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The Hepatoprotection Effect of the Asymmetric Curcumin Analogue Synthetic Product in Male Rat Abstract (*Rattus norvegicus* L.)

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ABSTRACT

The asymmetric curcumin analogue synthesized from cullilawan oil has a dioxolane active ring, so it can function as hepatoprotection. The chemical compound structure of the synthetic product influences the pharmacological properties. This study aimed to determine the hepatoprotection effect of the asymmetric curcumin analogue synthetic product in male rat (*Rattus norvegicus* L.) induced by carbon tetrachloride (CCl_4). Turmeric extract and Hepa-Q commercial drug products are used as a comparison. The method used is the in vivo method using male rats by looking at Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvate Transaminase SPGT) values and histopathological analysis using the H&E method. The results showed that the asymmetric analogue of curcumin synthesized using the microwave method gave better results than conventional methods. The effective dose of hepatoprotection in male rats is 52 mg/200 g of bodyweight.

Keywords: Asymmetric curcumin analogues, in vivo, hepatoprotector

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INTRODUCTION

Curcumin analogous compounds (AKAS) are compounds that have the same or even better pharmacological properties when compared to parent compounds [1]. Curcumin compound is a yellow powder from the Curcuma longa (Linn) plant which has acted as a drug for diabetes [2] and anti-cancer [3]. Curcumin as a chemopreventive compound that aims to slow down, block, or restore the carcinogenesis process [4]. Curcumin and curcumin analogues compounds have biological activities as an anti-inflammatory, antioxidant, antitumor, and anticancer (digestive, breast, ovarian, lung, nerve) [5,6,7,8,9]. The effect of these activities is influenced by the substituent and structure of curcumin analogue compounds. The asymmetric analogue curcumin product (5-benzo [1,3] dioxol-5-yl-1-phenyl-penta-2,4-dien-1-one, AKAS) can be synthesized from cullilawan oil [10] and cytotoxic activity tested in breast cancer cell cultures gives positive results [11]. Curcumin analogue synthesis method can influence the product both yield and biological activity. Curcumin compounds are often used as compounds that function as hepatoprotection that can protect and repair damaged liver cells [12]. The effectiveness of asymmetric curcumin analogue compounds synthesized from cullilawan oil as a hepatoprotection needs to be further investigated. The purpose of this study was to determine the hepatoprotection effect of asymmetric curcumin analogues as in male rats (*Rattus norvegicus* L.) induced by CCl_4 and to know the effective doses.

RESEARCH METHODS

Materials: The materials used in this study were male rats (*Rattus norvegicus* L.), asymmetric curcumin analogue synthetic product (AKAS-k and AKAS-m), methanol, turmeric extract, ethanol, CCl_4, Hepa-Q, buffer neutral formalin, turmeric extract, ethanol, CCl_4, Hepa-Q, buffer neutral formalin (BNF), haematoxylin-eosin dyes, phosphate buffer, trichloroacetic acid (TCA) 10%, solution 1.1.3.3-tetrametoxipropane (TMP), Tris-HCl, 5% BHT, NMPI (N-methyl-2-phenyl-indole), HCl concentrated, reduced GSH, dTNB and DiaSys® reagent kits.

Tools

The equipment used in the study includes enclosures, humidity measuring devices, thermometers, capillaries (Marienfed), Eppendorf tubes, centrifuges, micropestles, homogenizers, vortices, incubators, glassware, FTIR, and LCMS.

Experimental procedure

Male rats were acclimatized for 7 days in a room with a 12-hour cycle (light/dark), the humidity of 70±2%, the temperature of 22±2 °C. Male rats are grouped into 10 with 8 animals each according to Table 1 and then given treatment for 1 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 an Eppendorf tube, then centrifuged (Hettich centrifuge 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 a UV-Vis spectrophotometer (Genesis 10 UV). For histopathological analysis of the liver using hematoxylin-eosin (H&E) staining. The liver is fricated with formalin neutral buffer solution (BNF). Before staining, it is preceded by a process of deparaffinization.

Table 1. Group division and treatment of male rat

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	Hepa-Q drug	60	Positive control
M-1	CCl_4	No treatment	Negative control
MN	No CCl_4	No treatment	Normal control

RESULTS AND METHODS

The administration of asymmetric curcumin analogue (AKAS) before CCl_4 was shown to be able to increase the body weight of male rat model animals. At the time of administration of the toxic substance CCl_4 on day 0, the percentage change in body weight was still increasing and it continued for the 6th and 12th days. The trend of weight gain for each treatment group is the same and only differs for negative control (M-1). The administration of CCl_4 affects liver function. The percentage change in body weight after treatment for all groups is shown in Figure 1. Statistical analysis showed that there was no significant difference between each treatment for the presentation of changes in bodyweight of animal models with sig values (p = 0.001). The asymmetrical analogue of curcumin products, which were synthesized by the microwave method for all three doses, gave a better percentage of weight change compared to conventional methods.

