Full Length Research Paper

Evaluation of the hepatoprotective activity of standardized ethanolic extract of *Curcuma xanthorrhiza* Roxb.

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*Curcuma xanthorrhiza* is widely used in Indonesia folk medicine to treat liver disorders. This study has evaluated the hepatoprotective activity of standardized ethanolic extract of *C. xanthorrhiza*. The extract was standardized using GC-MS. The hepatoprotective activity of this extract was studied using ethanol-induced liver toxicity in rats. This respective activity was assessed through monitoring liver function tests through the measurement of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and protein content. Further, hepatic tissues were also subjected to histopathological studies. Pretreatment of the standardized *C. xanthorrhiza* ethanolic extract (500 mg/kg) reduced the fatty liver symptoms and significantly (p < 0.05) inhibit the increase of respective serum enzyme levels. The results of the present study indicated that *C. xanthorrhiza* possess hepatoprotective effects which could act as an effective treatment for acute hepatic diseases.

**Key words:** *Curcuma xanthorrhiza*, hepatoprotective activity, ethanol, serum enzyme.

**INTRODUCTION**

The liver plays an astonishing array of vital functions in the maintenance and performance of the body. Some of these major functions include carbohydrate, protein, and fat metabolism, detoxification and secretion of bile. Today, with the extensive use of hepatotoxicants in daily routine life, it has become imperative to safeguard human populations inhabiting poverty against liver diseases because mammalian liver is a highly toxicity sensitive organ and responsible for drug metabolism (Eaton et al., 1995). Alcohol abuse is one of the major health problems worldwide. Scientific research in herbal medicine with hepatoprotective activity may be a great benefit as an alternative therapy in alcohol induced liver disease.

*Curcuma xanthorrhiza* is a member of the ginger family (Zingiberaceae) which is a native Indonesian plant. It is known as ‘Temu Lawak’ in Malaysia (Sears, 2005). *C. xanthorrhiza* is a low growing plant with root (rhizome) that is similar to ginger with aromatic, pungent odor and bitter taste. The large leaves and the outsized rhizome of the plant contain various herbal qualities (Sears, 2005). In South-East Asia, it is traditionally utilized for a range of illness including hepatitis, liver complaints, and diabetes, rheumatism, cancer, hypertension and heart disorders.

The anti-hepatotoxic effects of *C. xanthorrhiza* have long been recorded in Asia, and are beginning to be recognized in the West. It is known to be hepatoprotective among Indonesians, and is used particularly in cases of Hepatitis. The effectiveness of *C. xanthorrhiza* in lowering the serum enzyme levels of alanine aminotransferase (SGPT), aspartate aminotransferases (SGOT) and y-glutamate transferases indicates the hepatoprotection of this plant against cisplastin induced hepatotoxicity (Seong et al., 2004). It also has the function of preventing liver adipose (fatty degeneration of the liver) which can cause breakdown of function that is irreversible, and leads to inevitable death.
This is often associated with high alcohol consumption. However, only few studies have been carried out so far on the hepatoprotective effects of this particular plant. Most of the studies have been focused on the other pharmacological properties of this *C. xanthorrhiza* such as its antibacterial and antiviral activities (Hwang and Shim, 2000). Besides, to date, no work has been done in *C. xanthorrhiza* using ethanol as the hepatotoxicant. Therefore, this study aimed to investigate the hepatoprotective activity of *C. xanthorrhiza* against ethanol-induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Plant material identification**

*C. xanthorrhiza* plants were obtained from Johor Plantation, Malaysia. A voucher specimen (11022) was authenticated and deposited at the Herbarium Unit of the School of Biological Sciences, Universiti Sains Malaysia.

**Preparation of the extracts**

The rhizome portion of *C. xanthorrhiza* was purchased in powder form from Chemical Engineering Pilot Plant (CEPP), UTM, Skudai, Johor, Malaysia. The coarsely powdered material (800 g) was macerated with 8 L of absolute ethanol for 72 h with occasional shaking. The maceration was repeated thrice. The extract was filtered and concentrated at reduced pressure on rotary evaporator resulting in dark yellow colored mass (yield 5.2%).

**Experimental animals**

Male Sprague-Dawley rats (180 - 200 g) were obtained from the Animal House, Universiti Sains Malaysia. The animals were acclimatized to laboratory conditions for seven days prior to the experiments. Six rats were housed per polycarbonate cage, with free access to food (normal laboratory chow, Gold Coin) and tap water *ad libitum*. The animals were maintained at room temperature under a light/dark cycle of 12 h. All experiments were performed between 9.00 a.m. to 2.00 p.m. in order to prevent confrontation with circadian rhythm. Experimental protocols and procedures employed in this study were approved by the Animal Ethics Committee of the University Sains Malaysia with the reference number USM/PPSF/50(054) Version 2.

