

NEPHROPROTECTIVE ACTIVITY OF ETHANOL EXTRACT OF *CURCUMA MANGGA* VAL. IN PARACETAMOL-INDUCED MALE MICEROSITA¹, YUANDANI^{1,2*}, MARIANNE¹

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ABSTRACT

Objective: This study aimed to evaluate nephroprotective activity of *Curcuma mangga* in paracetamol-induced male mice.

Methods: Male mice were divided into several groups including normal control, negative, positive, and treatment groups. Treatment groups were orally administered with *C. mangga* extract at doses of 100, 200, and 400 mg/kg bw for 7 days. On day 7 after 1 h of treatment, the mice were induced with paracetamol 1.05 g/kg bw. Serum creatinine level measurement and histopathology study were performed at the end of experiment.

Results: *C. mangga* extract was able to inhibit the increase of creatinine level and showed a significantly different from negative control ($p < 0.05$) and did not differ significantly from positive control ($p > 0.05$). The result was supported by histopathology examination which did not show any cell damage. The nephroprotective effect of *C. mangga* was in a dose-dependent manner. *C. mangga* extract at dose of 400 mg/kg bw depicted the strongest nephroprotective effect.

Conclusion: *C. mangga* extract was able to protect mice kidney induced by paracetamol.

Keywords: *Curcuma mangga*, Serum creatinine, Histopathology.

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INTRODUCTION

Acute renal failure accounts for about 2% of all paracetamol poisoning events and 10% of patients with severe poisoning. In therapeutic doses, paracetamol poisoning in the kidneys occurs after the end of glutathione (due to chronic alcohol consumption, hunger, or fasting) or due to the consumption of drugs that stimulate the P-450 microsomal oxidase enzyme (e.g., anticonvulsant drugs). Acute kidney failure due to paracetamol occurs in the form of acute tubular necrosis (ATN). ATN can occur singly or together with liver necrosis [1].

The involved P-450 microsomal enzyme is found in the liver and kidneys, although it is somewhat different in each organ. The severity of kidney damage and the quantity of reactions in tissue can be significantly reduced when inhibitors of the P-450 cytochrome are available [2].

The essential oil composition of the *C. mangga* Val. consist of a monoterpen hydrocarbon class, consisting of 4 main components identified as mirsen (78.6%), β -osimen (5.1%), β -pinen (3.7%), and α -pinen (2.9%) [3]. Pharmacological studies on *C. mangga* revealed that this plant has immunomodulatory activity and did not cause short term toxicity [4-5]. Curcumin is able to prevent kidney damage in paracetamol-induced rats [6]. *Curcuma longa*, *Nigella sativa* and *Phyllanthus acidus* had been proven to have nephroprotective effect [7-9]. However, the nephroprotective activity of the ethanol extract of *C. mangga* was seldom reported.

In the present study, the nephroprotective activity of ethanol extract of *C. mangga* on to paracetamol-induced male mice was investigated. Renal function was evaluated by measuring serum creatinine level and performing histology of the kidney.

METHODS

Plant materials

Fresh rhizomes (10 kg) of *C. mangga* Val. were collected from Berastagi, North Sumatera, then authenticated and deposited in Herbarium Medanense, University of Sumatera Utara (USU).

Plant extraction

The air-dried rhizomes of *C. mangga* (500 g) were soaked at room temperature in ethanol (3 × 3 days). The extracts were filtered and evaporated to yield crude extracts (38.4 g, 10.95% w/w). The extracts were stored at 4°C until tested for bioassay.

Nephroprotective effect

All procedures involving the use of laboratory animals were reviewed and approved by the Animal Ethics Committee of USU. 30 male mice (25–30 g) were obtained from USU Animal Resource Unit (approval number 581/I[EPH-FMIPA/2017]). The animals were fed mouse pellet and fresh water *ad libitum* for a week before experiments. Mice were randomly divided into six groups containing five animals each and all treatments were given daily for 7 days. All groups administered orally. The selected doses of *C. mangga* extract were based on Khorsahandi [7] who showed the nephroprotective effects of aqueous extract of *C. longa* (Zingiberaceae family) on the paracetamol (PCT)-induced nephroprotective. Mice in Group I served as the control group. Group II served as the negative group which orally administered with carboxymethylcellulose (CMC) Na 0.5%. Group III served as the positive group and treated with Curcuma[®] 58 mg/kg of body weight. Groups IV–VI, mice were treated with extract of *C. mangga* 100, 200, and 400 mg/kg bw. All mice were induced by PCT 1 g/kg of body weight. On day 8, all animals were weighed and anesthetized with diethyl ether.

Blood collection and histological study

Blood was collected through tail of mice using a 10 mL syringe after 24 h of last treatment. Creatinine level was determined according to Jaffe's reaction on Medan Regional Health Laboratory. The kidney was obtained after the mice were sacrificed, then washed in NaCl to remove blood. For histological studies, the kidney was preserved in 10% formalin for biochemical analysis. The kidney was determined on Pathology Laboratory in Murni Teguh Hospital.