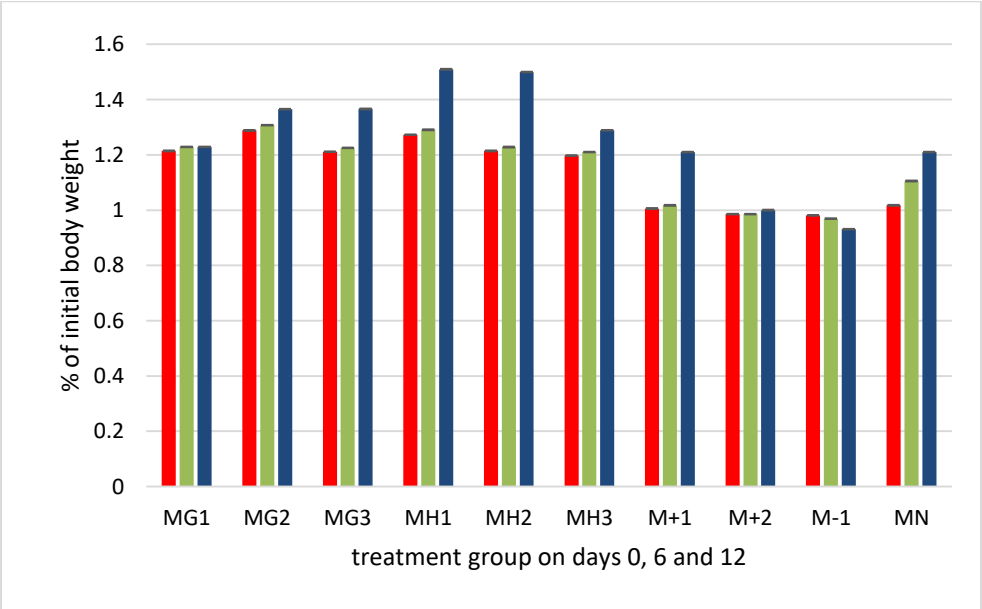


Figure 1. Graphic representation of the percentage of the initial bodyweight for each treatment group on days 0 (red), 6 (green) and 12 (blue). Standard deviation bars show minimal variation between male rat

The percentage change in body weight of the animal models for all treatment groups was also seen in line with the percentage of SGOT and SGPT values. In general, the treatment for each group significantly influenced the SGOT value with sig value (p = 0.001). The results of blood biochemical analysis for SGOT levels showed that the percentage value that gave the smallest value was the MH3 group which was given the analogue curcumin asymmetric analogue method (AKAS-m) at a dose of 52 mg/200 g bodywight (Figure 2). Post hoc analysis showed the percentage of SGOT change in MH3 group was not significantly different from the positive control for turmeric extract (M+1) treatment (sig, p = 0.8681) and Heap-Q (M+2) drug treatment (sig, p = 0.428).

