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Effect of Yellow Root Ethanol Extract (Fibraurea tinctoria Lour.) on Liver Histopathology of Paracetamol-Induced Male Swiss Webster Mice

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Abstract

Paracetamol, a commonly used analgesic and antipyretic, can cause liver damage at toxic doses due to excessive formation of N-acetyl-p-benzoquinone-imine (NAPQI), which depletes glutathione. This study aimed to evaluate the hepatoprotective effect of ethanol extract from yellow root (Fibraurea tinctoria Lour.) on paracetamol-induced liver damage in male Swiss Webster mice (Mus musculus). Thirty mice were divided into six groups: a healthy control, a negative control (paracetamol only), a positive control (curcuma extract), and three treatment groups receiving yellow root extract at doses of 75, 150, and 300 mg/kg BW. The treatment was administered orally for seven consecutive days, followed by paracetamol induction on day eight. Liver histopathology was assessed using hematoxylin-eosin staining, focusing on indicators such as hepatocyte damage, necrosis, and inflammation. The results showed that an ethanol extract of yellow roots (Fibraurea tinctoria Lour.) at a dose of 300 mg/kg BW positively affected the liver histopathology in male Swiss Webster mice (Mus musculus). This dose appeared to reduce hepatocyte necrosis and inflammation while maintaining liver structure, suggesting a dose-dependent protective effect on liver tissue at 300 mg/kg BW.

Keywords: Hepatoprotector, Histopathology, Yellow Root (*Fibraurea tinctoria Lour.*)

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Introduction

The liver is a vital organ responsible for essential metabolic processes, including macronutrient metabolism, blood detoxification, and homeostasis of lipids and cholesterol. However, liver damage can occur when detoxification processes fail, potentially leading to conditions like liver cirrhosis and failure[1]. Liver cirrhosis, characterized by fibrous tissue formation and regenerative nodules, impairs liver structure and function, reducing regenerative capacity and causing hepatocyte death[2]. Globally, cirrhosis ranks as the 14th leading cause of death, with 1.03 million annual fatalities, and has a prevalence of 3.5% in Indonesian hospitals[3].

Drug-induced liver injury (DILI) is a major cause of liver damage, often triggered by oxidative stress, mitochondrial dysfunction, and inflammation(1). Paracetamol, a commonly used over-the-counter drug, becomes hepatotoxic at high doses due to excessive N-acetyl-p-benzoquinone-imine (NAPQI) formation, which depletes glutathione and damages hepatocytes through oxidative stress[4,5].

Antioxidants mitigate liver damage by counteracting oxidative stress, serving as hepatoprotectors. Among natural sources, *Fibraurea tinctoria Lour*. (Yellow Root) has shown significant potential due to its phytochemical composition, including alkaloids, flavonoids, saponins, and tannins[6,7]. What makes *Fibraurea tinctoria Lour.* unique among hepatoprotective agents is its multifaceted mechanisms of action and dual therapeutic potential. The plant is rich in bioactive compounds such as berberine, jatrorrhizine, and palmatine, which exhibit antioxidant, anti-inflammatory, antidiabetic, and anticancer properties[8–11].

Despite its traditional use in Southeast Asia for liver ailments, *Fibraurea tinctoria Lour.* remains underexplored in modern research, particularly in dose-dependent studies and its effects on histopathological changes induced by hepatotoxic drugs like paracetamol. This study aims to evaluate the hepatoprotective potential of ethanol extract from *Fibraurea tinctoria Lour.* in paracetamol-induced liver damage in male *Swiss Webste*r mice, focusing on histopathological changes to validate its efficacy and underlying mechanisms.

Method

Preparation of Research Samples

This study used 30 male *Swiss Webster* mice (Mus musculus), each weighing 20-30 grams and aged 2-3 months. Inclusion criteria included healthy mice with active movements and normal appearance. Mice were excluded if they were found dead or exhibited signs of illness, such as inactivity, lack of appetite, dull fur, or fur loss.

