k (MA) analogs in increasing weight gain (Fig. 3b), as administration of AKS-k analogs had an effect on body weight that was inversely proportional to dose.

Liver Enzymes

CCl_4 administration was associated with increased serum ALT and AST levels in all groups by day 17 as compared to the normal untreated control group MN. In the negative control group M-1 (administered CCl_4 but not treated with any compound), ALT and AST values remained elevated through day 29.

In all treated groups of mice, AST values decreased significantly from day 17 to days 21 and 29, suggesting an improvement in liver function ($F_{(8,18)} = 2.611$; $p = 0.049$) (Fig. 4a). Among mice treated with curcumin analogs, the lowest AST levels were

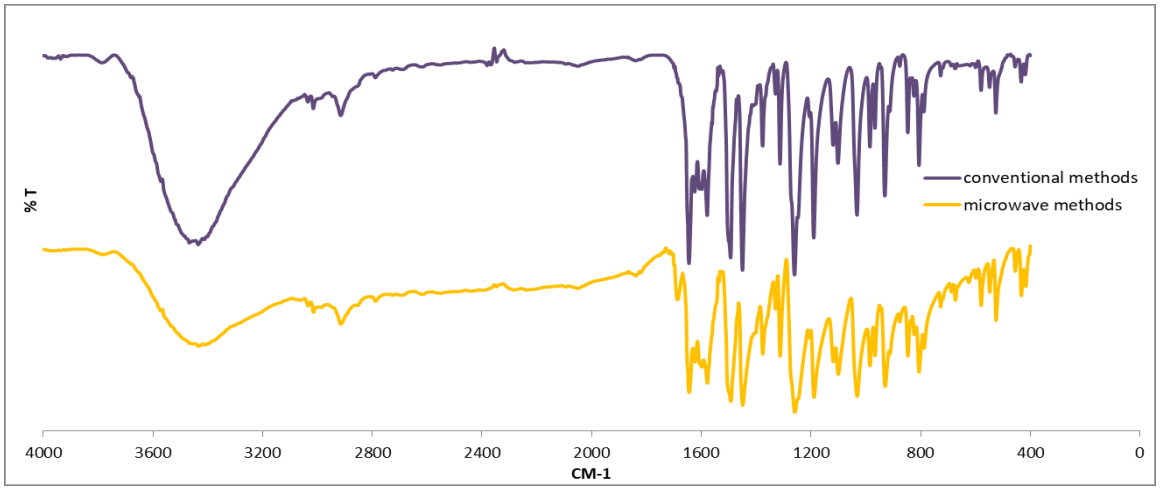


FIGURE 1. FTIR spectrum of curcumin analog compounds (AKS)

Table 2
Results of FTIR spectrum analysis

Functional groups	Wavelength	
	Product AKS-k	Product AKS-m
C=C stretching aromatic	1607 cm^{-1} dan 1493 cm^{-1}	1598 cm^{-1} dan 1492 cm^{-1}
C=C stretching aliphatic	2915 cm^{-1}	2914 cm^{-1}
C-O-C ether	1259 cm^{-1}	1258 cm^{-1}
$-CH_3$ bending	2915 cm^{-1}	2914 cm^{-1}
$-CH_2$- bending methylene	1449 cm^{-1}	1448 cm^{-1}
Carbonyl	1645 cm^{-1}	1688 cm^{-1}

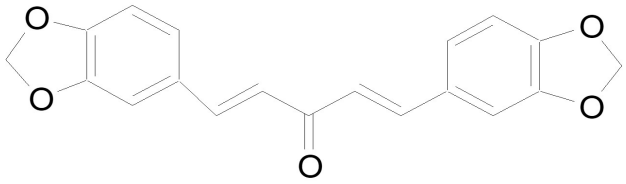


FIGURE 2. Chemical structure of curcumin analogs

observed in the MA1 group (AKS-k dose 13 mg/200 g body weight) and were statistically equivalent to those in the positive control group M+2 (Hepa-Q drug 60 mg/200 g body weight) ($p = 0.668$). MA1 was not significantly more effective in lowering AST levels than MA2 ($p = 0.989$).

AST values on day 29 are shown as a percentage of pretreatment values in Fig. 4b. Mice in the MA3 group had similar values ($p = 1.000$) to those in the Hepa-Q positive control group (M+2). The MA1 group (AKS-k dose 13 mg/200 g body weight) had the lowest AST values on day 29 and differed significantly from all other groups.