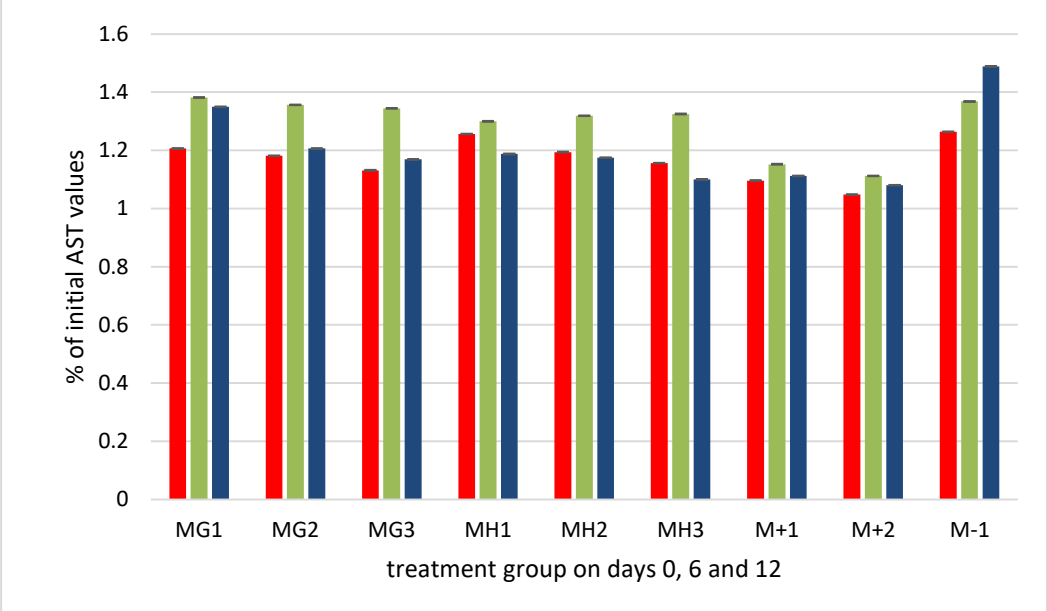


Figure 2. Graphic representation of the mean percentage of initial pretreatment AST values for each treatment group as measured on days 0 (red), 6 (green) and 12 (blue). Standard deviation bars show minimal variation between male rat

SGPT values for curcumin analogue products synthesized by the microwave method give better results when compared to conventional methods. In Figure 3 it can be seen that AKAS-k products at low doses do not decrease the SGPT value but at a dose of 52 mg/200g bodyweight there is a decrease in the percentage change in SGPT value. AKAS-m for each dose gives a trend of decreasing the percentage of decrease in SGPT value and for a dose of 52 mg/200 g bodyweight gives better results. The administration of curcumin analogue has a significant influence on the percentage change in SGPT values with sig values ($p = 0.009$). Post hoc analysis for the AKAS-m (MH3) group which had a better percentage change in SGPT values of AKAS products compared to the positive control, it was seen that there was no significant difference with sig> 0.05.

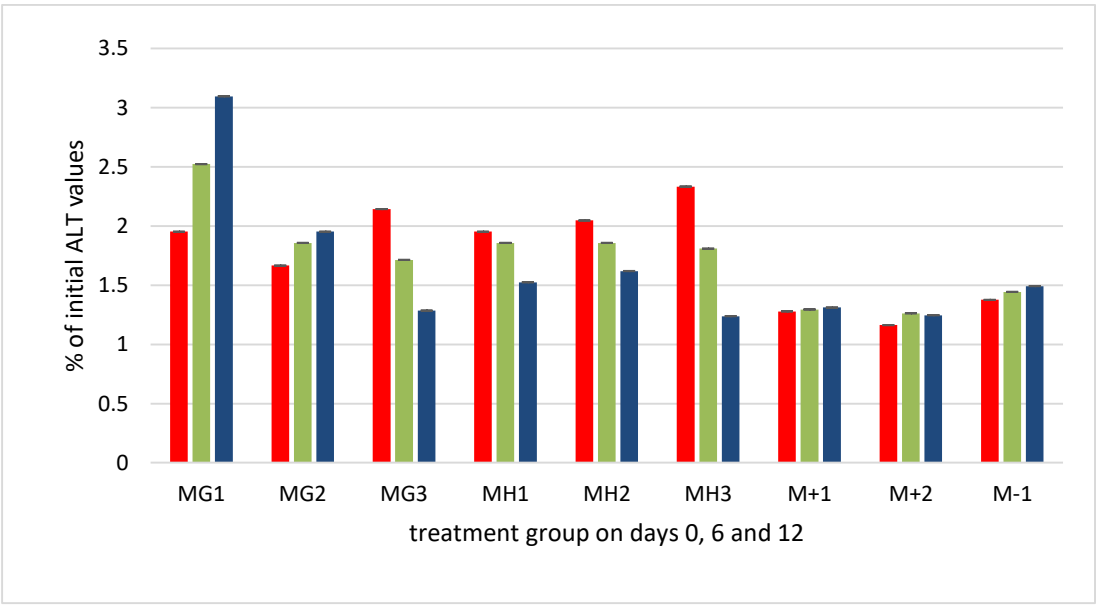
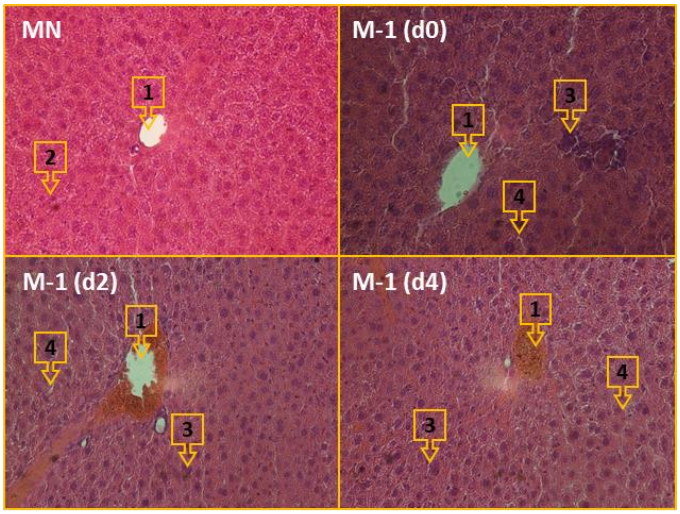


