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Effect of Process Methods on the Preventive Effects of Asymmetric Curcumin Analogues as Hepatoprotectors in Male Mice (Mus Musculus L.) Induced by CCl_4

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Abstract

One of the synthetic products obtained from culilawan oil with a very active dioxolane ring is asymmetric curcumin analogue compound (AKAS). AKAS products can be synthesized use conventional methods and the microwave method. The synthesis process method can influence physical properties and geometry of compounds as well as pharmacological effects. The purpose of this study was to determine the effect of the process method on the preventive effect of asymmetric curcumin analogues as hepatoprotectors in male mice (Musmusculus L.) induced by CCl_4 and to know the effective dose as hepatoprotector. The method used is in vivo using mice and as a comparison ingredient of turmeric extract (curcumin) and Heap Q. The parameters observed are the SGOT and SGPT values and histopathological analysis using the H&E method. The results showed that the method for the synthesis process of asymmetric curcumin analog products from culilawan oil had an effect on the hepatoprotector effect. Products synthesized using the microwave method (AKAS-m) give better results when compared to conventional methods (AKAS-k) and the effective dose as a hepatorotector is AKAS-m dose 26 mg / 200g bw.

Keywords: asymmetric curcumin analogues (AKAS), in vivo, hepatoprotector

1. Introduction

Culilawan oil is an essential oil derived from distillation from Cinnamomum culilawan Blume with a yield of 0.94% (Kapelle et al. 2016). Culilawan oil contains safrol compound which has a very active dioxolane ring and can be used as a precursor of synthetic drug. Compounds derived from safrol that can be synthesized are symmetric curcumin analog compounds and asymmetric curcumin analogues (Kapelle et al. 2015a,b). The curcumin analog compound has been tested for cytotoxic activity for breast cancer cell cultures and method of the process also influences the activity (Kapelle et al.2015c) [4]. The drug nature is caused by itsfunctional group which act as antioxidant. Curcumin and analogue curcumin have similar structure and have the possibility of pharmacological properties or have better pharmacological properties (Yang et al.2015). Curcumin is a component of turmeric which has functions to restore the carcinogenic process (Johnson et al. 2007). Curcumin is also often used as hepatoprotector that can protect and repair

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damaged liver cells (Khan et al. 2012). The effectiveness of asymmetric curcumin analog compounds synthesized from cullilawang oil as a hepatoprotector needs to be further investigated.

Liver is an organ that is very susceptible to the influence of chemical compounds and often damaged due to the entry of toxic substances. Blood supply to the liver comes from the digestive tract, so the toxic substances absorbed by the intestine will be carried to liver through the portal vein. Toxic substances that enter the liver can cause various effects such as steatosis, necrosis, cholestasis and cirrhosis. Carbon tetrachloride (CCl_4) is a hepatotoxin that causes liver damage (Burt et al.2007). The purpose of this study was to determine the effect of the process method on the hepatoprotective effect of curcumin analogue products from mace oil to male mice (*Mus musculus L.*) induced by CCl_4 and to know the effective dose as hepatoprotector

2. Method

2.1 Materials

The materials used in this study were male mice (*Mus musculus L.*), *Sprague Dawley* (SD) strains, synthesized asymetric analogous curcumin (AKAS-k and AKAS-m), methanol, turmeric extract, ethanol, CCl_4, Heap-Q, buffer neutral formalin (BNF), hematoxylin-eosin dye, phophate buffer, trichloroatic acid (TCA) 10%, 1.1.3.3-tetrametoxipropane (TMP), Tris-HCl, BHT 5%, NMPI (N-metil-2-fenil-indol), concentrated HCl, reduced GSH, dTNB, and DiaSys® reagents.

2.2 Equipments

The equipment used in the study included cages, humidity measuring devices, thermometers, capillary pipes (marienfed), eppendorf tubes, centrifuges, micro pestles, homogenizers, vortices, incubators, and glassware, FTIR and LCMS.

2.3 Experimetal Procedures

Mice were acclimatized for 7 days in a room with a 12 hour cycle (light/dark), humidity 70% ± 2%, temperature 22 °C ± 2 °C. After being acclimatized, mice were grouped to ten groups with eight, respectively according to Table 1, and then treated for one week every day. On the following day CCl_4 was given and the parameters seen were animal body weight, blood biochemistry of SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvate Transaminase). The animal blood was taken and collected in eppendorf tube, then centrifuged (Hettich zentrifugen micro 22R) at a speed of 10000 rpm for 10 minutes at 4 °C to obtain blood serum.Biochemical measurements of blood using the DiaSys® reagent-kit and measured with UV-Vis spectrophotometer (Genesis 10uv). For histopathological analysis of the liver using hematoxylin-eosin (H&E) staining.Liver organ is fricated with formalin neutral buffer solution (BNF). Before staining, it is preceded by deparafinization process.

