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Protective Activity of Turmeric (*Curcuma longa*) in Paracetamol-induced Hepatotoxicity in Rats

^{1,2}M.N. Somchit, ¹A. Zuraini, ¹A. Ahmad Bustamam, ³N. Somchit, ^{1,2}M.R. Sulaiman and ¹R. Noratunlina

¹Department of Biomedical Sciences, Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

²Laboratory of Natural Resources, Institute of Bioscience,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

³Universiti Industri Selangor, Jalan Zirkon A 7/A, Seksyen 7,
40000 Shah Alam, Selangor Darul Ehsan, Malaysia

Abstract: The hepatoprotective activity of the ethanol extract of *Curcuma longa* was investigated against paracetamol (acetaminophen, 4-hydroxy acetanilide)-induced liver damage in rats. Paracetamol at 600 mg kg⁻¹, induced liver damage in rats manifested by statistically increased serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Histologically, livers from these rats revealed parenchymal necrosis and massive inflammation. Pretreatment of rats with the ethanolic extract of *Curcuma longa* (100 mg kg⁻¹) prior to paracetamol dosing lowered serum liver enzyme activities. Livers of these rats showed normal histology. These results suggested that ethanolic extract of *Curcuma longa* has potent hepatoprotective effect against paracetamol-induced liver damages in rats.

Key words: *Curcuma longa*, hepatoprotective, paracetamol

INTRODUCTION

Curcuma longa or turmeric is a member of Zingiberaceae, a perennial herb with short and thick rhizomes. It is known as kunyit in Malaysia and Indonesia^[1], Haridra, Haladi or Rajami in India and Jianghuang in China^[2]. Turmeric has been used extensively in traditional Chinese and Ayurvedic medicinal systems^[2]. *Curcuma longa* rhizomes contain approximately 2% volatile oil, composed mainly of α - and β -turmerone, monoterpenes^[3], 5% curcuminoids, mainly curcumin^[4], demethoxycurcumin, bis-demethoxycurcumin (Fig. 1) and dihydrocurcumin, minerals, carotene and vitamin C^[2]. The active constituent of *Curcuma longa* is curcumin, which is the yellow substance which has been shown to have a wide range of therapeutic effects such as anti-inflammatory^[5], anti-tumour^[6], antioxidant^[7], anti-fungal^[8], anti-parasitic and antispasmodic^[9]. Luper^[10] reviewed turmeric as a potential agent for the treatment of liver diseases. In continuation of this study, we investigated the ethanolic extract of *Curcuma longa* rhizomes as a hepatoprotective agent against paracetamol-induced liver damage in rats.

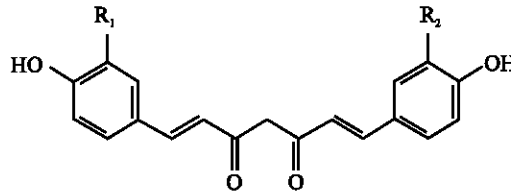


Fig. 1: Chemical structure of curcuminoids in *Curcuma longa*

Curcumin. R₁=R₂=OCH₃. Molecular weight = 368

Bis-demethoxycurcumin. R₁=R₂=H. Molecular weight= 308

Demethoxycurcumin. R₁= OCH₃, R₂= H. Molecular weight= 338.

MATERIALS AND METHODS

Plant and extraction: Fresh rhizomes of *Curcuma longa* were purchased from local wet market. The rhizomes were cleaned, cut into small pieces and oven dried at 45°C. Then it is powdered and macerated in 95% (v/v) ethanol for 48 h using Soxhlet apparatus. The extract was filtered using Wartman No. 1 filter and concentrated to dark yellow residue on a rotary evaporator, with the yield of 19%.

Materials and animals: Paracetamol and other chemicals were obtained from Sigma Chemicals (USA) and palm oil was purchased from local market. Paracetamol was suspended in 1% dimethyl sulfoxide (DMSO) in palm oil (150 mg mL⁻¹). Male Sprague Dawley rats (180-200 g, n= 6/group) were obtained from Institute for Medical Research (IMR), Kuala Lumpur and housed at the Animal House, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. They were divided randomly into four groups of six rats each. They were kept in polypropylene cages with wood shavings as bedding in 12 h light/dark cycle at 27±2°C. The animals were adapted to laboratory conditions for 7 days prior to the experiments and were given feed and tap water *ad libitum*. The experimental procedures were carried out in strict compliance with the Animal Ethics Committee's rules and regulation followed in this institute.

Pre-treatment was given orally daily for 7 days of either the rhizome extract (100 mg kg⁻¹) or normal saline. The animals were then treated with either paracetamol (600 mg kg⁻¹) or dosed vehicle (1% v/v DMSO in Palm oil) (Table 1). Animals were anaesthetized with pentobarbitone (70 mg kg⁻¹, ip) 48 h after the treatment and blood (3.0 mL) was collected by cardiac puncture using sterile disposable syringes. Serum was obtained by centrifugation (5000 rpm for 10 min) and stored at -20°C prior to analysis. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assayed spectrophotometrically using Sigma Diagnostic kits (USA). Animals were sacrificed by cervical dislocation. Sections from the middle lobe of the liver were fixed in formalin and processed for histopathology studies.