Figure 3. Graphic representation of the mean percentage of initial pretreatment ALT values for each treatment group as measured on days 0 (red), 6 (green) and 12 (blue). Standard deviation bars show minimal variation between male rat

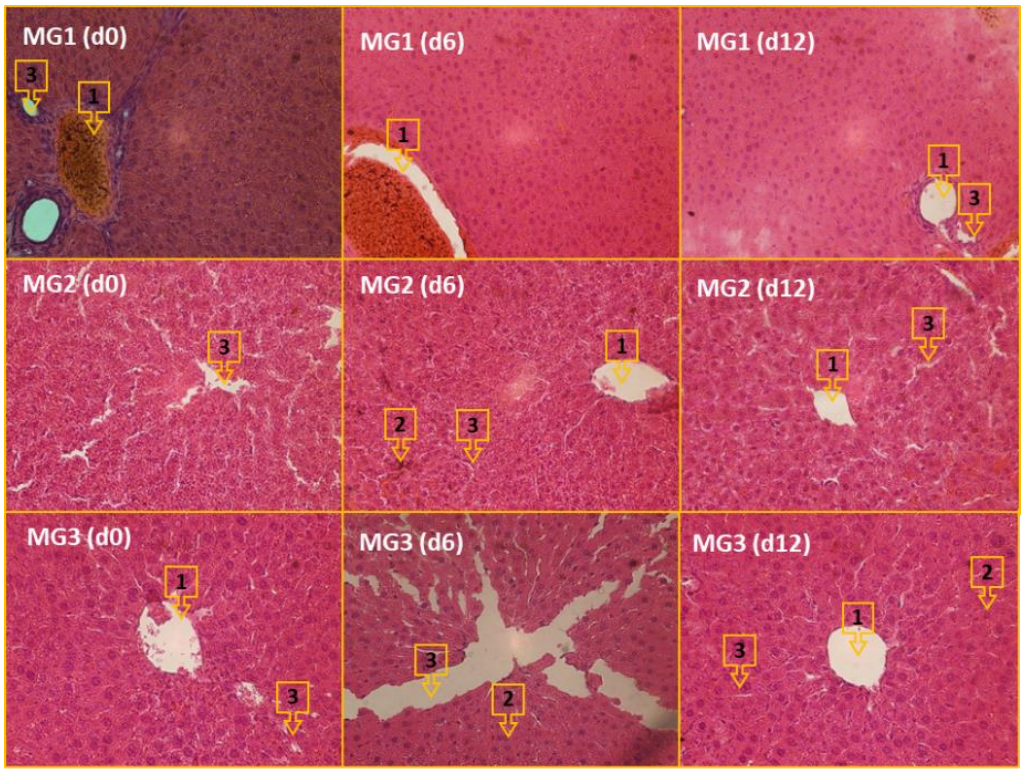
The effect of the treatment group analyzed by the H&E method showed a difference between the microscopic depiction of normal liver cells (MN) and the group given CCl_4 (M-1). Toxic carbon tetrachloride (CCl_4) can cause liver damage in the form of degeneration and necrosis with the initial stages of cell damage in the form of hydropic degeneration and continued with fat degeneration, and cell death or necrosis (Weber et al., 2003). Cells that have been induced by carbon tetrachloride have been seen to have weakened in the central venous region which is different from normal cells that are still visible with normal cells that are still visible in the central venous veins as round and empty centres (Figure 4).