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Table 1.Group division and treatment of mice

Group	Treatment	Dose mg/200 g body weight	
MC1	AKAS-k	13	
MC2	AKAS-k	26	
MC3	AKAS-k	52	
MD1	AKAS-m	13	
MD2	AKAS-m	26	
MD3	AKAS-m	52	
M+1	Turmeric extract	130	Positive control
M+2	Heap-Q drug	60	Positive control
M-1	CCl_4	No treatment	Negative control
MN	No CCl_4	No treatment	Normal control

3. Results and Discussion

Asymmetric curcumin analogue treatment (AKAS) before CCl_4 was shown to be able to maintain the body weight of animal models. Sampling for 7 days showed weight gain, then the next day (H0) given CCl_4 for all groups shows weight loss. The presentation of changes in body weight after treatment is shown in Figure 1. Positive control of both turmeric (M+1) extract and heap Q (M+2) drugs increase during the process, and an upward trend is also found in the MD3 sample code. In contrast to samples with other treatments, visible weight loss is the same as negative control. Statistical analysis showed that there was no significant difference between each treatment for the presentation of body weight changes of animal models with sig values ($p = 0.126$). Weight loss occurs due to the entry of toxic substances into the body. The provision of CCl_4 that has done continuously could influence liver function. Asymmetric analogue curcumin products which were synthesized by the microwave (MD3) method at a dose of 52 mg / 200g bw gave better results when compared to products synthesized by conventional methods.

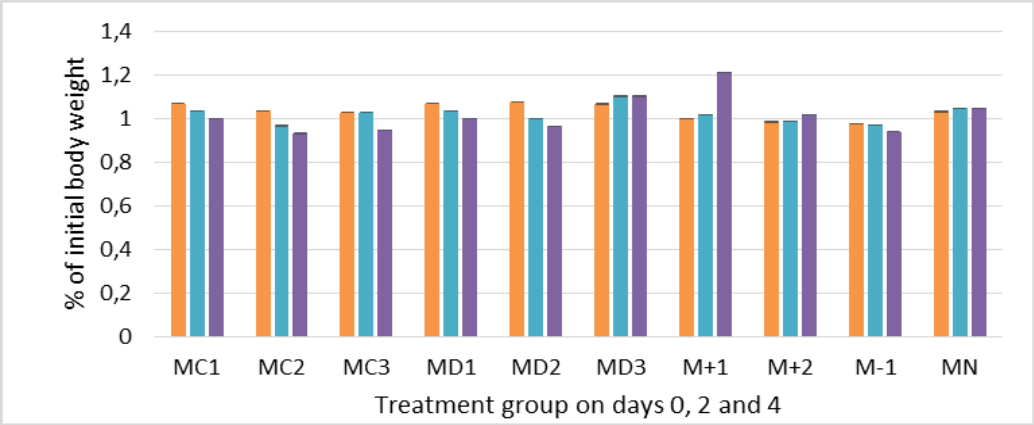

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Figure 1.Graphic representation of percentage of the initial body weight for each treatment group on Days 0(orange), 2 (blue) and 4 (purple).Standard deviation bars show minimal variation between mice.

AKAS treatment for 7 days affected SGOT levels after being given CCl_4. The presentation of changes in SGOT values after being given CCl_4 on days 0, 2 and 4 is shown in Figure 2. The trend of increasing SGOT levels as in negative control (M-1) is notoccur after being given AKAS treatment. Products that provide better SGOT change values when compared to other treatments are AKAS analogues which are processed using the microwave method at dose of 26 mg / 200 g bw. There was significant difference between the types of treatments and days of changes in the SGOT levels of animal models with sig values (p = 0.001).Post hoc analysis of the value changes in SGOT levels for the MD2 group significantly different from the other treatment groups (sig <0.05), but not significant from the positive control group.