Acclimatization and Maintenance of Test Animals

The mice were acclimated for seven days prior to the research procedure to familiarize them with the food and environment. During this period, the animals were housed in plastic cages with ram wire lids and seam-lined interiors. Each cage had a base area of approximately 148.4 cm^2 and a height of 17.8 cm^2. The mice were fed peeleet and provided with food and water ad libitum. The cage environment was maintained at a non-humid condition, with a temperature of approximately 25°C. Each group of mice was housed in separate cages, and their health was monitored daily in accordance with guidelines[12].

Treatment of Test Animals

The mice were divided into six groups and treated orally once per day for seven consecutive days[13]. On day eight, 24 hours

after the last treatment, a peroral dose of 250 mg/kg BW paracetamol[14] was administered to induce liver damage. On day nine, 24 hours after paracetamol administration, the mice were euthanized. The livers were dissected for macroscopic examination, liver weight measurement, and histopathological analysis.

Macroscopic Observation of Liver Organ

Macroscopic observations focused on the shape and color of the liver surface. The results were recorded and documented[5].

Preparation of Hispatology Preparations

The liver was cleansed using physiological NaCl solution, and its weight was measured. The tissue was fixed in 10% neutral buffered formalin to preserve its structure. The fixed liver tissue underwent a dehydration process using graded alcohol concentrations (70%, 80%, 90%, and 95%) for 24 hours, followed by immersion in 100% alcohol for 1 hour, repeated three times to ensure complete dehydration.

Subsequently, the tissue was cleared using xylol for 1 hour, with this process also repeated three times to remove any residual alcohol. The cleared tissue was then infiltrated with paraffin and embedded in paraffin media to create tissue blocks. These blocks were sectioned into thin slices with a thickness of 4–5 microns using a microtome. The tissue sections were then stained with Hematoxylin and Eosin (H&E) to visualize cellular and structural details. The prepared slides were finally examined under a light microscope to evaluate histological features[12].

Result and Discussion

This study adhered to ethical guidelines for the care and use of laboratory animals, ensuring the humane treatment of test subjects. Ethical approval for this research was granted by the Ethics Committee of Universitas Muhammadiyah Purwokerto, with an ethical clearance certificate number KEPK/UMP/181/IV/2024.

Quality Standard Testing of Yellow Root Extract

The quality standard test for yellow root extract (*Fibraurea tinctoria Lour*.) included specific and non-specific parameters. Specific parameters, as shown in Table 1 and Non-specific parameters, are presented in Table 2. Both of which met established standards of less than 10%[15,16].

The loss on drying reflects the amount of volatile compounds, including water, essential oils, and solvents, that evaporate during the drying process. A higher value may indicate the presence of residual solvents, potentially affecting the extract's quality[17], The moisture content indicates the residual water in the extract, which impacts its stability and susceptibility to microbial contamination. With a moisture content of 6.21%, the yellow root extract demonstrated high stability and resistance to enzymatic degradation or microbial spoilage.

Extraction Yield and Phytochemical Screening

From 2000 g of yellow root powder, the extraction process produced 227.16 g of condensed extract, resulting in a yield of 11.36%, which exceeds the minimum standard of 10%. This high yield suggests a significant concentration of active compounds in the extract[18].

Phytochemical testing confirmed the presence of various bioactive compounds in the yellow root extract, each contributing to its hepatoprotective properties. Flavonoids were identified by the formation of a deep red color during testing with Mg + HCl. These compounds, including quercetin and

luteolin, are potent antioxidants that neutralize free radicals, reduce oxidative stress, and enhance endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase[19]. Alkaloids showed positive results in tests using Wagner, Mayer, and Dragendorff reagents, characterized by brown, white, and red precipitates, respectively. Notably, alkaloids like berberine protect hepatocytes by reducing inflammation and boosting antioxidant defenses[8]. Tannins were identified by a greenish-brown color in $FeCl_3$ tests, demonstrating their antioxidant properties, including chelating metal ions, inhibiting lipid peroxidation, and mitigating oxidative stress[20]. The presence of terpenoids was confirmed by a brownish-red color during testing, highlighting their role in modulating metabolic pathways and enhancing liver regeneration, as evidenced by studies on *Panax notoginseng*[21]. Saponins, identified through the formation of stable foam, have shown the ability to ameliorate toxin-induced liver damage by reducing oxidative stress and promoting cellular repair[22]. Together, these compounds contribute synergistically to the hepatoprotective potential of yellow root extract.