Changes in ALT were generally less dramatic than those for AST. ALT levels decreased in all treated groups of mice as measured on days 17, 21, and 29 (Fig. 5a) and significant differences were observed in efficacy ($F_{(8,18)} = 7.136$; $p = 0.001$). Treatment group MB3 (AKS-m dose 52 mg/200 g body weight) had significantly lower ALT values than those of the positive control groups (M+1 and M+2, $p = 0.059$ and $p = 0.036$, respectively). Treatment group MA1 (AKS-k dose 13 mg/200 g body weight) had significantly lower ALT values than those of the positive control groups (M+1 and M+2, $p = 0.010$ and $p = 0.185$, respectively). However, the MA1

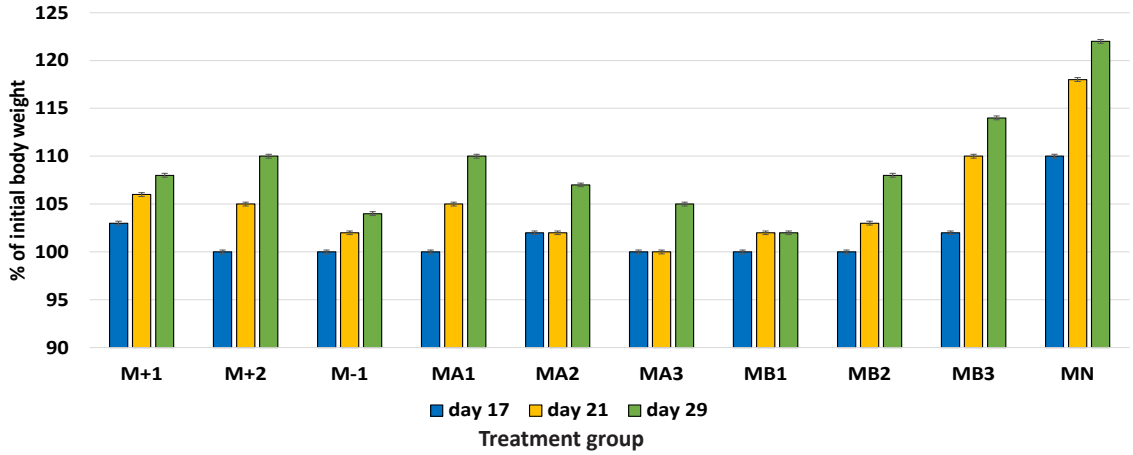


FIGURE 3a. Percentage of the initial pre-treatment body weight (day 14) for each treatment group on days 17 (blue), 21 (yellow), and 29 (green). Standard deviation bars show minimal variation between mice.

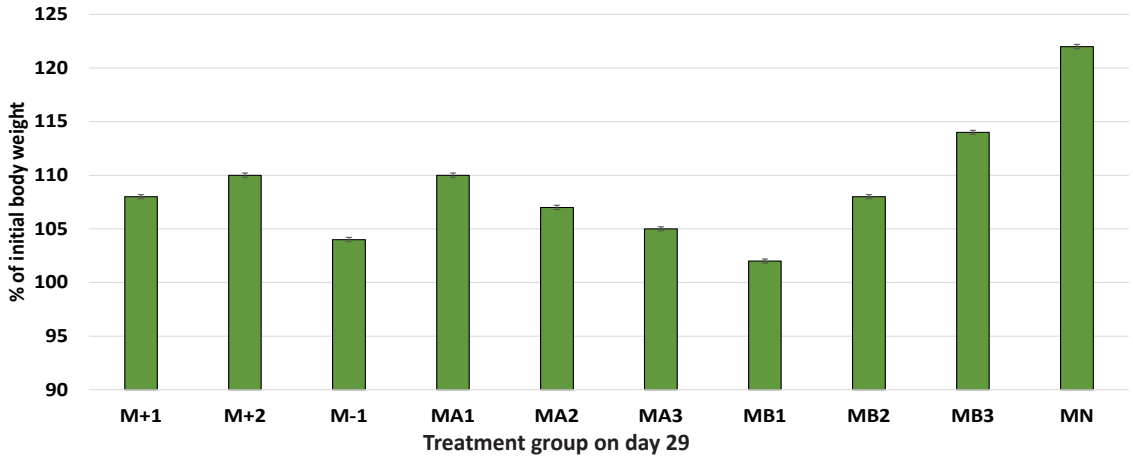


FIGURE 3b. Percentage weight gain between day 14 and day 29 (ANOVA $F_{(9,19)} = 38933.33$; $p = 0.001$)

