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Research Article

Hepatoprotective Effect of Corn Silk Infusion in Male Wistar Rats

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Abstract: The use of medicinal plants in treating various disease has been reported since long time ago, including for hepatic disease. Corn silk contains phytochemicals of medical benefit such as flavonoids compounds which act as antioxidant agents and has been widely reported possess hepatoprotective effect. Using a model of carbon tetrachloride (CCl_4)-induced hepatotoxicity in 36 male Wistar rats, this study investigated the effect of corn silk infusion and assessed using enzymes produced by the liver in plasma [alkaline phosphatase (ALP) and liver glutathione (GSH)]. The corn silk infusion (in 50, 100, and 200 mg/kg BW doses) were administered 24 hours after CCl_4-induction liver damage with 3 mL/kg BW CCl_4 in olive oil (1:1, v/v), intraperitoneally for seven days. Along with corn silk groups, distilled water (0.2 mL/kg BW) and Curcumin (100 mg/kg BW) were given for induction and drug control, respectively. In the end of the study (8^{th} day), the level of both ALP dan GSH were measured. The differences among groups for GSH and ALP level were statistically calculated using ANOVA method. The result showed that the corn silk infusion is active at 200 mg/kg BW based on both ALP (18.74% decreased) and GSH (5-7% increased) level. Moreover, the flavonoid compound was detected on the infusion that may contribute on its hepatoprotective activity. In conclusion, corn silk infusion owned hepatoprotective effect in male Wistar rats.

Keywords: corn silk, hepatoprotective, ALP, GSH

Introduction

Medicinal plants have been use traditionally for preventing and treating various disease and contribute to the development of human health and welfare [1]. Moreover, medicinal plants are the most affordable treatment [2] for population. Corn silk (Zea mays, L.), is part of the corn with limited exploration on its potential activities. Traditionally, it uses for relieving pain and other medical purposes. Meanwhile, based on previous research, corn silk possess anti-fatigue activity [3], immunostimulant activity [4], antimicrobial activity [5], and antioxidant activity [6]. Furthermore, another research described that the active compound of corn silk are phenolic compounds such as flavonoid which act as an antioxidant [7] and elicit hepatoprotective effect on its ethanolic extract using MDMA-induced model [8]. The liver function can be evaluated from the level of enzyme produced by the liver, alkaline phosphatase (ALP) and glutathione (GSH). Alkaline phosphatase is related to bile production and its augmentation indicate liver problem [9]. Glutathione in reduced form (GSH) is an oxidative stress indicator which act as protective mechanism against oxidative damage. The low level of GSH indicate of high level of free radical that induce liver damage [10]. Since the MDMA (3,4-methylendioxymetammfetamine) are strictly distributed for drug abuse reason, carbon tetra chloride become a choice for inducing liver damage. Therefore, this study was focus on hepatoprotective effect of corn silk infusion (regarding the traditional uses on population) using CCl_4-induced liver damage of male Wistar rats.

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Materials and Methods

Materials

The materials included corn silk, distilled water, ALP reagent (Fluitest® ALP DGKC, Analitycon), GSH reagent (Glutathione Assay Kit, Sigma-Aldrich®), filter paper (20-25 μm of pore size), CCl_4, olive oil (Brataco®), standard curcumin (pharmaceutical graded), Carboxymethyl Cellulose (Brataco®), ketamine (pharmaceutical graded), standard animal pellets, animal bedding, xylazine (pharmaceutical graded), heparin (pharmaceutical graded), H_2SO_4 (pharmaceutical graded), and 1% v/v of $FeCl_3$ (pharmaceutical graded). Each material were obtained from laboratory of Pharmacology, Department of Pharmacy, Universitas Islam Indonesia.

Equipments

The equipment included laboratory glassware (Pyrex®), cabinet dryer, grinder, mesh no. 40, microcuvette, micropipette, centrifuge (bioSan®), analytical balance (Ohaus®), spectrophotometer (Shimadzu®), plate reader (Thermo Scientific, Multiskan FC®), waterbath, animal cage, gavage syringe, syringe injection, rotary evaporator, animal balance.

Plant Extraction and Phytochemical Identification

The corn silk has been locally collected from corn field in Sleman district, Yogyakarta, Indonesia. The silk for this research was the silk of the corn that remain inside of the cornhusk to minimize the contamination. Its authentication process was done in Laboratory of Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta. The collected corn silk then washed using running water to diminish the dirt, dried in cabinet dryer for 15 hours (50°C), and grounded using grinder and sieved using mesh no 40. Starting the extraction, 100 grams of grounded corn silk was boiled with 1.1 L distilled water (15 minutes, 90°C) followed by filtration and evaporation using rotary evaporator. The active compound was identified by using colour-reaction method. Identification of polyphenol compound was made by mixing 2 mL of the extract with 1% of ferric chloride which elicit a blue, dark blue or dark green colour. For assessing flavonoid content, it used Mg powder mixed with HCl solution that produced red or violet colour. While foaming test was used for saponin identification, hydrochloric acid addition formed red-colour layer in the bottom of the flask as the occurrence of steroid in the extract.