Statistical analysis

Statistical analysis was performed using SPSS version 17.0. Data were analyzed using Shapiro–Wilk normality test and one-way analysis of

variance was used for comparison between groups and followed by *post hoc* Tukey test. Data were expressed as mean \pm SEM and $p < 0.05$ showed statistical significance.

RESULTS AND DISCUSSION

Serum creatinine levels

Table 1 summarizes the levels of serum creatinine after treatment with extract of *C. mangga*. The ethanol extract of *C. mangga* was administered in various doses to know the ability of *C. mangga* to neutralize kidney damage due to toxic doses of paracetamol with indicator of decreased serum creatinine levels and microscopic of renal kidney organs.

The serum creatinine levels of *C. mangga* group were significantly different with those of negative control group. The serum creatinine levels of the normal group were 0.3700 mg/dl which remained in the normal range of serum creatinine mice (0.3–1.0 mg/dL) [10].

CMC Na 0.5% ($p < 0.05$), signifying that *C. mangga* extract displayed nephroprotective effect. Of all the samples, *C. mangga* at dose of 400 mg/kg bw showed the highest nephroprotective activity which was comparable to those of positive control (Curcuma[®] 58 mg/kg bw). Curcuma tablet contains curcumin which has been proven to prevent kidney damage in paracetamol-induced rats [2]. The nephroprotective effect of *C. mangga* was in a dose-dependent manner. The increment of dose of *C. mangga* extract demonstrated the enhancement of nephroprotective activity.

The toxic doses of paracetamol cause significant tissue damage associated with glutathione depletion and lipid peroxidation resulting in intracellular accumulation and high reactive metabolite binding N-acetyl-p-benzoquinone imine, liver cell damage, and often end with death. Similar effects also occur in kidney tissue [11]. This results in the accumulation of paracetamol which results in a biochemical chain reaction and culminates in acute and chronic nephropathy [12]. In addition, paracetamol also triggers apoptosis in the liver and kidney cells [13,14].

The nephrotoxic effect of paracetamol toxic dose is associated with metabolic disorders of serum electrolytes, blood urea nitrogen, and serum creatinine [11]. Creatinine is derived from endogenous tissue keratin cleavage [15].

Table 1: Serum creatinine levels of male mice (mean \pm SEM, n=5)

Groups	Serum creatinine level (mg/dL)
Normal	0.37 \pm 0.57
Negative control (paracetamol)	1.28 \pm 0.08*
Positive control (curcuma 58 mg/kg bw)	0.45 \pm 0.04
Treatment group (<i>C. mangga</i> 100 mg/kg bw)	0.81 \pm 0.07 ^{a, b*}
Treatment group (<i>C. mangga</i> 200 mg/kg bw)	0.72 \pm 0.072 ^{a, b*}
Treatment group (<i>C. mangga</i> 400 mg/kg bw)	0.53 \pm 0.08 ^{a*}

Data were analyzed by one-way ANOVA, and followed by Tukey *post hoc* test.

^a $p < 0.05$ compared to negative control. ^b $p < 0.05$ compared to positive control

* $p < 0.05$ compared to from the normal, *C. mangga*: *Curcuma mangga*

Based on data analysis of serum creatinine level performed on control group, it was found that toxic dose of paracetamol in negative control group increased serum creatinine level as compared to normal group. The result was in agreement with previous studies which showed significant increase in serum creatinine in the administration of toxic doses of paracetamol [15-16].

Creatinine is a muscle degradation product that indicates a renal dysfunction if the levels exceed the normal limits. Serum creatinine is a strong indicator of renal function and its concentration is relatively constant from day to day [17]. Curcuminoid as an antioxidant in *C. mangga* neutralizes kidney damage due to toxic doses of paracetamol with indicator of decreased serum creatinine levels [18].

Microscopic examination

The nephroprotective effect of *C. mangga* was supported by microscopic examination as shown in Table 2.

The histopathological changes were observed after induced by paracetamol toxic dose. The ethanol extract of *C. mangga* prevented the damages of kidney. However, dilatation of tubular lumen was still observed at dose of 100 and 200 mg/kg bw. The complete protection on kidney was observed after treatment with *C. mangga* at dose of 400 mg/kg bw as compared to positive control (Fig. 1).

Paracetamol-induced nephrotoxicity may incorporate several molecular pathways of apoptosis including removal of intracellular protective molecules and caspase activation. Although paracetamol does not alter the expression of messenger ribonucleic acid in the antiapoptotic Bcl-xL gene, it can lower Bcl-xL protein, which means it can increase apoptotic activity [19].