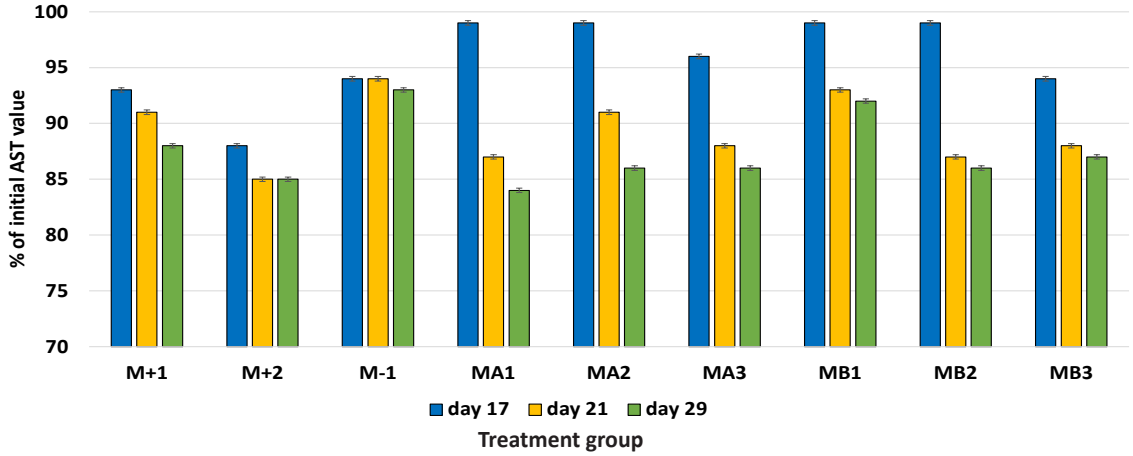


FIGURE 4a. Mean percentage of initial pretreatment AST values for each treatment group as measured on days 17 (blue), 21 (yellow), and 29 (green). Standard deviation bars show minimal variation between mice.

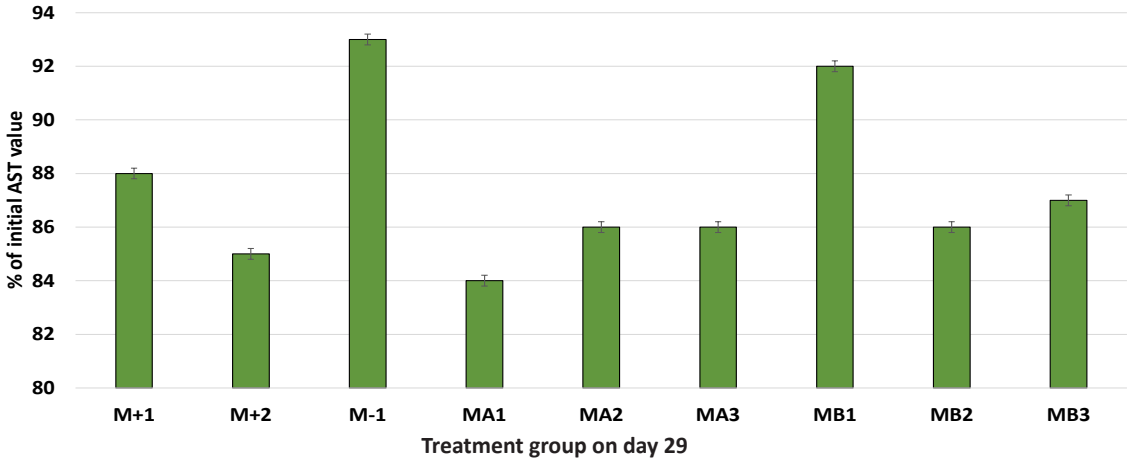


FIGURE 4b. Mean percentage of initial pretreatment AST values measured on day 29 (ANOVA $F_{(8,17)} = 11433.33$; $p = 0.001$)

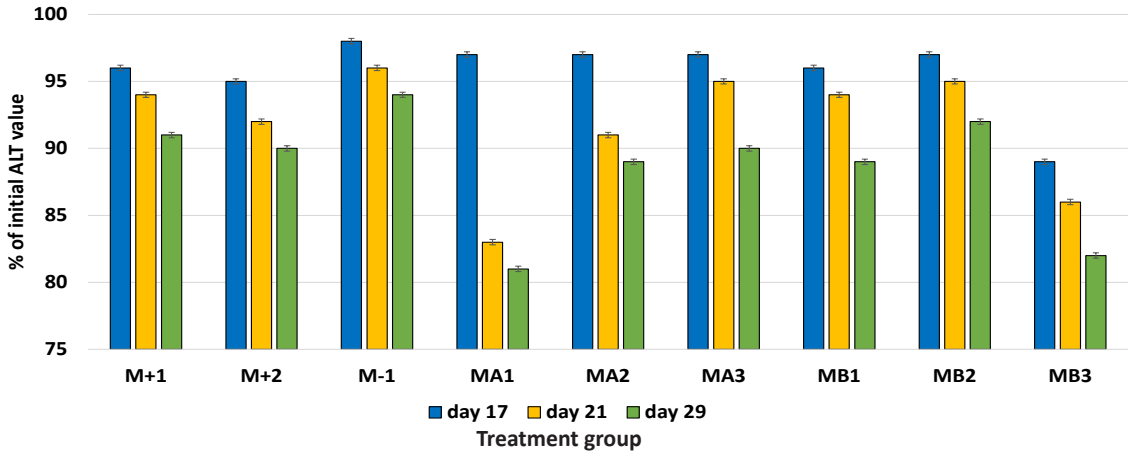


FIGURE 5a. Mean percentage of initial pretreatment ALT values for each treatment group as measured on days 17 (blue), 21 (yellow), and 29 (green). Standard deviation bars show minimal variation between mice.

