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Research Article

EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF HEXAGAMAVUNON-1 AGAINST CARBON TETRACHLORIDE-INDUCED HEPATIC INJURY IN RATS

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ABSTRACT

The present study was carried out to investigate the hepatoprotective activity of hexagamavunon-1 (HGV-1) and its antioxidant property. The groups of animals were administered with HGV-1 at the doses 5, 10, and 20 mg/kg BW, p.o. once in a day for 6 days and at day 7 the animals were administration with carbon tetrachloride (CCl_4) (20%, 2 ml/kg BW in liquid paraffin, i.p.). The effect of HGV-1 on serum transaminase (SGPT), alkaline phosphates (ALP) and total bilirubin were determined in the rat before and after treatment. In addition, the effects of HGV-1 glutathione (GSH) content and catalase (CAT) were also performed. The result demonstrated that HGV-1, 10 and 20 mg/kg demonstrated significant reduction in serum hepatic enzymes which were increased by CCl_4. In addition, HGV-1, doses 10 and 20 mg/kg significantly increased liver GSH and CAT when compared to those in rats treated by carbon tetrachloride. In order to know the mechanism of hepatoprotective, the antioxidant of HGV-1 was assayed using free radical NO scavenging activity and resulted a potent radicals scavenging or antioxidant. Taking together, HGV-1 is capable to protect the liver from carbon tetrachloride-induced liver damage. Probable mechanism of its action is through its antioxidant property

Key words: Hepatoprotective, Hexagamavunon-1 (Hgv-1), Ccl_4, Antioxidant

INTRODUCTION

Liver is an important organ that regulates many important metabolic functions including drug elimination and detoxification[1]. Some of drug or xenobiotics can damage the liver through the metabolism via P450. Carbon tetrachloride (CCl_4) is one of the toxicant that widely used as a hepatotoxic agent for screening the anti-hepatotoxic/hepatoprotective activity in experimental model, because the CCl_4-induced hepatotoxicity is regarded and similar as and analogue of liver injury caused by variety of hepatotoxins in human. The oxidative damage through free radical generation and decreased activities of antioxidant enzymes is one of the mechanism involved in the hepatotoxic effect of CCl_4[2]. Free radicals generation from CCl_4 are also capable covalently binds to proteins or lipids, and then initiates the lipid peroxidation in the cellular membrane and liver damage[3-5]. Hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis[1]. Antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect of indigenous substance[6]. The consideration of the terapeutic strategy against hepatotoxicity and liver injury is find the antioxidant compounds that are capable to protect liver injury through free radical scavenging or antioxidant.

Curcumin (diferuloylmethane) is a yellow pigment and the active constituents of turmeric. In animal studies turmeric demonstrated the hepatoprotective effects against variety of hepatotoxin such as carbon tetrachloride (CCl_4)[7,8], galactosamine[9], acetaminophen (paracetamol)[10], and Aspergillus aflatoxin[11]. Our group has been synthesized hexagamavunon-1 (HGV-1), one of analog curcumin (Fig.1). HGV-1 has demonstrated the biological activities such as antioxidant, anti-inflammatory [12] and inhibited cyclooxygenase enzyme[13]. The derivatives also less effects of ulcer and did not significantly shown toxic effects on acute and subchronic toxicity testing[11].

The Study of potential effects of HGV-1 have been conducted related into their potential pharmacological effect in human. One of their main activities in this regard is their antioxidant activity[12,14]. Antioxidant plays an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infection and degenerative diseases. Regarding to the important role of antioxidant activity of HGV-1, the present study was conducted to evaluate the antioxidant and hepatoprotective activity of HGV-1 against CCl_4-induced liver damage in rats.

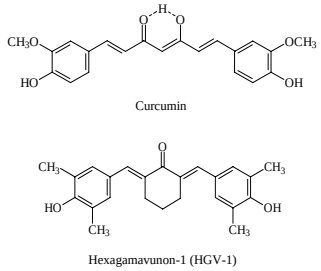


Fig. 1: Structure of curcumin and its analog hexagamavunon-1 (HGV-1)

MATERIALS AND METHODS

Materials

Hexagamavunon-1 (HGV-1) was obtained from Curcumin Research Center, Faculty of Pharmacy, Gadjah Mada University. Carbon tetrachloride (CCl_4), glutathione, and 5,5'-dithio *bis*-2-nitrobenzoic acid (DTNB) were purchased from E. Merck, Darmstadt, Germany. Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (SALP), total bilirubin were analyzed using reagent kits (DIALAB). All other reagents were of analytical grade.