The concentration of corn silk infusion in this study were 50, 100, and 150 mg/kg BW. With maximum volume administration in rats is 2 mL (standard 200 grams – rats body weight), 5 mg of corn silk infusion in one mL of water (5mg/mL) exhibited the concentration of 50 mg/kg BW. Meanwhile, for 100 and 150 mg/kg BW, it needs 10 and 20 mg of corn silk infusion on each mL of water respectively (10mg/mL and 20 mg/mL).

Laboratory Animals

This study used healthy male Wistar rats weighed 200 – 300 grams, 2 – 3 months old, with normal range of ALP (44-147 IU/L)[11] and GSH level (1.3–1.6 nmol/mL)[12]; determined at day-0 by blood identification. The animals that indicate stressed, sick, having normal liver function after CCl4 induction and or death during study was excluded. The animal study was purchased from Department of Pharmacology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. All animals were maintained under standard temperature (23 ± 2°C), 12 hours light cycle (dark and light), fed with standard diet, and drinks ad libitum. The animal procedures have been authorized by the ethical committee of Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia (Ref. 32/Ka.Kom.Et/70/KE/II/2017). The animal study was acclimatized for 7 days prior to the study.

Hepatoprotective assay

The animals study was randomly divided into 6 groups (6 rats each group) as follow: Group I was control normal animals, without CCl_4-induction and treatment except food and drinks ad libitum, Group II was induced by CCl_4 and given with distilled water (0.2 mL/kg BW), Group III was administered with

Curcumin (100 mg/kg BW) as a drug control after CCl_4-induction, while Group IV, V, and VI were administered with corn silk infusion in 50, 100, and 200 mg/kg BW doses after CCl4-induction, respectively. The liver damage induction by CCl_4 (3 mL/kg BW in olive oil (1:1, v/v), intraperitoneally) were carried out at the first day of experiments and followed by treatments administration at second to 8th days of experiments [8]. Blood were collected via orbital puncture (anesthetized with the combination of ketamine and xylazine) at day 0, 2 and 8, and sacrificed with high dose of ketamine for termination in the end of the study [8]. Fresh blood collection was further examined for its liver enzyme level using kit reagent ALP (Fluitest® ALP DGKC, Analitycon) and GSH (Glutathione Assay Kit, Sigma-Aldrich®) adhere on its reagent manual instruction. The ALP identification was made by mixing 2 µL of serum with 1000 µL of ALP reagents, followed by absorbance determination (λ=405 nm) using spectrophotometer. While for GSH level was measured by the level of enzyme resulting in the absorbance. The measurement was performed in triplicate for each concentration.

Data Analysis

The ALP level was calculated using formula:

$$\text{ALP (IU/L)} = 2757 \times \Delta A_{405}\ \text{nm/min} \quad (1)$$

Where:

2757 = extinction coefficient
ΔA_{405} nm/min = mean of absorbance
ALP (IU/L) = Alkaline Phosphatase (International Units per Liter)

While, for the GSH level was calculated using formula:

$$\text{GSH (nmoles/mL)} = \frac{\Delta A412/\min(\text{sample}) \times \text{dil}}{\Delta A412/\min(1\ \text{nmole}) \times \text{vol}} \quad (2)$$

Where:

ΔA_{412}/min (sample) = slope generated by sample (after subtracting the values generated by the blank reaction)
ΔA_{412}/min (sample) = slope calculated from standard curve for 1 nmole of GSH
dil = dilution factor of original sample
vol = volume of sample in the reaction in mL

The data of both ALP and GSH level were analyzed using SPSS® 16 with ANOVA statistical method to compare the differences between groups ($p<0.05$).

Results and Discussions

Phytochemical Identification

In this part, the corn silk infusion was investigated on its phytochemical compounds as shown in Table 1. Using colour-reaction methods, the result showed that corn silk infusion possesses flavonoid, polyphenol, saponin, and steroid. This finding supports previous result that also found similar compounds [13-17]. Moreover, previous research also stated that antioxidant properties of corn silk showed free radical scavenging activity, inhibition of lipid peroxidation, and catalytic activity of chelating metal ion [18]. Particularly on flavonoids, another study proved that flavonoid as phenolic compounds own strong antioxidant activity [7] which plays an important role on hepatoprotective activity since it delays oxidation process at lower concentration [19]. Polyphenol, as non-enzymatic antioxidant, possess aromatic ring and water-soluble properties. It works by interrupting free radical chain reaction [16]. While saponin are well-known as an effective scavenge activity that can inhibit progressive autoxidative damage [17], steroid plays an important role on biochemical pathways of antioxidant [18].