Paracetamol also induces the stress of the endoplasmic reticulum on renal glomeruli, which causes oxidative stress and inflammation in podocyte cells as well as mesangial glomeruli [19]. The reactive oxygen species compound, which is the result of paracetamol metabolism, can also cause glomerular damage that begins with leukocyte infiltration [21]. One of the adverse effects of paracetamol overdose is renal tubular necrosis [22]. Damage of renal organ cells in the group with ethanol extract of *C. mangga* at dose of 100 and 200 mg/kg bw extract showed less damage as compared to control group. The ethanol extract of *C. mangga* at dose of 400 mg/kg bw did not showed any renal cell damage when compared with other treatment groups.

CONCLUSION

The ethanol extract of *C. mangga* was able to protect kidney damage due to toxic dose of paracetamol. The strongest protection shown by serum creatinine level was observed at dose of 400 mg/kg bw. The result was supported by histopathological study which showed no damage on kidney organ. However, further studies are required to elaborate the molecular mechanism of *C. mangga* as nephroprotective agent.

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Table 2: Effect of *C. mangga* extract on kidney

Groups	Glomerular atrophy	Dilatation of lumen tubulus	Necrosis
Normal	-	-	-
Negative control (CMC Na 0.5%)	++	++	++
Positive control (curcuma 58 mg/kg bw)	-	-	-
Treatment group (<i>C. mangga</i> 100 mg/kg bw)	-	+	-
Treatment group (<i>C. mangga</i> 200 mg/kg bw)	-	+	-
Treatment group (<i>C. mangga</i> 400 mg/kg bw)	-	-	-

-: Normal, +: Light, ++: Medium, CMC: Carboxymethylcellulose, *C. mangga*: *Curcuma mangga*

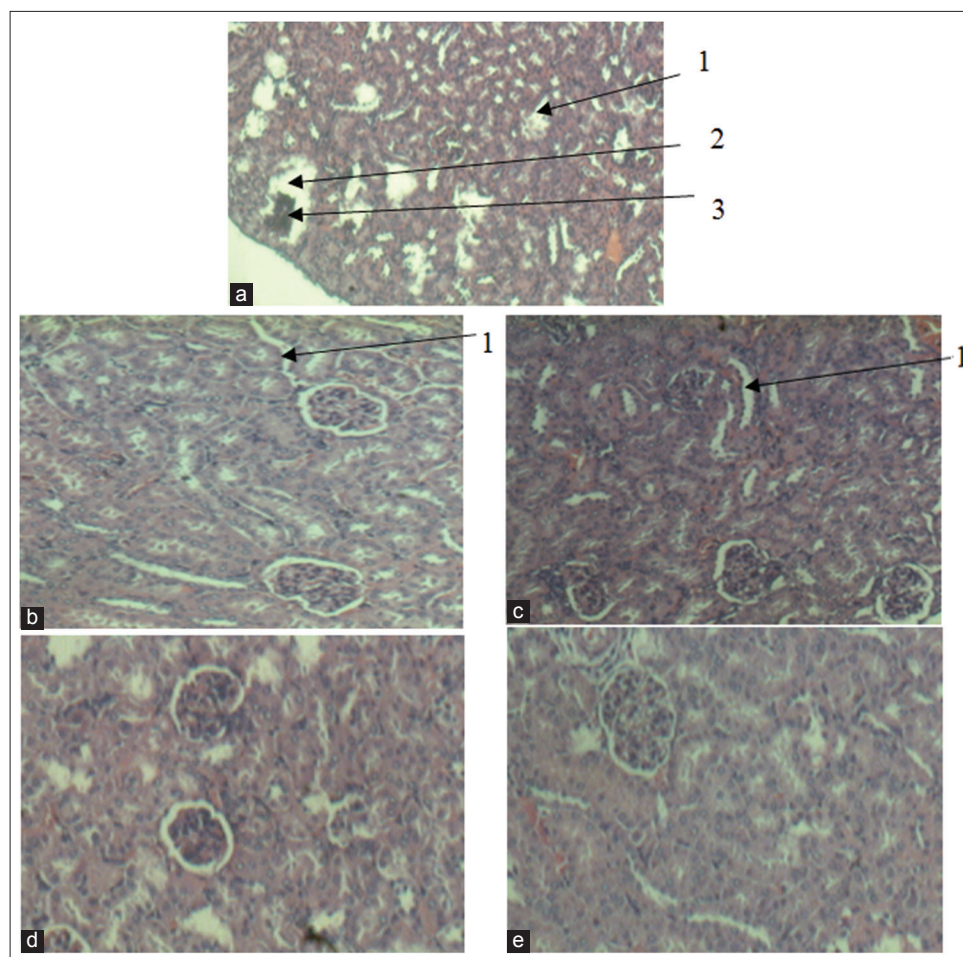


Fig. 1: Microscopic examination on kidney (a) carboxymethylcellulose Na 0.5% (b) *Curcuma mangga* extract 100 mg/kg bw (c) *C. mangga* extract 200 mg/kg bw (d) *C. mangga* extract 400 mg/kg bw (e) curcuma tab. (1) Glomerular atrophy (2) Dilatation of lumen tubulus (3) necrosis

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