Statistical analysis: The results are expressed as mean±SD. One-way analysis of variance was performed and sequential differences among the means were calculated at the level of p<0.05 using Tukey-Kramer post test analysis.

RESULTS

Paracetamol at the dose of 600 mg kg⁻¹ p.o. induced significant increase in serum liver enzymes. Control (Group 1) which received normal saline and dosed vehicle serum value of ALT, AST and ALP in rats were found to be 89.3±10.5, 47.2±15.9 and 25.4±12.4 IU, respectively (Fig. 2). Rats in group 2, which received normal saline and paracetamol had significantly increased (p<0.05) liver enzyme activities with values 1354.6±217.3, 862.4±157.3 and 487.3±98.6 IU, respectively. Rats in Group 3 which received the rhizome extract and dosed vehicle had similar

Table 1: Treatment groups of rats

Groups	Pre-treatment	Treatments
1	Normal saline	1% DMSO/Palm oil
2	Normal saline	600 mg/kg paracetamol in 1% DMSO/Palm oil
3	100 mg kg ⁻¹ <i>Curcuma longa</i> extract	1% DMSO/Palm oil
4	100 mg kg ⁻¹ <i>Curcuma longa</i> extract	600 mg kg ⁻¹ Paracetamol in 1% DMSO/Palm oil

Each group (n=6 rats). Pre-treatment administered orally daily for 7 days prior to the treatment

values (p>0.05) of serum liver enzymes as the controls (Group 1) (Fig. 2). Rats in Group 4, which received the plant extract (100 mg kg⁻¹ orally, daily for 7 days) and 600 mg kg⁻¹ paracetamol were found to be 156.7±23.5 (ALT), 112.4±35.2 (AST) and 135.5±38.8 (ALP). These values were significantly lower (p<0.05) than the values of the toxic control group (Group 2) and were similar (p>0.05) to the control values (Group 1).

Histologically, paracetamol induced liver necrosis and massive inflammation of parenchyma (Fig. 3A and B) of rats in Group 2. Interestingly, *Curcuma longa* pretreated rats prior to paracetamol treatment (Group 4) had normal liver histology (Fig. 3C). Livers from rats of control group (Group 1 and Fig. 3D) and Group 3 (Rats received extract only, data not shown) showed normal liver histology.

DISCUSSION

Paracetamol-induced liver injury is commonly used as models for investigating the efficacy of hepatoprotective drugs^[1]. The raised serum liver enzymes such as ALT, AST and ALP in intoxicated rats can be attributed to the damage in the histostructural integrity of the liver cells/hepatocytes^[12]. The crude extract of *Curcuma longa* rhizomes used in this study protected the structural integrity of hepatocyte membrane. This was evident from the hepatoprotection provided by *Curcuma longa* rhizomes to rats given paracetamol, which inhibited the rise in serum liver enzymes. Many natural nonnutrient compounds present in vegetables and spices display a series of important biological properties helpful for human health including antioxidant and anti-inflammatory effects^[13] and turmeric is an excellent example of a spice with a long list of medicinal properties.

Paracetamol is mainly metabolized in the liver by glucuronidation and sulfation^[14]. However, hepatotoxicity of paracetamol has been attributed to the formation of a toxic reactive metabolite where a part of paracetamol is activated by the hepatic Cytochrome P450^[15]. This highly reactive metabolite, N-acetyl-p-benzoquinoneimine^[16] binds covalently with cellular macromolecules (proteins,

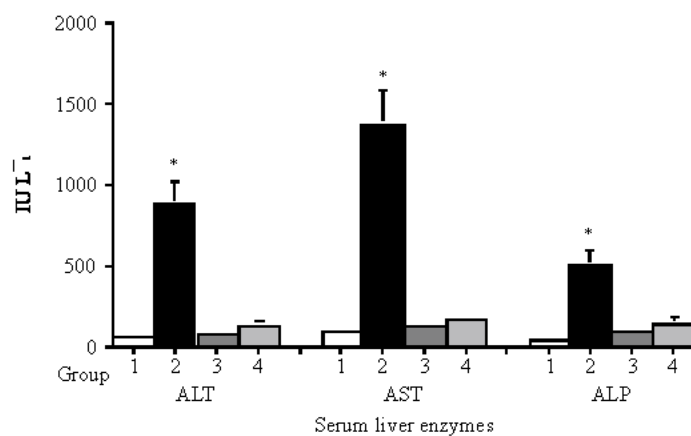


Fig. 2: Effects of ethanolic extract of *Curcuma longa* rhizomes on paracetamol-induced rise in serum liver enzymes in rats
*: Statistical difference ($p < 0.05$)