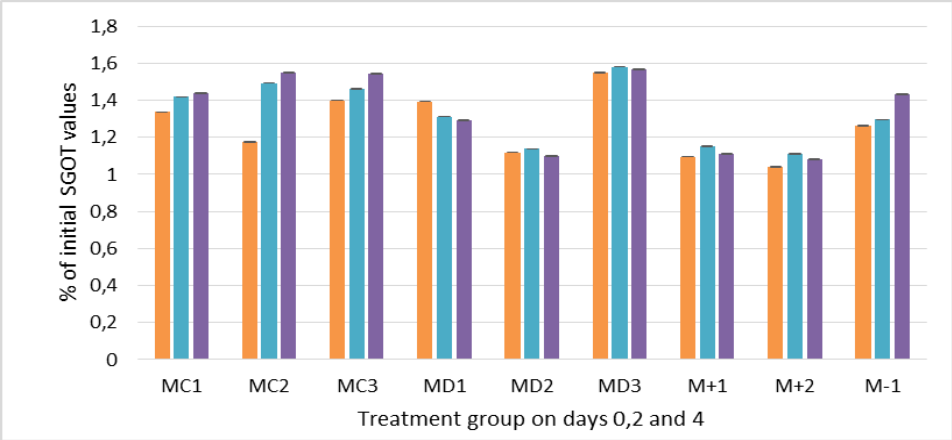

Figure 2.Graphic representation of the mean percentage of initial pretreatment SGOT Values for each treatment group as measured on Days 0 (orange), 2 (blue) and 4 (purple). Standard deviation bars show minimal variation between mice

Biochemical analysis of blood for SGPT values after being given CCl_4 on days 0, 2 and 4 is shown in Figure 3. The trend of increasing SGPT levels as in negative control (M-1) did not occur after AKAS treatment. The treatment group that gave the best SGPT change value was MD2, which is AKAS-m product with a dose of 26 mg / 200g bw. Statistical analysis showed that there was a significant difference between the types of treatments and days of changing SGPT levels with sig values (p = 0.001). Post hoc analysis the value of changes in SGOT levels for the MD2 group differed significantly from the M+2 positive control treatment group but did not differ significantly from the M+1 positive control group.

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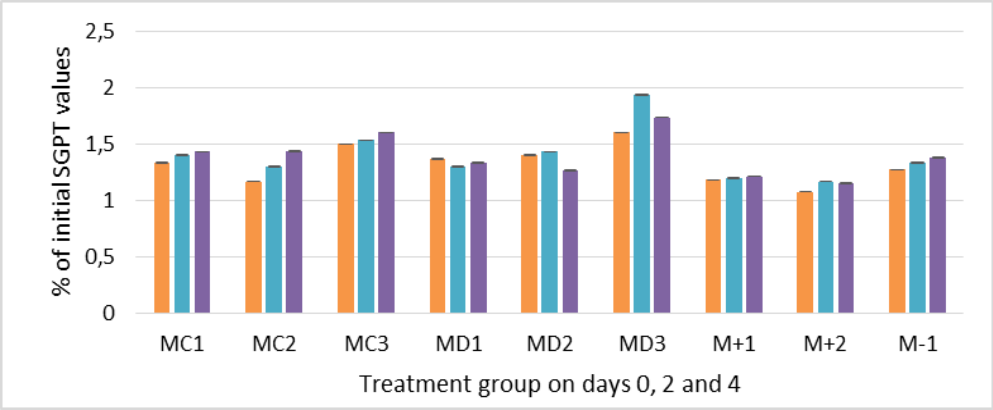

Figure 3.Graphic representation of the mean percentage of initial pretreatment SGPT Values for each treatment group as measured on Days 0 (orange), 2 (blue) and 4 (purple). Standard deviation bars show minimal variation between mice.

Biochemical analysis of blood can be supported by looking at liver cells damage with histopathological analysis. Microscopic features for normal liver cells in mice and negative control groups provide a difference in cell damage (Figure 4). Carbon tetrachloride (CCl_4) compounds are chemicals that are toxic and can cause liver damage in the form of degeneration or necrosis. In the initial stages of liver cell damage in the form of hydrophic degeneration and continued with fat degeneration, and cell death or necrosis occurs (Weber *et al*., 2003). Normal liver cells can be seen as a network structure of cells that are still regular because there is no damage due to the influence of substances or toxic substances. In contrast to cells that have been induced with CCl_4, microvescular and macroecular fatty acids were seen in the central venous region. Damage to liver cells is also marked by the presence of vacuoles due to swollen hepatocytes which causes the sinosiod to narrow. For normal cells, the central venous vein is clearly seen as a round and empty center.

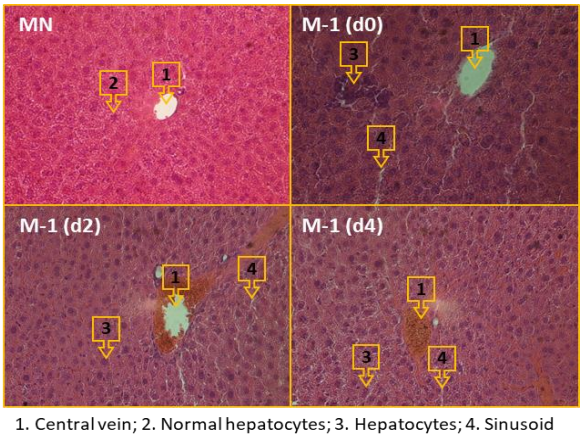

Figure 4. Microscopic images of normal liver structure of mice (MN) and liver structure induced by CCL_4 (M-1). H & E method, Magnification 400x

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The administration of asymmetric analogue curcumin analog products using the conventional method (AKAS-k) and the microwave method (AKAS-m) gives different microscopic picture for each dose and sampling time (Figure 5 and Figure 6). At the beginning of CCl_4 administration for day 0, there was less cell damage, as seen from the presence of a central vein that still appeared round and had not occurred. After days 2 and 4, there began to be visible liver cells damage from dilated sinusoids and more fat in the central venous region.

