

The effect of single-bulb garlic oil extract toward the hematology and histopathology of the liver and kidney in mice

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Single garlic has been used as a traditional medicine for hypercholesterolemia and increasing stamina. The aim of this research was to analyze the effect of single-bulb garlic oil (SGO) extract toward hematology and histopathology liver and kidney in mice. Male mice (strain Balb-C, 12 weeks old, weight of 28 ± 3 g) was treated by SGO for 28 days (once per days) with various doses K- (Normal); K+ (SGO 0.0 mg/kg BW); T1 (SGO 6.25 mg/kg BW); T2 (SGO 12.5 mg/kg BW); T3 (SGO 25.0 mg/kg BW); T4 (SGO 50.0 mg/kg BW). The end of the treated mice was sacrificed. Blood was collected to analyze hematology. Liver and kidney were collected then made microanatomy slide with Hematoxylin-Eosin staining. Parameters of hematology are the levels of hemoglobin, hematocrit, number of erythrocytes, and number of leukocytes. Histopathology of liver and kidney determine by cell necrosis. The result of the research showed that SGO increased the number of erythrocytes and leucocytes but there is no effect on the levels of hemoglobin and hematocrit. Histopathology on liver and kidney showed there is no effect in necrosis. The conclusion is the single-bulb garlic oil extract safe if consumed continuously

Keywords: Hematology. Histopathology. Single-bulb garlic oil extract.

INTRODUCTION

Garlic (*Allium sativum*) belongs to the *Liliaceae* family that all have common characteristics, such as being herbaceous perennial plants that form a tuber (Arzanlou, Bohlooli, 2010). Garlic bulb are the part of the plant that many people use for treatment and health care. Some diseases commonly treated with garlic are cancer, diabetes mellitus, and hypercholesterolemia (Ried, Frank, Stocks, 2013). Hypercholesterolemia is an increase in cholesterol levels that can be accompanied by an increase in low-density lipoprotein (LDL). High LDL levels increase the risk of and stimulate some diseases, such as hypertension, diabetes, atherosclerosis and other cardiovascular conditions (Wright, Aronne, 2012).

The main active ingredients of garlic bulb are sulfur compounds (Lawson, Larry, 1998; Iciek, Kwiecień, Włodek, 2009; Touloupakis, Ghanotakis, 2010) including diallyl disulfide, diallyl trisulfide, S-allyl cysteine, allyl disulfide (Block, 1985; Yoo *et al.*, 2010; Block *et al.*,

2010), alliin (Chung, 2006), allicin; and a one (Ledezma, Apitz-Castro, 2006). Garlic has several important enzymes, namely allinase, peroxidase and myosinase (Bhandari, 2012). Some studies have shown that garlic has a high effectiveness against hypercholesterolemia (Gebreyohannes, Gebreyohannes, 2013), hypertension (Bhandari, 2012) and as an antioxidant (Rahman, Fazlic, Saad, 2012). Garlic is also potential as an antimicrobial (Benkeblia, 2004), anti-parasite and anti-diabetic agent (Gebreyohannes, Gebreyohannes, 2013). The use of garlic in maintaining stamina and against disease is through increased monocyte and macrophage phagocytosis activity (Agnesa, Susilo, Lestari, 2017), i.e., as an immunostimulant (Bayan, Koulivand, Gorji, 2014).

Garlic has several varieties, one of which is single-bulb garlic (Sethi *et al.*, 2014). In Indonesia, the single-bulb garlic variety is known as *Bawang lanang*. Single-bulb garlic is a garlic that only consists of one bulb when it grows in an inappropriate environment. Single-bulb garlic is only found in certain areas in Indonesia. One of these garlic-producing areas is Ngadas district in Malang Regency. Single-bulb garlic has a strong pungent and acrid taste compared with multi-bulb garlics (Bharat, Alankruta, Harischa, 2013). Single-bulb garlic is a plant

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usually consumed in Indonesia society to maintain stamina and prevent various diseases caused by hyperlipidemia. Studies of the effects of continuous single-bulb garlic consumption have not been reported. Based on that description, we analyze the effects of single-bulb garlic oil extract (SGO) on hematology and histopathology of liver and kidney when continuously consumed.