Animals

Studies were carried out using male Wistar albino rats (180–200 g). Rats were obtained from the animal house, Faculty of Pharmacy Gadjah Mada University, Yogyakarta, Indonesia. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than five animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light

cycle (12/12 h) and allowed free access to standard pellet diet (The National Agency of Drug and Food Control, Indonesia) and water ad libitum. The rats were acclimatized to laboratory condition for 1 week before commencement of experiment. All procedures described conducted in accordance with Guideline for Care and Use of Animals Laboratory of Faculty of Pharmacy, Gadjah Mada University.

Hepatoprotective study

Healthy thirty Wistar albino rats were divided into 6 groups each containing 5 animals. Before treatment, all animals were determined SGPT, ALP and total bilirubin. Group 1 (control), administered with vehicle (CMC Na 0.5 %, p.o.) for six days. Group 2 (control HGV-1), administered with HGV-1, 20 mg/kg, p.o. for six days. Group 3 (control hepatotoxin (CCl_4)), administered with vehicle (CMC Na 0.5 %, p.o.) for six days. Groups 4-6 administered with HGV-1 at a dose of 5, 10, and 20 mg/kg, p.o., respectively for six days. On day 7, the groups 3-6 treated with carbon tetrachloride (CCl_4, 2.0 ml/kg, i.p.). Forty eight hours after CCl_4 administration the animals were fasted and blood sampling for determination of SGPT, ALP and total bilirubin.

After collection of blood samples the rats were sacrified and their livers excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer. A part of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for estimation of glutathione by the method of Ellman (1959)[15]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of catalase (CAT) activities was measured by the method of Aebi (1974)[16].

Estimation of GSH

The procedure to estimate the reduced glutathione (GSH) level followed to the method as described by Ellman (1959)[15]. The homogenate (in 0.1 M phosphate buffer, pH 7.4) was added with equal volume of 20 % trichloroacetic acid (TBA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200 µl) was then transferred to a new set of test tubes and added 1.8 ml of the Ellman's reagent (5, 5'-dithio *bis*-2-nitrobenzoic acid) (0.1 mM) was prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes make up to the volume of 2ml. After completion of the total reaction, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH. The glutathione level in liver was calculated as micromol/g liver.

Estimation of CAT

Catalase activity was measured by the method of Aebi (1974)[16]. Supernatant (0.1 ml) was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein.

Assay of NO radical scavenging activity

Various concentrations of HGV-1 and SNP (10 mM, final concentration) in phosphate buffered saline (PBS) in a final volume of 2 ml were incubated at 25 °C for 150 min. A control experiment without tested compounds but with the equivalent amount of vehicles was conducted in an identical manner of control. After incubation, 1.0 ml samples of reaction mixtures containing nitrite were removed and diluted with 1.0 ml of Griess reagent. The absorbance of these solutions were measured at 540 nm against the corresponding blank solutions. The ES_{50} value is the concentration of sample required to scavenge 50% of the NO radicals. Addition of varied concentrations of HGV-1 into the reaction mixture affected an increase in total absorbance upon treatment with Griess reagent. It was indicated that the HGV-1 interfered with the absorbance value of nitrite detection. Therefore, in these experiments we had to exclude this interference by subtracting their absorbance at each concentration.

Statistical analysis

The data are presented as the mean ± S.E.M. The statistical significance of differences between the groups were assessed with a one-way ANOVA, followed by Bonferroni/Dunn post-hoc test analysis using rel 13.0 software SPSS (Chicago, IL, USA).