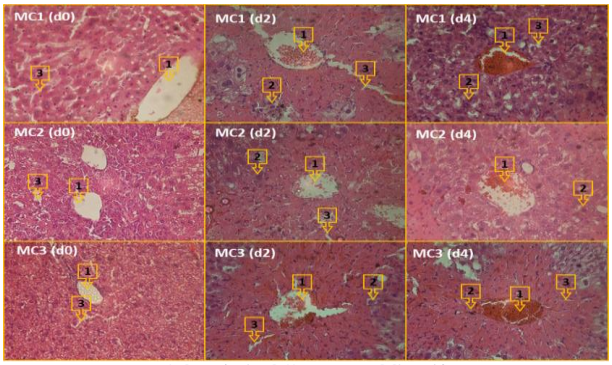

Figure 5. Microscopic images of mice liver structure for three doses of AKAS-k treatment. H & E method, Magnification 400x

From the microscopic picture of liver cells, it was shown that the dose of asymmetric analogue curcumin synthetic products which had the effect of preventing the deterioration of liver function was 26 mg / 200g bw for the MD2 group. This is in suitable with blood biochemical analysis data for SGOT and SGPT values. A central vein was seen clearing from fat for the 4th day after CCl_4 (Figure 6). For lower doses and the timing of CCl_4, hepatocytes, sinusoids and Central veins are seen which are clearly visible with fatty. When compared with positive control (Figure 7), AKAS products have the potential to be hepatoprotector because they are able to give good results even with lower doses. For curcumin itself, it appears that there is still fat in central veins and hepatocytes despite using higher doses.

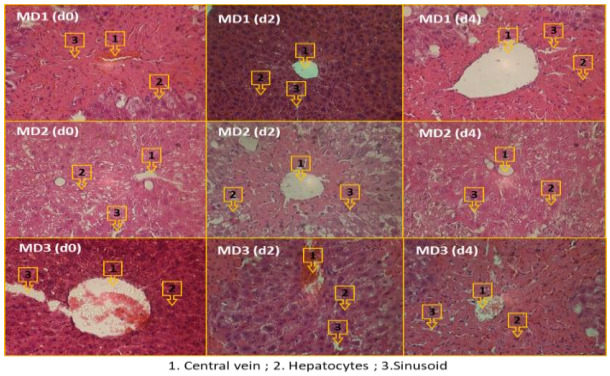

Figure 6. Microscopic images of mice liver structure for three doses of AKAS-m treatment. H & E method, Magnification 400x

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The protective effect on liver for the asymmetric curcumin analog compound is due to the active group in the compound. AKAS products have negatively charged alkene, benzene, carbonyl and ether groups making it possible to capture free radicals and become neutral molecules. If free radicals in the body have been captured, then by itself the body can repair itself. This is seen from the regeneration of damaged liver cells, if it can be concluded that the curcumin analog product is an antioxidant and has a hepatoprotective effect [10].

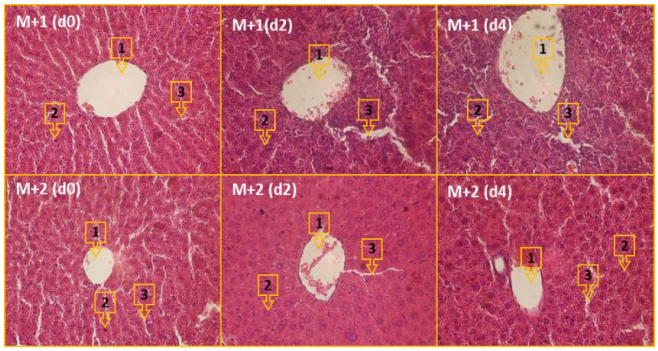

Figure 7. Microscopic images of mice liver structure for positive control mice.H & E method, Magnification 400x

4. Conclusion

The method for the synthesis process of asymmetric curcumin analog products from culilawan oil had an effect on the preventive of liver damage. Products synthesized using the microwave method (AKAS-m) give better results when compared to conventional methods (AKAS-k) and the effective dose as a hepatorotector is AKAS-m dose 26 mg / 200g bw.

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