MATERIAL AND METHODS

Preparation of Single Garlic Oil Extract (SGO)

Single-bulb garlic oil extract was obtained by Soxhlet extraction with *n*-hexane at UPT *Material Medica Batu*. A total of 1 kg of garlic was dried at 50 °C, mashed, and resulting in 0.3 kg of dried garlic powder. The garlic powder was put into thimble extractor, then 1 L hexane was added as a solvent. The soxhletation process was run for 18 hours; the result was evaporated using a rotary evaporator for 3 hours. The result of the soxhletation process was 3 mg of garlic oil extract. The dose of SGO for treatment was 6.25 mg/kg BW, 12.5 mg/kg BW, 25.0 mg/kg BW, and 50.0 mg/kg BW. SGO extract was diluted with corn oil for treatment.

LC-MS-MS analysis

A 1 mL sample of SGO was inserted into a 25 mL centrifuge tube. Then 14 mL of 2.5% formic acid in water was added, and the sample homogenized using a vortex for 30 minutes. After that, the sample was centrifuged at 4000 rpm for 10 minutes at room temperature. The supernatant was transferred in aliquots of as much as 1.4 mL to 1.5 mL centrifuge tubes and centrifuged again at 13000 rpm for 30 minutes. The supernatant was transferred into a micro-tube and the extract diluted with acetonitrile (50 μ L extract + 950 μ L acetonitrile), homogenized, and centrifuged at 5900 rpm for 30 minutes. The supernatant was then transferred to the micro-tube auto-sampler for injection into the LC-MS machine.

Experimental design and treatment

This study used *Balb-C* male mice aged 12 weeks with a weight of 28 \pm 3 g as experimental animals. The mice were acclimatized for 7 days and given standard feed. During acclimatization, the body weight and the behavior of mice were evaluated. Thirty treatment animals were divided into six treatment groups: K- (normal), K+ (SGO 0 mg/kg BW); T1 (SGO 6.25 mg/kg BW), T2 (SGO 12.5 mg/kg BW), T3 (SGO 25.0 mg/kg BW), T4

(SGO 50.0 mg/kg BW). The doses used in this study were based on the dose levels of daily consumption of humans (Lash *et al.*, 1998; Koscielny *et al.*, 1999; WHO, 1999). The SGO was given orally, as much as 0.3 mL per day for 28 days (BPOM, 2017). On the 29th day, the mice were sacrificed using inhalation anesthesia (isoflurane 4%). The animal treatment protocols were approved by Ethical Clearance of Republic Indonesia No. 880-KEP-UB.

Blood hematology analysis

Analysis of blood hematology included hemoglobin level indicator, hematocrit level, erythrocyte number, and leukocyte number. Hemoglobin level (%) was measured using a hemoglobin Tall Quist scale. Hematocrit level (%) was measured when collecting blood put in micro-hematocrit heparinized tubes then centrifuged. The erythrocytes and leukocytes were counted directly under 400x magnification using a Neubauer chamber (Bastidas, 2017).

Histopathology of liver and kidney

Histopathological measurement, liver, and kidney were fixed with 10% formalin solution. The liver and kidney of the mice were embedded in paraffin, sectioned into 5 μ m-thick sections and stained with hematoxylin-eosin (HE). The amount of necrosis was evaluated using semi-quantitative methods by a NASH CRN scoring system (0–3), in which 0 = normal, 1 = <25% necrosis, 2 = 25–50% necrosis, and 3 = >50% area of necrosis (Palipoch, Punsawad, 2013; Brunt, 2016).

Data analysis

Data on the hemoglobin level, total erythrocyte, total leukocyte, necrosis on liver cells, kidney tubules and kidney glomerulus were analyzed by one-way ANOVA ($p < 0.01$) followed by a Tukey post-hoc test. Data are represented as the mean \pm standard deviation (SD)

RESULTS AND DISCUSSION

SGO active compounds

The results of LC-MS analysis showed that the most abundant active compounds in the SGO extract were alliin, allicin, and ajoene (Z and E), with concentrations of 41 mg/mL, 26 mg/mL, 25 mg/mL, and 11 mg/mL, respectively. The thiosulfate compounds are a much-studied compound of the *Allium* species. The compound

is formed from S-alk(en)yl-l-cysteine-S-oxide contained in the cytoplasm, which then undergoes an enzymatic reaction catalyzed by allinase. Thiosulfate compounds have different components in each species of allium according to their precursor compound. The most commonly found compound is alliin; in garlic, alliin is the dominant compound found in garlic, which is the allicin precursor. The amino acid alliin is an alkyl amino extracted from cysteine sulfoxide. Garlic contains alliin compounds 0.6–14% of fresh garlic weight, and about 2,500–4,500 mg allicin per gram of fresh garlic weight (Mardomi, 2017).