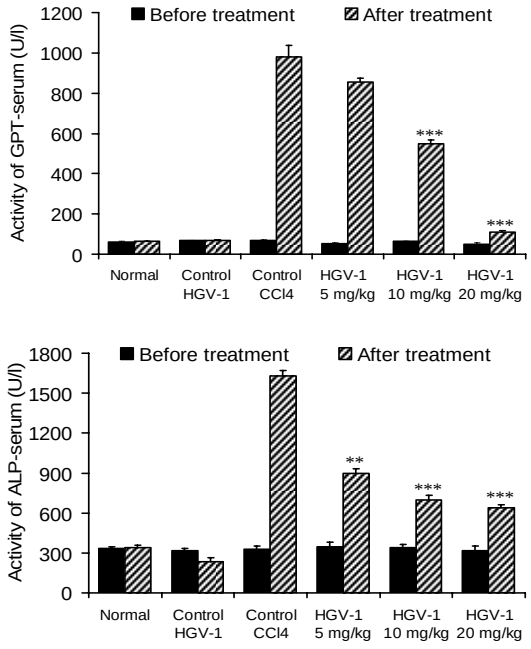
RESULTS

Effect of HGV-1 on SGPT, ALP and total bilirubin

The effect of HGV-1 on SGPT, ALP and total bilirubin of the serum of CCl_4-induced liver damge rats are presented in Fig 2. The result demonstrated that SGPT, SALP and total bilirubin were found to be significantly increased in rats treated with CCl_4 when compared with the normal group. The administration of HGV-1 in six consecutive day at the dose of 10 and 20 mg/kg showed significantly decreased the activity of serum transaminase, SALP and total bilirubin and increased protein content in CCl_4-induced liver damage in rats compared to that of control hepatotoxin (CCl_4) groups.

GSH level in liver tissues

The effect of HGV-1 on glutathione content in the liver is shown in Fig 2. The content of GSH level of liver homogenate in CCl_4 control group was found to significantly lower than in normal group. The administration of HGV-1 at doses 10 and 20 mg/kg capable to increase the GSH content of liver. These results were increased by 26.83 and 72.36 % respectively as compared to CCl_4 control group. Therefore HGV-1 capable to protect the depletion of GSH content against CCl_4-induced liver damaged.



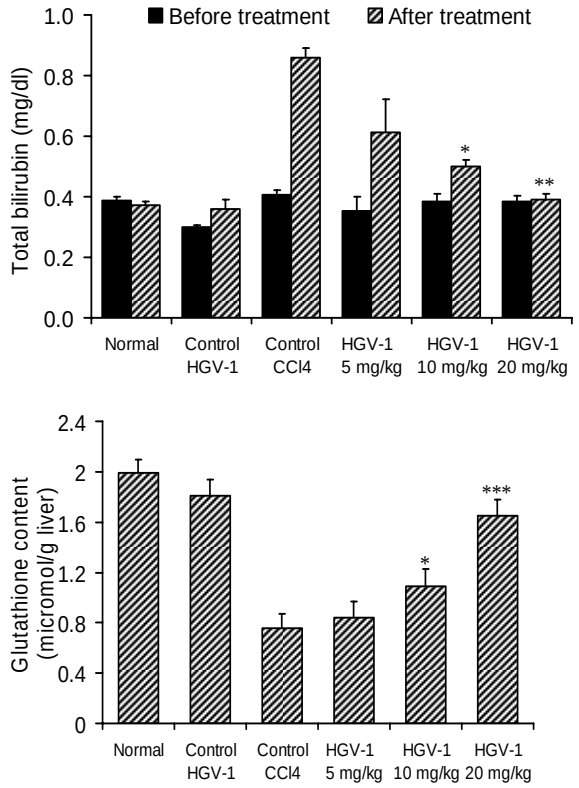


Fig. 2: Effect of administration of HGV-1 on serum GPT level, ALP, total bilirubin and glutathione content against CCl_4-induced liver damage in rats.

Data were expressed as mean ± SEM. * p<0.05; ** p<0.01; *** p<0.001 significantly different to control CCl_4.

Catalase activity in liver tissues

The activity of CAT in liver homogenate is shown in Fig 3. CAT activity of liver total homogenate in CCl_4 control group was found to be lowers than in normal group (Fig.3). The administration of HGV-1 10 and 20 mg/kg significantly increased CAT activity compared to CCl_4 control group. The administration HGV-1, 20 mg/kg completely restored the CAT activity to the normal level. The results suggest that HGV-1 capable to protect the reduction of CAT against CCl_4-induced liver damaged.