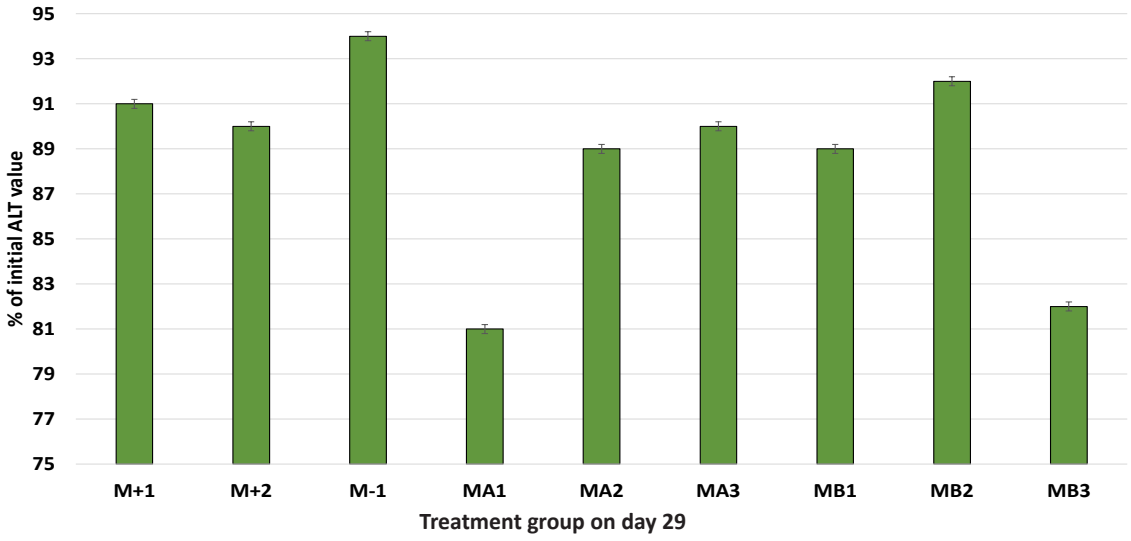


FIGURE 5b. Mean percentage of initial pretreatment ALT values measured on day 29 (ANOVA $F_{(8,17)} = 22800$; $p = 0.001$)

group (AKS-k dose 13 mg/200 g body weight) had similar ALT values to the MB3 group (AKS-m dose 52 mg/200 g body weight) ($p = 0.989$).

ALT values on day 29 are shown as a percentage of pretreatment values in Fig. 5b. Mice in the MA3 group had similar values ($p = 1.000$) to those in the Hepa-Q positive control group (M+2). Group MA2 had similar ALT values as group MB1 ($p = 1.000$). The MA1 group (AKS-k dose 13 mg/200 g body weight) had the lowest ALT values on day 29 and differed significantly from all groups.

In all treated groups of mice, improvement in health of test animals over time was evidenced by a decrease in AST values by day 21 and (except for the M+2 group) further decrease through day 29. A lesser effect with a similar trend was noted for ALT where the predominant pattern observed was that ALT values for all AKS compounds were similar or better compared to both positive controls.

AST and ALT levels for mice receiving the same dose level of AKS-m (produced by microwave) differed from those receiving the AKS-k (derived from conventional methods), with AKS-k being effective at a lower dose. From this, it can be inferred that the synthesis process influences the structure and physical-chemical properties of the compound. The geometry of the compound can have an effect on the antioxidant properties of the compound and morphological changes seen in response to CCl_4 in the liver.

Liver Morphology

Administration of CCl_4 led to expected progressive morphological changes in hepatocytes of untreated mice (Fig. 6).

Normal hepatocytes have a regular, lobular arrangement with central veins which are uniform and empty. CCl_4 induced vacuolar and microvacuolar cytoplasmic changes in hepatocytes. In some cases fatty debris was seen in the central venous region, and swollen hepatocytes caused narrowing of the sinusoids (Fig. 6). In contrast, microscopic images of livers of mice given various treatments are shown in Figs. 7, 8, and 9.