Histopathological Analysis

Figure 1 illustrates liver morphology across treatment groups. The negative control (Na CMC) and Treatment 1 (75 mg/kg BW) showed pale red livers with white spots, indicating significant damage. In contrast, the positive control (Curcuma), Treatment 2 (150 mg/kg BW), and Treatment 3 (300 mg/kg BW) displayed improved morphology, with Treatment 3 closely resembling the healthy group. This suggests a dose-dependent hepatoprotective effect of yellow root extract, with the highest dose providing the greatest protection.

Paracetamol-induced liver damage primarily results from the formation of free radicals during its biotransformation, leading to the disruption of endoplasmic reticulum membranes and oxidative stress. This process depletes glutathione (GSH), a critical antioxidant, allowing NAPQI) to bind to hepatocytes, forming toxic protein adducts that trigger cellular damage and necrosis. The interaction between free radicals and unsaturated fatty acids in cell membranes further generates unstable peroxides, exacerbating membrane degradation and structural damage[23].

Figure 1 illustrates liver morphology across treatment groups. The negative control (Na CMC) and Treatment 1 (75 mg/kg BW) showed pale red livers with white spots, indicating significant damage. In contrast, the positive control (Curcuma), Treatment 2 (150 mg/kg BW), and Treatment 3 (300 mg/kg BW) displayed improved morphology, with Treatment 3 closely resembling the healthy group. This suggests a dose-dependent hepatoprotective effect of yellow root extract, with the highest dose providing the greatest protection.

The hepatoprotective effects of yellow root (*Fibraurea tinctoria Lour.*) stem from its bioactive compounds, particularly flavonoids, alkaloids, saponins, and terpenoids. Flavonoids act as potent antioxidants by neutralizing free radicals, preventing lipid peroxidation, and enhancing endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase[24]. Alkaloids, such as berberine, further support hepatoprotection by reducing pro-inflammatory cytokines, increasing glutathione levels, and modulating signaling pathways like NF-κB and Nrf2 to combat oxidative stress[9]. Saponins and terpenoids complement these effects by promoting liver regeneration and stabilizing cellular membranes through their antioxidant and anti-inflammatory properties[21,22].

The antioxidant mechanism of yellow root involves multiple pathways: preventing oxidant production, directly scavenging free radicals by donating electrons, and indirectly enhancing the activity of natural antioxidants. This multifaceted mechanism resembles that of curcumin, a well-documented hepatoprotective agent, which enhances glutathione synthesis, limits lipid peroxidation, and reduces hepatocyte apoptosis. These findings suggest that yellow root extract, particularly at higher doses (300 mg/kg BW), effectively mitigates liver damage induced by paracetamol[11,25].

Limitations

This study has limitations, including a small sample size, lack of toxicity profiling at higher doses, and absence of biochemical markers like ALT and AST to complement histopathological findings. Future research should address these gaps by increasing sample size, conducting toxicity assessments, and incorporating biochemical analyses for a more comprehensive evaluation of the extract's safety, efficacy, and mechanisms.

Conclusion

The results demonstrated that an ethanol extract of yellow roots (*Fibraurea tinctoria Lour*.) at a dose of 300 mg/kg BW positively influenced liver histopathology in male Swiss Webster mice (*Mus musculus*). The extract reduced hepatocyte necrosis and inflammation while maintaining liver structure, indicating a dose-dependent hepatoprotective effect. These findings highlighted the potential of yellow root extract as a natural therapeutic agent for managing liver damage caused by oxidative stress or drug-induced hepatotoxicity.

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Conflict of Interest

The authors declare no conflict of interest related to this study.

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Table 1. Results of Specific Parameter Measurements.

Standard Parameters	Type	Characteristics	Results
Specific Parameters	Organoleptics	Shape	Thick sticky
		Smell	Typical
		Taste	Bitter
		Color	Dark brownish yellow

Table 2 Results of Non-Specific Parameter Measurements.