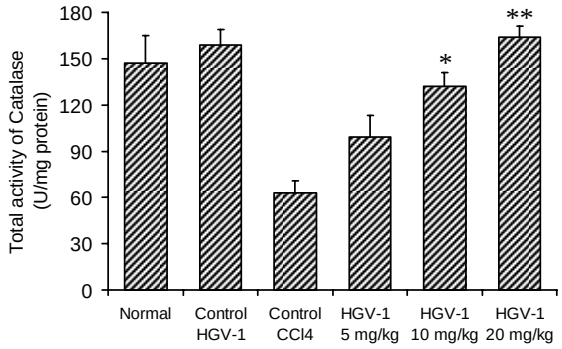


Fig. 3: Effect of administration of HGV-1 on catalase against CCl_4-induced liver damage in rats.

Data were expressed as mean ± SEM. * p<0.05; ** p<0.01 significantly different to control CCl_4.

***In vitro* NO free radical scavenging activity**

Effect of HGV-1 on NO free radical scavenging activity is presented in Fig 4. In order to know the mechanisms of hepatoprotective of HGV-1, it necessary to investigate the antioxidant effect of HGV-1. The result demonstrated that HGV-1 capable to scavenge NO free radical by ES (32.68 ± 0.40).

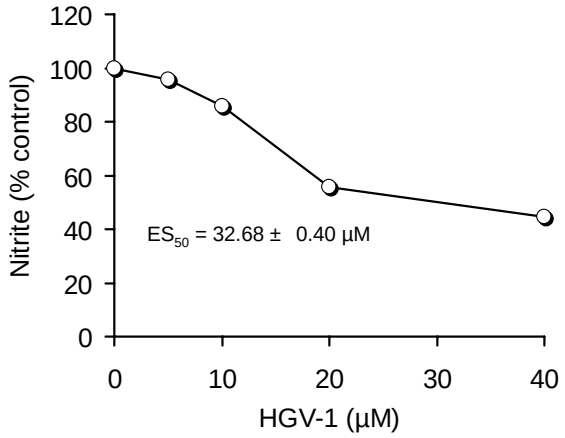


Fig. 4: The effects of HGV-1 on the accumulation of nitrite upon decomposition of sodium nitroprusside (10 mM).

The incubation time was 150 min and temperature 25°C. Each point represent the mean ± SEM (n = 5).

DISCUSSION

Hepatocellular damage or necrosis, lead to elevation of serum marker enzymes, which are released from liver into the blood[1]. The determination of enzyme levels in assessment of CCl_4-induced liver damage, such as SGPT and SGOT is largely used. The membrane damage releases the enzyme into circulation. SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. However, GPT is more specific to the liver, and is thus a better parameter for detecting liver injury[17]. Our study using the model of CCl_4-induced liver damage in the rats demonstrated that HGV-1 at the different doses caused significant inhibition of SGPT levels. In other hand, serum ALP and bilirubin levels are related to the hepatic function. The present study using the model of CCl_4-induced liver damage in rats demonstrated that HGV-1 caused significant inhibition of SGPT, SALP and bilirubin levels. Therefore, the administration of HGV-1 capable to relief and improve of the functional status of the liver from the CCl_4-induced liver damage.

It well accepted that a deficiency of GSH within living organisms can lead to tissue disorder and injury. The liver injury induced by hepatotoxic agents such as paracetamol and CCl_4 are known to be correlated with low tissue levels of GSH[18]. Accordingly, the present results were suggested that the administration HGV-1 might provide a mean for recovery of reduce GSH levels and to prevent tissue disorders and liver damages. In present study, we have demonstrated the effectiveness of low doses of HGV-1 (10 and 20 mg/kg) by using model CCl_4-induced liver damaged in rats.

Another antioxidant defense system in the body is catalase (CAT). The reduction of this protective defense system results in increased sensitivity to free radical-induced cellular damage. The administration of CCl_4 lead to generation of free radical such as trichloromethyl free radical ($\bullet CCl_3$) and trichloromethylperoxy free radical ($CCl_3O_2\bullet$)[19,20] which is associated with inactivation of CAT. The excessive and accumulation of free radicals may result in alterations in the some biological activity of cellular macromolecules. The administration of HGV-1 increases the activities of CAT and protect from the accumulation of free radical result from

metabolism of CCl_4. Hence, it may be possible that the mechanism of hepatoprotective of HGV-1 is due to its antioxidant effect.