**Standardization of *C. xanthorrhiza* ethanolic extract**

The standardization of *C. xanthorrhiza* ethanolic extract was performed based on the validated method described by Devaraj et al. (2010).

**Hepatoprotective activity**

**Study design**

After one week of acclimatization, animals were divided into three groups of six rats (n = 6) in each. Group A and B were administered with cosolvent 5 ml/kg (p.o.). Group C was given 500 mg/kg of standardized *C. xanthorrhiza* ethanolic extract (p.o.). The vehicle or extract were administered orally for 7 days. By referring to related literatures regarding the dose of animal (Zeng et al., 2008; Zhao et al., 2008; Khanal et al., 2009), 50% ethanol 12 ml/kg was administered orally to groups B and C at 2 h after the administration of vehicle and extracts, respectively for 7 consecutive days.

**Biochemical parameters examination**

Six hours after the last alcohol treatment, blood samples were collected from all groups through cardiac puncture. After overnight fasting (at least 16 h), all rats were briefly anaesthetized by diethyl ether inhalation. Blood withdrawal was performed through cardiac puncture by using a needle (size 0.50 x 16 mm, Terumo). Blood samples obtained were left to clot at room temperature for 60 min. Then, the clotted blood were centrifuged at 3,000 rpm for 15 min to obtain blood serum and analyzed for various biochemical parameters. All serum biochemical testing were conducted at Lam Wah Ee Hospital, Penang, Malaysia using Roche (Integra700®) machine (Orhan et al., 2003).

**Histopathological observation**

Immediately after sacrifice of rats, the livers were removed and fixed in 4% formalin. After processing, the tissues were embedded in paraffin wax with Histo-Centre II- N (Barnstead/ Thermolyne, Iowa, USA) and sectioned into 4-5 µm thickness using microtome. The sections were stained with hematoxylin and eosin (H and E) for microscopic observation, which includes fatty changes, hepatocytes disarrangements and necrosis symptoms.

**Statistical analysis**

The statistical analysis was performed by one-way ANOVA followed by Dunett’s multiple comparison tests in SigmaStat® version 3.5 Software. The results were expressed as mean ± S.E.M to show differences in groups. The differences are considered significant when p < 0.05.

**RESULTS**

**Standardization of *C. xanthorrhiza* ethanolic extract**

Xanthorrhizol was identified and quantified at 9.58 min retention time. 0.1238 mg of xanthorrhizol was quantified in 1 mg of *C. xanthorrhiza* ethanolic extract (Devaraj et al., 2010).

**Biochemical analysis**

Based on the results in Figure 1, rats treated with 5 g/kg of ethanol has significant elevation of ALT, AST, ALP and total protein levels compared to those groups pretreated with standardized *C. xanthorrhiza* ethanolic extract. Treatment of standardized *C. xanthorrhiza* ethanolic extract for 7 consecutive days has provided a significant (p < 0.05) protection to the liver, preventing the raise of the liver enzymes activities.
Figure 1. Effects of the standardized ethanolic extracts of *C. xanthorrhiza* on serum liver enzyme levels. 500 mg/kg represent the dose of *C. xanthorrhiza*. Data are mean ± SEM; * and ** indicate significant difference as compared to the alcohol-treated group at p < 0.05 and p < 0.001 respectively; # indicate significant difference as compared to the control group at p < 0.05 respectively (ANOVA, Dunnett's test, n= 6 rats).

**Histopathological observation**

The histological features of the normal liver as Figure 2a indicated a normal liver lobular architecture and cell structure, each hepatocyte has nuclei that are distinctly rounded. A majority of hepatocytes have single nucleus but binucleates cells are also common. The Reticular Fibers (RF) around the Central Vein (CV) form most of the supporting connective tissue of the liver. In this normal liver tissue, the reticular fibers are clearly seen around the CV. There were no pathological changes in healthy control livers. In ethanol-treated group, the reticular fibers have become thinner around the CV compared to the normal liver section indication liver injury as in Figure 2b (i). Figure 2b (ii) exhibited clearly the macrovesicular steatosis associated with hepatocytes ballooning and Mallory bodies resulting in liver damage. Increase in the size of Kupffer cells which appears clearly within the lumen of sinusoids also can be seen in Figure (b) ii. Distorted hepatocytes and undefined cell lining can be observed in the alcohol treated group as well. The histopathological changes induced by alcohol were significantly improved by 500 mg/kg of standardized *C. xanthorrhiza* ethanolic extract, showing lesser hepatocytes ballooning as in Figure 2c compared to the alcohol treated group. The liver section displayed the recovery of the hepatocytes into normal polyhedral shapes with some cell linings almost similar to the healthy liver group.