Livers of positive control mice, treated with turmeric extracts in group M+1 (turmeric extract 130 mg/200 g body weight), had debris in the central vein and fatty tissue in the hepatocytes despite using a higher dose than that needed for AKS; however, reparation of microscopic liver changes was evident on day 29 (Fig. 9). Morphological effects of synthetic curcumin analog compounds were most obvious in MA3 group mice (AKS-k dose 52 mg/200 g body weight) on day 29, where the central vein was clear of cellular debris and there was no evidence of hepatocellular degeneration or necrosis (Fig. 7). At lower doses of both curcumin analog compounds (13 mg/200 g body weight), some hepatocytes remained fatty, and sinusoids or central veins were compressed or contained debris (Figs. 7 and 8); however, fewer microscopic lesions were observed than in untreated

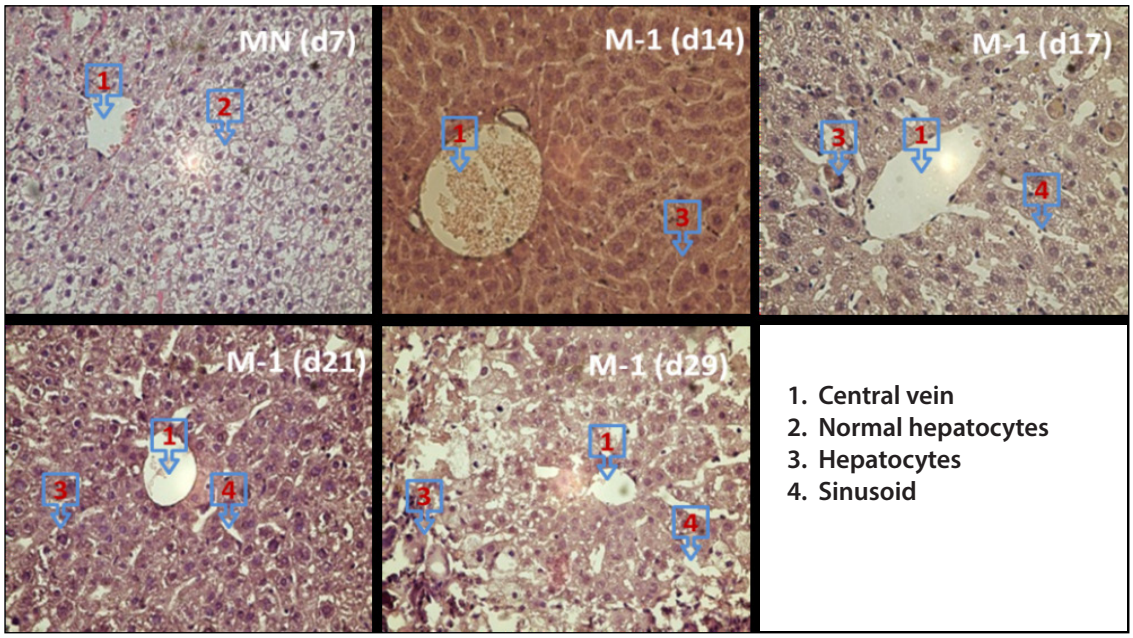
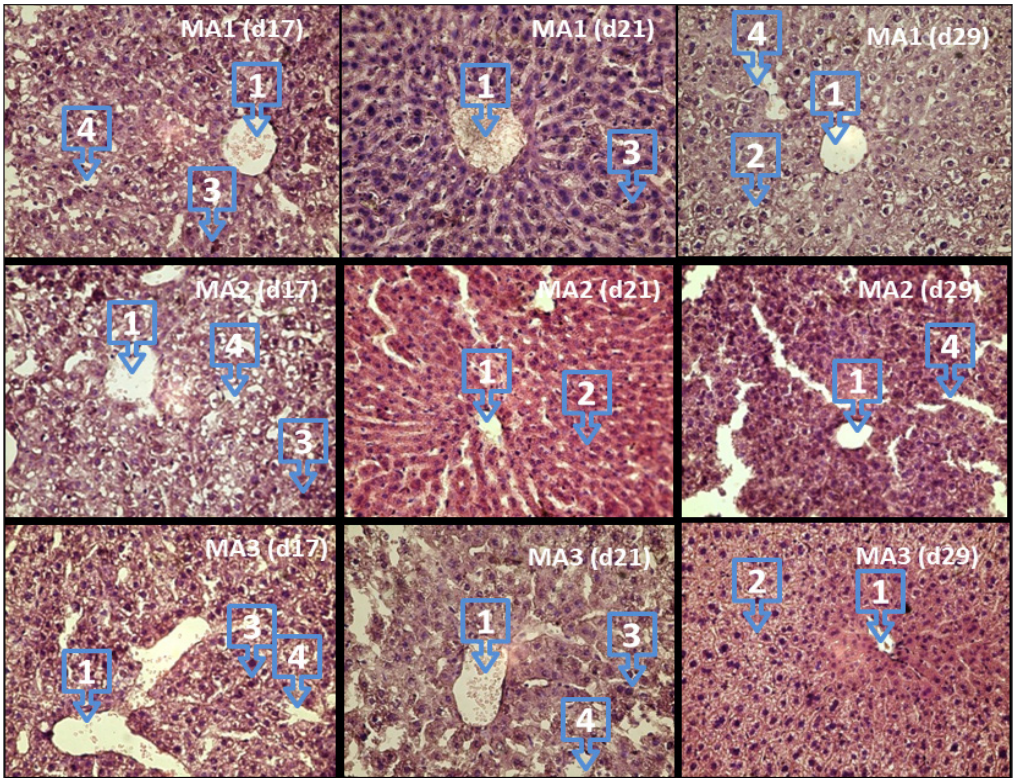
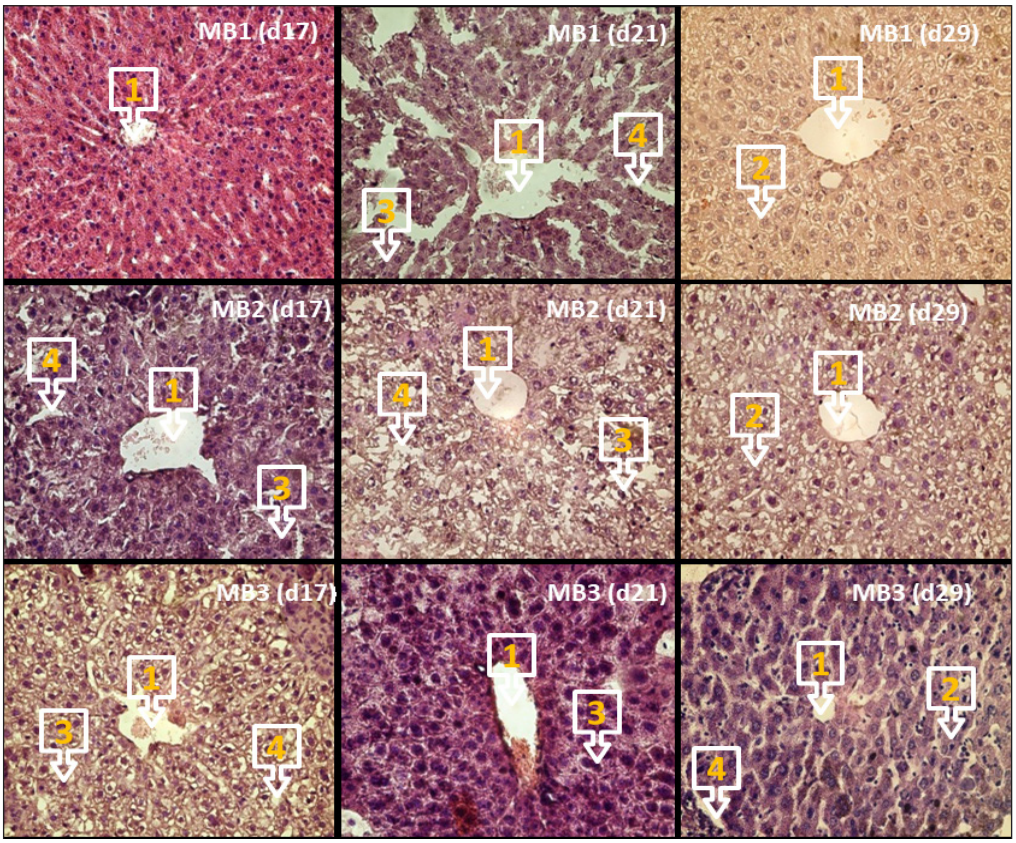