1. Central vein; 2. Normal hepatocytes; 3. Hepatocytes; 4. Sinusoid

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

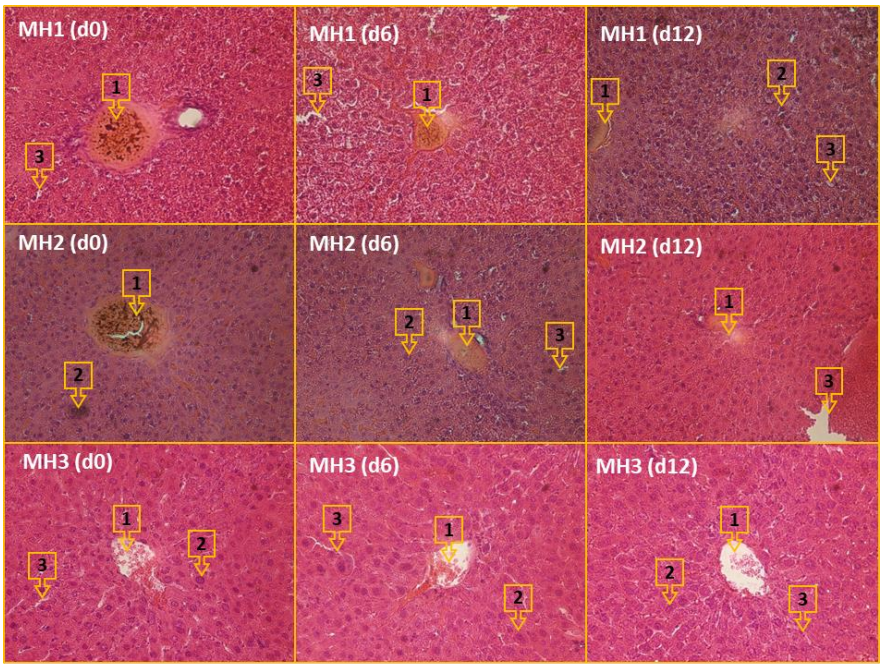
Treatment of asymmetric analogue curcumin analogue products with conventional methods (AKAS-k) for groups of MG1, MG2, MG3 with different doses showed microscopic differences. The greater the dose given, there is an improvement in liver cells, where in the central vein on day 12 seems to have begun to improve. The process of cell regeneration that occurs is getting better day by day, although on days 0 and 6 there is still visible fatty and widening of the sinusoid (Figure 5).



1. Central vein ; 2. Hepatocytes ; 3.Sinusoid

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

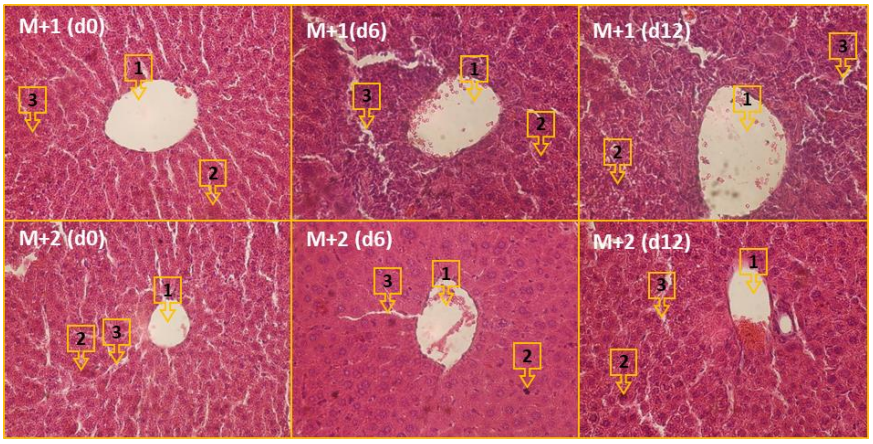
The asymmetric analogue of curcumin analogue products by the microwave (AKAS-m) method shows the same thing as the AKAS-k group where the greater the dose, provides a microscopic picture of liver cell repair (Figure 6). The MH3 group given the highest dose was the best dose among the asymmetric curcumin analogue synthesis products. This is in line with the SGPT and SGOT biochemical analysis data, where the MH3 group gave better results among other product groups.



1. Central vein ; 2. Hepatocytes ; 3.Sinusoid

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

Microscopic images for positive control of the turmeric extract group showed a weakening in the central vein and hepatocytes (Figure 7). The same thing with AKAS products, so it is said that AKAS products can function as hepatorotectors. The influence of the group contained in AKAS can capture free radicals from the toxic substance CCl_4 so that it becomes neutral. AKAS have a very active dioxolane ring and also influences the alkene, carbonyl, and ether groups.



1. Central vein ; 2. Hepatocytes ; 3.Sinusoid

Figure 7. Microscopic images of male rat liver structure for positive control. H&E method, magnification 400x

CONCLUSION

The asymmetric curcumin analogue synthetic products from cullilawan oil exert a hepatoprotective effect on male rats (Rattus norvegicus L.) induced by CCl4. Products synthesized using the microwave method (AKAS-m) give better results when compared to conventional methods (AKAS-k). The effective dose as a hepatoprotection is 52 mg/200g bodyweight.

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