Table 1. Phytochemical identification of corn silk infusion

Sample	Flavonoid	Polyphenol	Saponin	Steroid
Corn silk infusion	+	+	+	+

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Evaluation of Liver Function

Alkaline Phosphatases (ALP) are homodimer enzymes that can be found in several tissues but abundant in liver [20, 21]. It is a canicular enzyme that plays a role in bile production with its elevation level may indicate liver problem, cholestasis [9]. Meanwhile, glutathione (GSH) is a vital substance in detoxification and cell physiology [22]. Low level of GSH indicate a liver problem. Carbon tetrachloride can elucidate liver damage with the mechanism of action such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity [23] that alter the biomarker level of the liver, increasing ALP level and lowering GSH level.

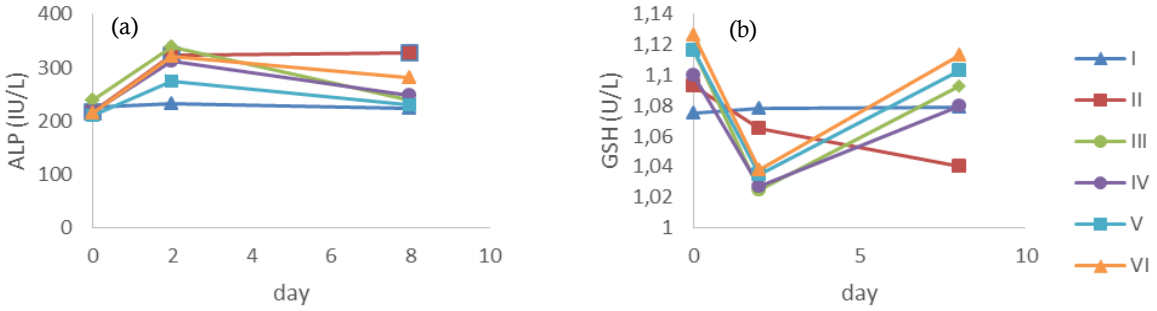

Figure 1. Result of ALP (a) and GSH (b) activity

Group I (control normal); II (CCl_4-induction, 3 mL/kg BW in olive oil); III (CCl_4 + curcumin 100 mg/kg BW); IV (CCl_4 + 50 mg/kg BW of corn silk infusion); V (CCl_4 + 100 mg/kg BW of corn silk infusion); VI (CCl_4 + 200 mg/kg BW of corn silk infusion), (n=6).

The effect of corn silk infusion treatment on liver function was described by the level of ALP and GSH level (Fig. 1). The enzyme level on day 0 was a baseline, while the high level of those enzyme on day 2 indicated the successful CCl4-induction liver damage. Following the treatment administration, the enzyme level on day 8 resemble the hepatoprotective effect of the corn silk infusion. Among three concentrations tested, 200 mg/kg BW of corn silk infusion represented the most effective concentration on lowering ALP (267.71±71 U/L) significantly and elevating GSH (1.103±0.038 nmoles/mL) level (compared to day 2; 335.02±56.30 U/L and 1.035±0.021 nmoles/mL, respectively). Both ALP and GSH level on all concentration were significantly different ($p<0.05$) against negative control although there were no significantly differences among groups ($p>0.05$). This findings was supporting previous research published the effect of corn silk ethanol extract elevate and maintain GSH level which is strongly related to its antioxidant activity via up regulation of Nrf2 [24]. Other study defined the antioxidant properties of corn silk was connected to diminishing level of ALT and augmenting level of GSH [25]. Flavonoid is one of active compound on corn silk which evidently having high antioxidant level and uppermost DPPH scavenging activity [26]. Based on those previous studies, the activity of corn silk infusion on enzyme alteration in this study may connected on its active compound that has been identified in this study, a flavonoid compound. A study mentioned that flavonoid can neutralize free radicals, CCl_4 in this regard, since it has aromatic ring as an electron donor that stabilize the free radicals ion [27].

Conclusion

The corn silk infusion possesses hepatoprotective activity with flavonoid as a major compound that responsible on its activity in CCl_4-induced male Wistar rats. The best activity was obtained at 200 mg/kg BW of corn silk infusion. It decreased the ALP level by 18.74% and increased GSH level by 7.210%.

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