FIGURE 6. Microscopic images of normal liver structure of the normal control mice (MN) and the liver in mice with damage induced by CCl_4 (M-1). H&E method, magnification 400×.



1. Central vein; 2. Tissue repair; 3. Hepatocytes; 4. Sinusoid

FIGURE 7. Microscopic images of mouse liver structure for 3 doses of AKS-k treatment on days (d) 17, 21, and 29. H&E method, magnification 400×.



1. Central vein; 2. Tissue repair; 3. Hepatocytes; 4. Sinusoid

FIGURE 8. Microscopic images of mouse liver structure for 3 doses of AKS-m treatment on days (d) 17, 21, and 29. H&E method, magnification 400×.

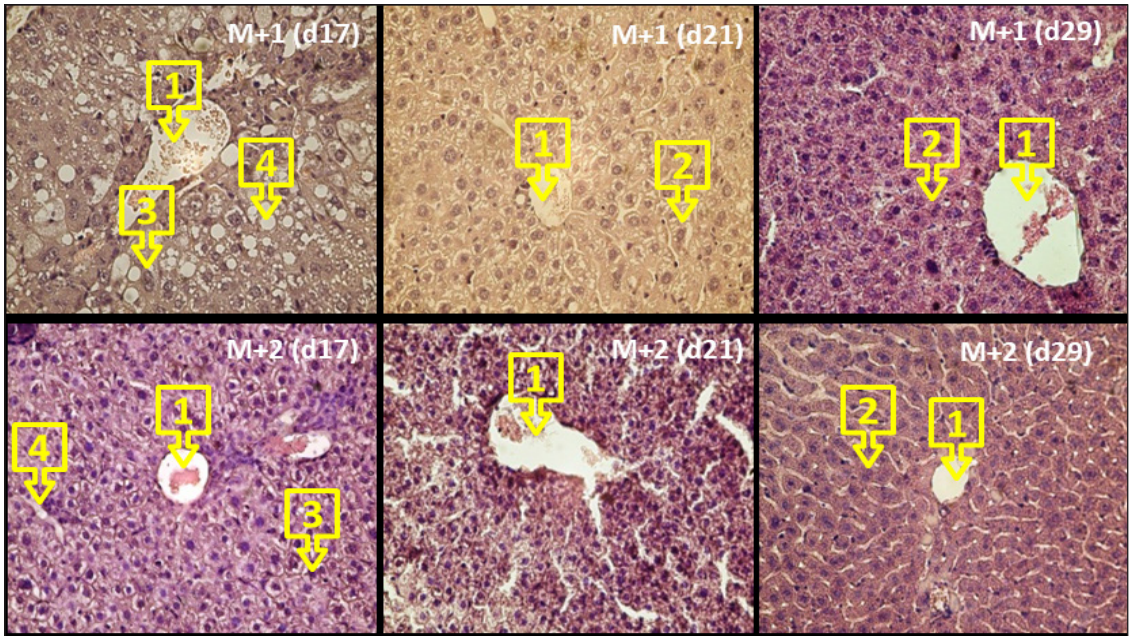


FIGURE 9. Microscopic images of mouse liver structure for the 2 positive control groups—Hepa-Q drug (M+2) and turmeric extract (M+1)—on days (d) 17, 21, and 29. H&E method, magnification 400×.

controls. Continued improvement was seen in mice across all synthetic curcumin analog treatment groups terminated on days 21 and 29. Thus, curcumin analogs have the potential to be hepatoprotectors because they can reduce microscopic liver lesions at lower doses than those needed for turmeric effects.

Both analogs were similar in that longer duration of treatment was associated with more clearing of the central veins and absence of fatty hepatocytes, indicating damage repair. Histological differences for both analogs were related to dose levels. In MA groups (AKS-k), even at large doses, the cell damage remains, sinusoids still look wide. By contrast, in high dose MB group (AKS-m) treated mice, the central vein looks cleaner and fatty deposits have diminished.

The effectiveness of the synthetic curcumin analog compounds in hepatoprotection can be attributed to the active group in the compound. Curcumin analogs have negatively charged alkene, benzene, carbonyl, and ether groups, making it possible to capture free radicals and convert them into neutral molecules (Fig. 2). If free radicals are captured, then further cellular damage can be averted and natural processes of healing can occur. ***This study demonstrated hepatic repair following curcumin analog treatment.*** This study's findings support those of Ruhe et al. (2001) that curcumin analog products led to improved serum liver enzyme values and decreased hepatocellular degeneration and necrosis.

CONCLUSION

Processing method affects the hepatoprotective ability of curcumin analog compounds, produced from culilawan oil, in mice with CCl_4 induced liver damage. Following a 7-day acclimation period, mice received CCl_4 to induce liver damage. After 7 additional days mice received treatment. Mice which received AKS-k treatment at a dose of 13 mg/200 g body weight exhibited improved weight gain, lowered AST and ALT levels, and repaired microscopic liver lesions. These results rivaled or exceeded the effects of the known hepatoprotectors turmeric and Hepa-Q. However, effects at higher AKS-k doses were often inferior. Higher doses of the AKS-m product were required to achieve the same or better level of weight gain and reduction in liver enzymes as seen with 13 mg/200 g body weight AKS-k. But effects of AKS-m more closely followed a dose-response curve. Therefore, ***curcumin analog compounds that are processed by a conventional heating method may be more hepatoprotective—and at a lower dose—than are products processed by the microwave method, presumably due to differences in compound geometry.***