Decomposed Allicin will produce several organosulfur products one of which is ajoene (Mardomi, 2017). Allicin compounds are considered to have many benefits such as being antibacterial, antiviral, antifungal and anti-parasitical agents. Derivative compounds such as ajoene can inhibit the growth of gram-positive and gram-negative bacteria (Cavallito, Bailey, 1944).

Blood haematology of mice

The results of the body weight, the calculation of hemoglobin level, total erythrocyte and total leukocyte are

shown in Tables I and II. Table I shows that there was not any difference in the body weight of mice at the end of the treatment in all groups. Table II shows that the provision of SGO did not have a significant effect on the percentage of Hb levels; the higher the SGO doses given did not effect an increase of Hb percentage. The average hematocrit of mice on the various treatments also showed no difference; the SGO gave neither have any effect on the volume of the precipitating red blood nor the plasma volume. Thus, SGO given to the mice for 28 days did not appear to have a harmful physiological effect (Ried, Travica, Sali, 2016; Haseeb *et al.*, 2018).

The average number of erythrocytes in mice given SGO showed a significant difference compared with normal group ($p < 0.01$). The mice were treated with SGO 6.25 mg/kg BW showed an increase in the number of erythrocytes compared to the normal and untreated mice. The higher of SGO doses were given, the higher the number of erythrocytes. The increasing number of erythrocytes did not affect the blood hematocrit condition. Thus, the provision of SGOs did not have any adverse effects. As long as the blood hematocrit is usually maintained, the mice should be in good condition (Restell *et al.*, 2014).

TABLE I - The average of mice body weight (initial, final, and difference)

Treatment	Average of initial body Weight (g)	Average of final body Weight (g)	Average of difference body Weight (g)
K- (Normal)	26.2 ± 1.3 ^a	30.1 ± 1.4 ^a	3.9 ± 0.7 ^a
K+ (SGO 0 mg/kg BW)	26.6 ± 1.6 ^a	31.6 ± 2.1 ^a	5.0 ± 0.4 ^a
T1 (SGO 6.25 mg/kg BW)	28.6 ± 1.5 ^a	30.8 ± 1.3 ^a	4.0 ± 0.7 ^a
T2 (SGO 12.5 mg/kg BW)	27.6 ± 1.8 ^a	30.0 ± 1.8 ^a	2.4 ± 0.8 ^a
T3 (SGO 25 mg/kg BW)	27.0 ± 1.6 ^a	31.0 ± 1.3 ^a	4.0 ± 1.0 ^a
T4 (SGO 50 mg/kg BW)	27.0 ± 1.6 ^a	31.2 ± 1.3 ^a	4.2 ± 0.8 ^a

Description: K- (Normal); K+ (SGO 0 mg/kg BW); T1 (SGO 6.25 mg/kg BW); T2 (SGO 12.5 mg/kg BW); T3 (SGO 25 mg/kg BW); T4 (SGO 50 mg/kg BW). The same notation shows there is no difference result.

TABLE II - The average of hemoglobin level, blood hematocrit, total erythrocyte and total leucocyte in mice

Treatment	Average of Hb Level (%)	Average of Blood Hematocrit (%)	Average of total erythrocytes (mm ³)	Average of total leukocytes (mm ³)
K- (Normal)	60.0 ± 0.0 ^a	50 ^a	3.92.10 ⁶ ±1184 ^a	2600.0±605 ^a
K+ (SGO 0.0 mg/kg BW)	70.0 ± 0.0 ^a	52 ^a	3.94.10 ⁶ ±1150 ^a	2775.5±370 ^a
T1 (SGO 6.25 mg/kg BW)	67.5 ± 5.0 ^a	54 ^a	6.02.10 ⁶ ±4468 ^b	2900.0±653 ^a
T2 (SGO 12.5 mg/kg BW)	69.3 ± 1.5 ^a	52 ^a	9.79.10 ⁶ ±9688 ^c	2950.0±660 ^a
T3 (SGO 25.0 mg/kg BW)	60.0 ± 11.0 ^a	50 ^a	8.93.10 ⁶ ±2917 ^c	7337.5±765 ^b
T4 (SGO 50.0 mg/kg BW)	60.0 ± 0.0 ^a	50 ^a	8.08.10 ⁶ ±1106 ^c	10987.5 ± 189 ^c