HGV-1 effectively reduced the generation of NO radicals and scavenged NO radicals in a dose dependent manner. HGV-1 exhibited potent NO radical scavenging with ES_{50} values is 32.68 μM. The pretreatment with HGV-1 capable to prevent the accumulation of free radicals which is generated from CCl_4. This antioxidant mechanism probably contributes to the hepatoprotective of HGV-1 against CCl_4.

In conclusion, the results of the present study demonstrate that HGV-1 has a potent hepatoprotective action upon carbon tetrachloride-induced hepatic damage in rats. Our results indicated that the hepatoprotective effects of HGV-1 may be due to its antioxidant and free radical scavenging properties.

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REFERENCES

1. Meyer SA and Kulkarni AP. Hepatotoxicity. In: Hodgson E, Smart RC. (Eds.). Introduction to biochemical toxicology. 3rd ed. A. New York: John Wiley & Sons; 2001. p. 487-90.
2. Poli G. Liver damage due to free radicals. Br Med Bull 1993; 49: 604-20.
3. Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 1989; 43: 139-54.
4. Connor HD, Lacagnin LB, Knecht KT, Thurman RG, Mason RP. Reaction of glutathione with a free radical metabolite of carbon tetrachloride. Mol Pharmacol 1990; 37: 443-51.
5. Williams AT, Burk RF. Carbon tetrachloride hepatotoxicity: an example of free radical-mediated injury. Semin Liver Dis 1990; 10: 279-84.
6. Deleve LD, Kaplowitz N. Mechanisms of drug induced liver disease. Gastroenterol Clin North Am 1995; 24: 787-810.
7. Deshpande UR, Gadre SG, Raste AS, Pillai D, Bhide SV, Samuel AM. Protective effect of turmeric (*Curcuma longa* L.) extract on carbon tetrachloride-induced liver damage in rats. Indian J Exp Biol 1998; 36: 573-77.
8. Park EJ, Jeon CH, Ko G, Kim J, Sohn DH. Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. J Pharm Pharmacol 2000; 52: 437-40.
9. Kiso Y, Suzuki Y, Watanabe N, Oshima Y, and Hikino H. Antihepatotoxic principles of *Curcuma longa* rhizomes. Planta Med 1983; 49: 185-87.
10. Donatus IA, Sardjoko, Vermeulen NP. Cytotoxic and cytoprotective activities of curcumin. Effects on paracetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. Biochem Pharmacol 1990; 39: 1869-75.
11. Soni KB, Rajan A, Kuttan R. Reversal of aflatoxin induced liver damage by turmeric and curcumin. Cancer Lett 1992; 66: 115-21.
12. Reksohadiprodjo MS, Timmerman H, Sardjiman, Supardjan AM, Martono S, Sugiyanto et al. Inventors. Derivatives of Benzylidine cyclohexanone, Benzylidine cyclopentanone, and Benzylidine Acetone, and Therapeutics Uses Thereof. United State Patent US 6,777,447 B2; 2004 Aug. 17.
13. Nurrochmad A, Supardjan AM, Sardjiman. Inhibition of Cyclooxygenase by Cyclovalone and Its Three Analogue Compounds, Indonesian J Pharm 1998; 9: 180-85.
14. Sardjiman S, Reksohadiprodjo M, Hakim L, van der Goot H, Timmerman H. 1,5-Diphenyl-1,4-pentadiene-3-ones and cyclic analogues as antioxidative agents. Synthesis and structure-activity relationship. Eur J Med Chem 1997; 32: 625-30.
15. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959; 82: 70-77.
16. Aebi H. Catalase. In: Bergmeyer HV, editor. Methods in enzymatic analysis. Vol 2, New York: Acadamic press; 1974. p.674-84.
17. Willianson EM, Okpako DT, Evans FJ. Selection, preparation and pharmacological evaluation of plant material. England: John Wiley; 1996.
18. Leeuwenburgh C, Ji LL. Glutathione depletion in rested and exercised mice: biochemical consequence and adaptation. Arch Biochem Biophys 1995; 316: 941-49.
19. Recknagel RO, Glende EA. Carbon tetrachloride heptotoxicity: an example of lethal cleavage. Crit Rev Toxicol 1973; 2: 263-97.
20. Zhu W, Fung PC. The roles played by crucial free radicals like lipid free radicals, nitric oxide, and enzymes NOS and NADPH in CCl_4-induced acute liver injury of mice. Free Radic Biol Med 2000; 29: 870-80.