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LITERATURE CITED

Allegra A, Innao V, Russo S, Gerace D, Alonci A, Musolino C. 2016. Anticancer activity of curcumin and its analogues: preclinical and clinical studies. Cancer Invest. 35(1)1-22. https://doi.org/10.1080/07357907.2016.1247166

Johnson JJ, Mukhtar H. 2007. Curcumin for chemoprevention of colon cancer. Cancer Lett. 255(2):170-181. https://doi.org/10.1016/j.canlet.2007.03.005

Kapelle IBD, Manalu W. 2018. The preventive effect of curcumin analogs (AKS-k) from cullilawan oil as hepatoprotector in rats (*Rattus norvegicus* L.) induced by CCl_4. Int J Prog Sci Tech (IJPSAT). 11(2):81-85.

Kapelle IBD, Irawadi TT, Rusli MS, Mangunwidjaja D, Mas'ud ZA. 2016. Rekayasa proses sintesis piperonal kulit lawang (*Cinnamomum culilawan*, Blume) sebagai prekursor obet kanker. Jurnal Penelitian Hasil Hutan [Journal of Forest Products Research]. Kementrian Lungkungan hidup dan kehutanan, Puslitbang. 34(3):217-229. https://doi.org/10.20886/jphh.2016.34.3.217-229

Kapelle IBD, Irawadi TT, Rusli MS, Mangunwidjaja D, Mas'ud ZA. 2015a. Synthesis of new curcumin analogues from Kulit Lawang oils using the conventional method and microwave. Sci J Chem. 3(3):50-56. https://doi.org/10.11648/j.sjc.20150303.13

Kapelle IBD, Irawadi TT, Rusli MS, Mangunwidjaja D, Mas'ud ZA. 2015b. The influence of synthesis methods against anti-cancer activity of curcumin analogs. Cancer Res J. 3(4):68-75. https://doi.org/10.11648/j.crj.20150304.12

Kanitkar M, Gokhale K, Galande S, Bhonde RR. 2008. Novel role of curcumin in the prevention of cytokine-induced islet death in vitro and diabetogenesis in vivo. Brit J Pharmacol. 155:702-713. https://doi.org/10.1038/bjp.2008.311

Khan RA, Khan MR, Ahmed M, Sahreen S, Shah NA, Shah MS, Bokhari J, Rashid U, Ahmad B, Jan S. 2012. Hepatoprotection with a chloroform extract of *Launaea procumbens* against CCl_4-induced injuries in rats. BMC Complem Altern M. 12 Article No 114. https://doi.org/10.1186/1472-6882-12-114

Mansour HH, Hafez HF, Fahmy NM. 2006. Silymarin modulates cisplatin-induced oxidative stress and hepatotoxicity in rats. J Biochem Mol Biol. 39(6):656-661.

Maiti K, Mukherjee K, Gantait A, Saha BP, Mukhrejee PK. 2006. Curcumin-phospolipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharmaceut. 330(1-2):155-163. https://doi.org/10.1016/j.ijpharm.2006.09.025

Mehta A, Kaur G, Chintamaneni M. 2012. Piperine and quercetin enhances antioxidant and hepatoprotective effect of curcumin in paracetamol induced oxidative stress. Int J Pharmacol. 8(2):101-107. https://doi.org/10.3923/ijp.2012.101.107

RaoTS, Basu N, Siddiqui HH. 2013. Anti-inflammatory activity of curcumin analogues. Indian J Med Res. 137(4):841-845

Ruhe RC, McDonald RB. 2001. Use of antioxidant nutrients in the prevention and treatment of type 2 diabetes. J Am Coll Nutr. 20(Suppl 5):363S-369S. https://doi.org/10.1080/07315724.2001.10719169

Tang LX, He RH, Yang G, Tan JJ, Zhou L, Meng XM, Huang XR, Lan HY. 2012. Asiatic acid inhibits liver fibrosis by blocking TGF-beta/Smad signaling in vivo and in vitro. Plos One. 7(2):e31350. https://doi.org/10.1371/journal.pone.0031350

Weber LW, Boll M, Stampfl A. 2003. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol. 33(2):105-136. https://doi.org/10.1080/713611034

Yadav DK, Sharma K, Dutta A, Kundu A, Awasthi A, Goon A, Banerjee K, Saha S. 2017. Purity evaluation of curcuminoids in the turmeric extract obtained by accelerated solvent extraction. J AOAC Int. 100(3):586-591. https://doi.org/10.5740/jaoacint.17-0057

Yang CH, Yue J, Sims M, Pfeffer LM. 2013. The curcumin analog EF24 targets NF-κB and miRNA-21, and has potent anticancer activity in vitro and in vivo. Plos One. 8(8): e71130. https://doi.org/10.1371/journal.pone.0071130