Description: K- (Normal); K+ (SGO 0 mg/kg BW); T1 (SGO 6.25 mg/kg BW); T2 (SGO 12.5 mg/kg BW); T3 (SGO 25.0 mg/kg BW); T4 (SGO 50.0 mg/kg BW). The same notation shows there is no difference result.

The same trend occurred in the number of leukocytes. The higher the doses given, the higher the number of leukocytes produced ($p < 0.01$). The increase in the number of leukocytes due to the provision of garlic is associated with its function as immunostimulant (Nwabueze, 2012). Garlic contains sulfur compounds that act to initiate an increase in the immune system (Iciek *et al.*, 2009). The increasing number of leukocytes due to the provision of SGO is associated with an increase of the leukocyte phagocytic capacity (Santos *et al.*, 2012; Yamashita *et al.*, 2015; Agnesa, Susilo, Lestari, 2017). The provision of garlic can activate non-specific and specific defense mechanisms (Rahman, Billington, 2000). Several studies have shown that allicin from SGO may increase the production of interleukin-2 and IL-12, and TNF- α from splenocytes (Wang *et al.*, 2010; Schäfer, Kaschula, 2014; Kim, 2016; Lestari, Rifa'i, 2018)) and increase macrophage metabolism activity.

The number of necrosis cells in liver cells and kidney

The effect of single garlic extract on the damage of liver cells and kidney cells was determined by assessment of cell necrosis. The results of the calculation of the necrosis average on the liver cells, kidney tubular cells and kidney glomerulus cells are shown in Table III.

The average of scoring system cells undergoing necrosis in the liver was 19.9. While in the glomerulus cells and tubular cells, the highest average of necrosis was 23.1 and 21.1. The calculation of necrosis cells in the liver as well as the tubular and glomerular kidney tissue did not show any difference in the various treatments. Thus, the provision of SGO does not have any effect on the number of cells undergoing necrosis in various groups.

The microanatomy of the liver, tubular and glomerular kidney are shown in Figures 1 and 2.

After oral administration, the SGO will be absorbed by the intestine, passed through the blood vessel through the portal vein hepatica, then metabolized in the liver. The active compound from garlic extracts such as allicin, alliin, ajoene, and S-Allyl cysteine are found distributed in serum, liver, and kidney (Gao *et al.*, 2013). According to an analysis about safety and toxicity, active compounds from garlic extract can passed through blood-brain barrier permeability (BBB-permeability). Drugs and active compounds that can pass through the BBB have an ability to decrease residual disease and toxicity and increase the efficiency of active compound for pharmacology activity in the brain. However, active compounds from garlic extract show no side effects (Jouyban, Soltani, 2012; Shaik *et al.*, 2016). Shaik *et al.* (2016) showed that rats were given garlic for 6 months did not reveal any toxic effects.

Drugs that are administered orally will undergo biotransformation in the body. The process of drug biotransformation requires an enzyme from the Cytochrome P450 (CYP450s) (Gao *et al.*, 2013). Single-bulb active garlic compound is not as an inhibitor or substrate of CYP2D6 isoenzymes so these active compounds will be metabolize by the body without any obstacles. CYP2D6 is one of the isoenzymes of CYP450 responsible for the metabolism of the drug in the liver. A drug or compound may be a CYP isoenzyme inhibitor or a substrate for the isoenzyme. If the drug becomes an inhibitor and is a substrate for the same CYP isoenzymes, then drug metabolism will be delayed (Badyal, Dadhich, 2001).