**DISCUSSION**

Alcohol was recognized to be a cause of liver damage by the ancient Greeks, and it is currently the most common cause of liver disease other than paracetamol. The magnitude and range of the health and socio-economic problems attributable to alcohol abuse are enormous (Leibach, 1975). Here, “alcohol” refers exclusively to ethanol, the only alcohol present in commercially available alcoholic beverages. Alcohol metabolism takes place entirely in the liver, which contains several different high affinity enzyme systems such as alcohol dehydrogenase (ADH) capable of oxidizing alcohol (Caballeria et al., 1987). ADH catalyzes the oxidation of alcohol to acetaldehyde, transferring hydrogen to the cofactor nicotinamide adenine dinucleotide (NAD), which is converted to its reduced form, NADH. The resulting increase in the ratio of NADH/NAD, which is further increased by acetaldehyde oxidation, is responsible for the majority of the metabolic imbalances that occur following alcohol ingestion and plays a major role in the initial pathogenesis of alcohol-induced fatty liver diseases (Vallee and Bazzone, 1983). In addition, the metabolism of alcohol also induces a potentially injurious...
“hypermetabolic state”, and leads to the generation of toxic free radicals capable of damaging cell membranes, proteins and nucleic acids (Israel et al., 1975; Cross et al., 1987).

Alcohol-induced acute liver injury has significantly elevated the serum transaminases and ALP activities. The results in Figure 1 has showed a promising likelihood that standardized C. xanthorrhiza ethanolic extract has exhibited a hepatoprotective activity following the significant decrease in serum transaminases and ALP activities. Aminotransferases are the group of enzymes that catalyze the reversible transfer of amino acid group from alpha-amino acid to oxo acid (James et al., 2003). ALT enzymes or known as alanine transaminase is abundant in cytosol of hepatic parenchymal cells (Okuda, 1997). While, aspartate transaminase (AST) is found in cytosol
and mitochondria of hepatocytes. It is also distributed in cardiac muscle, skeletal muscle, pancreas and kidney (Shyamal et al., 2006). Hence, ALT measurement is more liver specific to determine hepato damage (Shyamal et al., 2006). However, AST is still being used in lab as a parameter to assess the liver damage since it is considered to be a sensitive indicator of mitochondria damage specifically in the centrilobular region of liver (Panthehghini, 1990). Alkaline phosphatase (ALP) is an enzyme in the cell linings of the biliary ducts of the liver. It is capable of hydrolyzing phosphate esters at alkaline pH and often used as marker for cholesstatic liver dysfunction (Gaw et al., 1998). Elevated enzyme levels are an important indicator of cellular leakage and loss of functional integrity of cell membrane in the liver (Ryan et al., 1990). Increase of ALT enzymes in ethanol-induced liver toxicity is due to the loss of structural integrity of liver (Chenoweth and Hake, 1962). Since this enzyme is located in the cytoplasm, it will be released into the blood circulation after cellular damage resulting in the elevation (Sallie et al., 1991). Increase of AST enzymes indicates that alcohol ingestion to rats causes both plasma membrane and organelle membrane (mitochondria) damage (Conda de la Rosa et al., 2008). Elevation of ALP enzyme levels can be related to the bile duct damage or cholestasis which will spill out the ALP into bloodstream indicating alcohol-induced liver damage (Nyblom et al., 2004). The elevation of these serum enzymes also gives rise to the total protein level. Therefore, the reduction of these enzyme levels in the treated group indicates the stabilization of plasma membrane and repair of hepatic tissues.

The benefits of Curcuma xanthorrhiza ethanolic extract has been further confirmed by histopathological observations. It was well-established that overdoses of ethanol lead to shrinkage of centrilobular reticular fibers, macrovesicular steatosis with ballooning of hepatic cells (fatty liver) and formation of Mallory bodies (Bujando et al., 2006). The classical appearance of macrovesicular steatosis is of a single large fat droplet displacing the nucleus occurring predominantly in perivenular hepatocytes (macrovesicular steatosis). Very rarely, the steatosis is panacinar and may be associated with severe cholestasis, cholangiolitis, and clinical presentation with hepatic failure (Morgan et al., 1978). Fatty change is characterized by the accumulation of triglyceride in hepatocytes (Salasapuro et al., 1981). The three main mechanisms which may play a role in the development of alcoholic fatty liver which increased substrate supply for fatty acid esterification, direct stimulation of the esterification pathway and decreased export from the liver of triglyceride as Very-Low-Density Lipoproteins (VLDL) (Salasapuro et al., 1981). These effects have been significantly reduced with the pretreatment of standardized Curcuma xanthorrhiza ethanolic extract. The macrovesicular inflammation evoked by ethanol considerably decreased following extract pretreatment. The possibility of steatosis (fatty liver) and hepatic cells standardized Curcuma xanthorrhiza ethanolic extract accelerated recovery of hepatic cells was evidenced by histopathological observation, which suggests protection against membrane fragility, thus decreases the leakage of the marker enzymes into the circulation.

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REFERENCES