Standard Parameters	Characteristics	Result (%)	Parameter (%)
Non-specific Parameters	Loss on Drying	13,53	<10
	Moisture content	6,21	<10

Table 3. Phytochemical Testing Results.

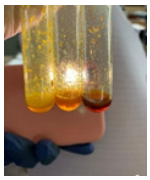
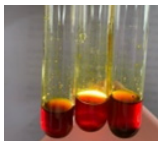
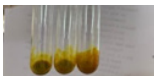
Test	Reagen	Result	Description	Observation
Flavonoids	Mg + HCl	Produces a deep red color	+	
Alkaloids	Wagner	Produces a brown precipitate	+	
	Mayer	Produces white precipitate	+	
	Dragendorf	Produces red precipitate	+	
Tannins	FeCl3	Produces a greenish brown color	+	
Terpenoids	Anhydrous Acetic Acid + Concentrated Sulfuric Acid	Produces a brownish red color	+	
Saponins	Hot Water + 2N HCl	Produces stable foam as high as 1.5 cm	+	

Table 4. Hispatology Test Results.

Organs	Group	Feeding	Description
Liver	Healthy	Without Treatment	Normal, no abnormalities
	Negative	Na CMC	Parenchymatous degeneration, hydropic degeneration, fatty degeneration
	Positive	Curcuma	Necrosis++
	Treatment 1	75 mg/Kg BB	Parenchymatous degeneration, hydropic degeneration, fatty degeneration
	Treatment 2	150 mg/Kg BB	Hydropic Degeneration, Fatty Degeneration, Necrosis++
	Treatment 3	300 mg/Kg BB	Hydropical Degeneration, Fatty Degeneration

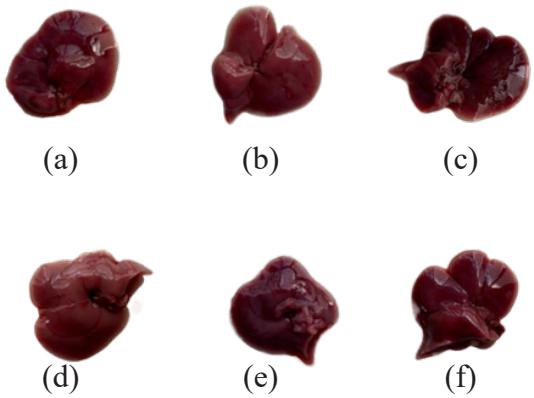


Figure 1. (a) Healthy Control, (b) Negative Control (c) Positive Control, Treatment 1 (75 mg/Kg BW) (d), Treatment 2 (150 mg/Kg BW) (e), Treatment 3 (300 mg/Kg BW) (f).

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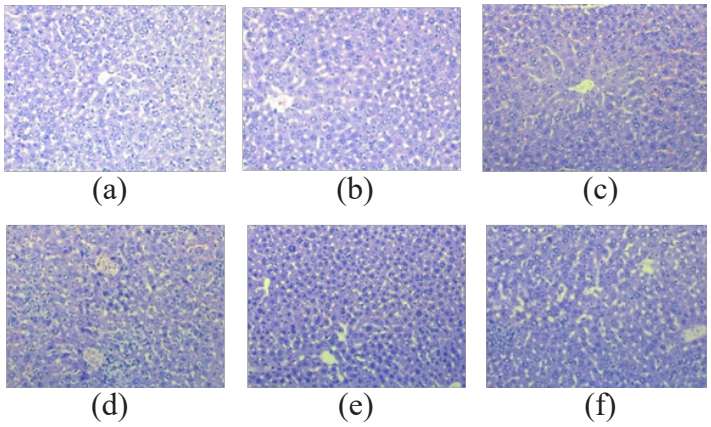

Figure 2. Liver Histopathology of Mice. Healthy Group (a), Negative Control (b), Positive Control (c), Treatment 75 mg/Kg BW (d), Treatment 150 mg/Kg BW (e), Treatment 300 mg/Kg BW (f).