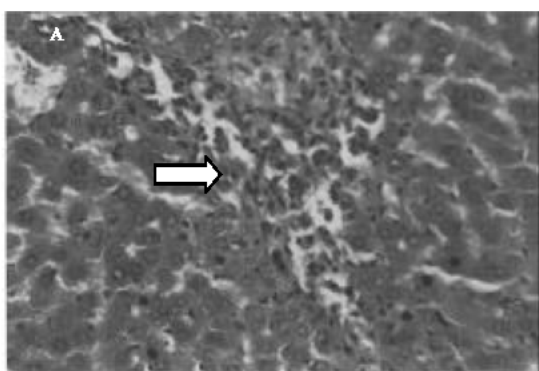


Fig. 3A: Histology of the liver from rat that received paracetamol treatment at 600 mg kg^{-1} showing necrosis of liver parenchyma. 400x.C-0.5% (w/v)

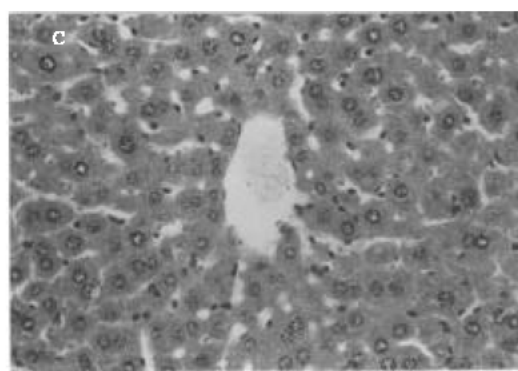


Fig. 3C: Histology of the liver from rat which received *Curcuma longa* extract and 600 mg kg^{-1} paracetamol showing normal liver histology. 400x

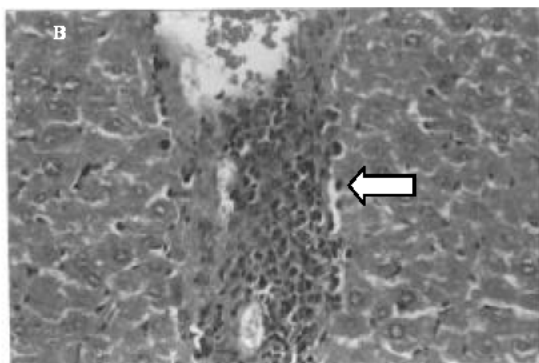


Fig. 3B: Histology of liver from rat that received paracetamol treatment at 600 mg kg^{-1} showing massive hepatitis. 400x

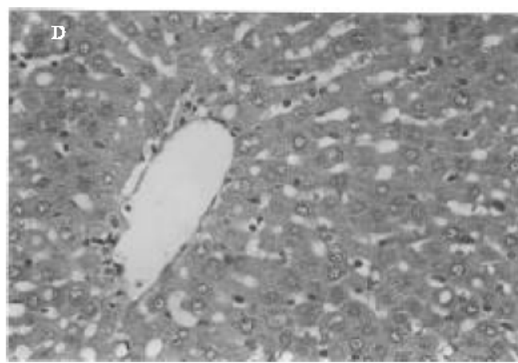


Fig. 3D: Liver histology of control rat which received normal saline and 1% DMSO/palm oil showing normal liver histology. 400x

DNA) to produce protein adducts. These modified proteins cause the dysfunction and death of hepatocytes leading to liver necrosis^[17].

The exact hepatoprotective mechanism of *Curcuma longa* extract is still unknown. The extract may either inhibit the formation of the toxic paracetamol metabolite or stimulate the hepatic regeneration. This type of stimulation appears to help the liver to become more resistant to damage caused by toxins^[18]. Furthermore, curcumin has been shown to provide protection against oxidative stress^[19]. It is believed that curcumin also has effect on the cellular oxidative metabolism^[20], which may improve the host hepatoprotective mechanism. Indeed, Donatus *et al.*^[21] demonstrated cytoprotective activity of curcumin in paracetamol induced cytotoxicity in rat hepatocytes *in vitro*. They concluded that curcumin possesses a strong anti-oxidant capacity.

Deshpande *et al.*^[22] reported that *Curcuma longa* extracts protected the liver from carbon tetrachloride toxicity (CCl₄) in rats. CCl₄ is another potent hepatotoxicant commonly used to induce liver injury. They reported all three serum parameters ALT, AST and ALP were reduced significantly when the rats were pretreated with extracts. These results were similar to the results reported in this current investigation using paracetamol as hepatotoxicant. Hepatoprotective effect of *Curcuma longa* rhizomes was observed in paracetamol-induced liver damage in rats. The present investigation reemphasizes the usefulness of *Curcuma longa* in traditional medicine as a hepatoprotectant agent used in traditional medicines.

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