Drugs are usually eliminated after metabolism in the body. Drug excretion can be determined by total clearance

TABLE III - The average number of necrosis cells in mice treated with single-garlic oil extract with various concentration

Treatment	Liver cells		Tubular cells		Glomerolus Cell	
	The average of necrosis cell	The average of normal cell	The average of necrosis cell	The average of normal cell	The average of necrosis cell	The average of normal cell
K- (Normal)	13.1 ^a	86.9 ^a	12.8 ^a	87.2 ^a	14.2 ^a	85.8 ^a
K+ (SGO 0.0 mg/kg BW)	19.9 ^a	80.1 ^a	21.1 ^a	78.9 ^a	23.1 ^a	76.9 ^a
T1 (SGO 6.25 mg/kg BW)	12.6 ^a	87.4 ^a	18.1 ^a	81.9 ^a	14.2 ^a	85.8 ^a
T2 (SGO 12.5 mg/kg BW)	13.2 ^a	86.8 ^a	17.1 ^a	82.9 ^a	12.2 ^a	87.8 ^a
T3 (SGO 25.0 mg/kg BW)	11.7 ^a	88.3 ^a	13.8 ^a	86.2 ^a	9.4 ^a	90.6 ^a
T4 (SGO 50.0 mg/kg BW)	9.8 ^{aa}	90.2 ^a	11.7 ^a	88.3 ^a	8.4 ^a	91.6 ^a

Description: K- (Normal); K+ (SGO 0 mg/kg BW); T1 (SGO 6.25 mg/kg BW); T2 (SGO 12.5 mg/kg BW); T3 (SGO 25 mg/kg BW); T4 (SGO 50 mg/kg BW). The same notation shows there is no difference result.

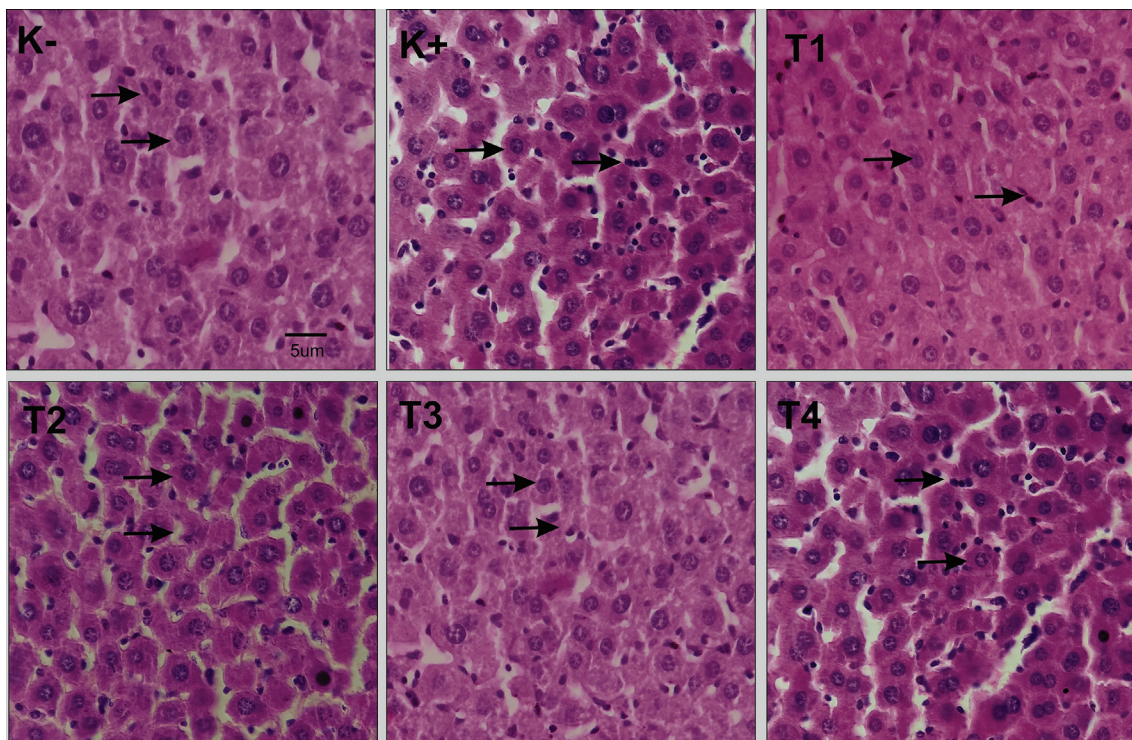


FIGURE 1 - Normal and necrosis cell liver, staining using Hematoxylin-Eosin. Arrow = necrosis cell and normal cell (400 X magnification).

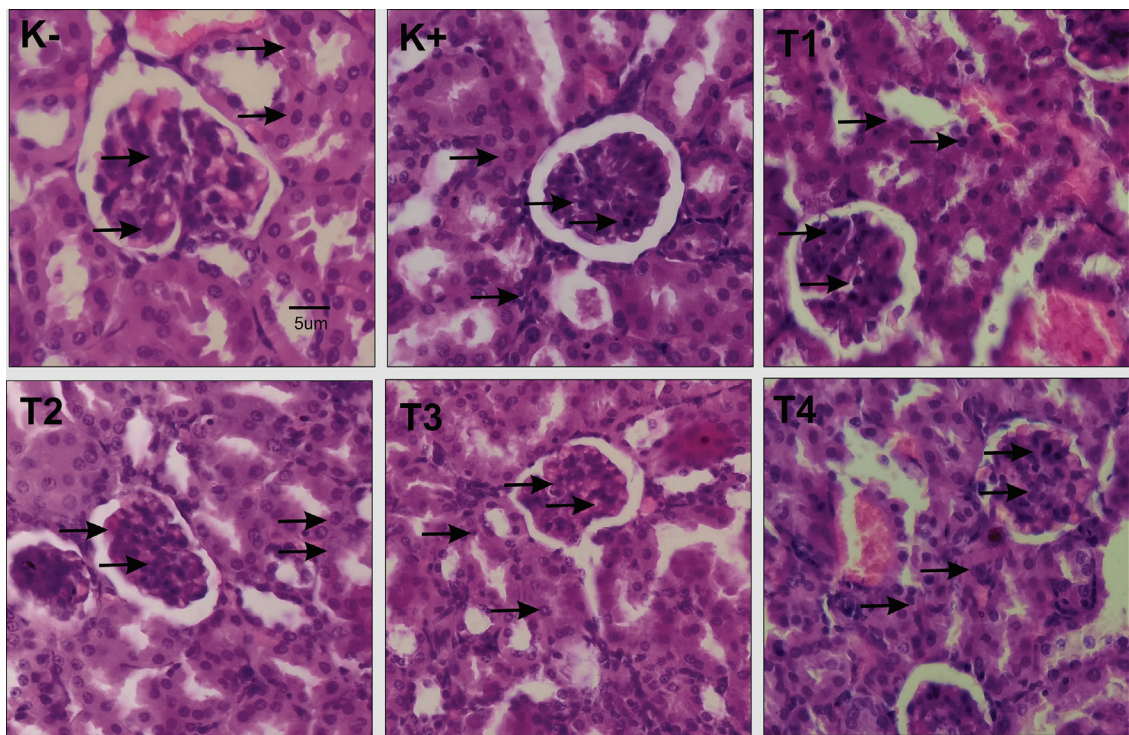


FIGURE 2 - Normal and necrosis cell glomerular and tubules in kidney, staining using Hematoxylin-Eosin. Arrow = necrosis cell and normal cell (400 X magnification).

and organic cation transporters 2 (OCT2) substrate (Beaumont *et al.*, 2014). Total clearance is drug excretion through a renal process in which the drug passes through

the kidney to the bladder and is removed through the urine. In the kidneys, there is a transporter to secrete drugs to urine, i.e., organic cation transporters (OCTs). Compounds

in garlic are not a substrate of Renal OCT. Organic cation transporters are essential transporters for drug elimination from plasma as well as in the kidney. Most drugs can be an OCT2 substrate or inhibitor. If a drug becomes a substrate of OCT, then the performance of OCT2 will be inhibited, so the drug cannot be eliminated thoroughly (Motohashi and Inui, 2013). This study suggests that single garlic might be considered as an alternative herbal treatment and safely used continuously.

CONCLUSION

This study showed that SGO extracts increase number of erythrocytes and leucocytes, but had no affect the levels of hemoglobin and hematocrit. Histopathology on liver and kidney after SGO consumption showed there is no effect on necrosis in these organs.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

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