Solanum nigrum L. as a Hepatoprotective Agent

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Abstract

Background: Liver damage may be caused by various factors. *Solanum nigrum* L. fruit is known to contain flavonoid antioxidant which is responsible for its hepatoprotective effect. A study was conducted to determine the protective effect of *Solanum nigrum* L. fruit infusion (SNFI) on CCl_4-induced hepatic cell damage in rats.
Methods: A complete randomized experimental study was conducted on 25 male Wistar strain-white rats (Rattusnorvegicus) which were divided into five groups during the period of September– October 2012. Group I (negative control) was given standardized food and water; group II (positive control) was induced by carbon tetrachloride (CCl_4) 10% paraffin intraperitoneally by 8 mL/kg body weight on the 8th day of the study; group III, IV, V (treated) were given *Solanum nigrum* L. fruit infusion (SNFI) by 22.5g/100mL, 45 g/100mL and 90g/100mL concentrations for 8 days, respectively, prior to CCl_4 induction. The calculation of necrotic liver cells was performed in 48 hours after induction. Data were statistically analyzed using Kruskal-Wallis test followed by Mann-Whitney post-hoc test.
Results: The percentage of necrosis liver cells in group III, IV, and V was smaller compared to the positive control group. The protective effect of SNFI against CCl_4-induced hepatotoxicity may be related to its ability to elevate the antioxidant agent in the body. There were significant differences in necrotic between Group II and group III, IV, V which were treated with SNFI.
Conclusion: Further investigation is required to characterize the active ingredients and the mechanism of SNF action to confirm the hepatoprotective and antioxidant effects.

Keywords: CCl_4, hepatoprotective, *Solanum nigrum* L.

Introduction

Liver diseases constitute a major problem of both Indonesia and worldwide proportions.[1,2] Liver plays many vital roles in metabolic processes, secretion of bilirubin, synthesis of plasma protein, and detoxification and excretion of xenobiotic (drugs, chemical agents, toxin) into the bile. Thus, damage that occurs on the liver can cause a very poor impacts and in some cases results in death. Hepatitis, for an example, has not only causes many deaths, but also brings on financial problems because of its high cost of treatment.[3] Moreover, treatments of hepatitis may cause many side effects.[4]

The use of traditional medicine including herbal medicines has been recommended by the World Health Organization (WHO) to maintain health, prevent and treat many diseases. In the case of hepatitis A, the herbal medicine can heal quickly and prevent post hepatitis syndrome, while in hepatitis B and C, herbal medicine can prevent disease progression to chronic stages.[5]

Solanum nigrum L. is one alternative of hepatoprotective herb. The most important antioxidant compounds contained in *Solanum nigrum* L. fruit is flavonoid which is known to have the ability to fight free radicals and prevent hepatotoxicity through various mechanisms.[6,7] It is an evident from the previous study conducted by Kuppuswamy Raju, *et al*.[8] that ethanol extract of dried fruits of *Solanum nigrum* L. has given remarkable hepatoprotective effect in CCl_4-induced liver damage in rats. Carbon tetrachloride (CCl_4) is known to causes the strongest hepatotoxicity. Within the body, carbon tetrachloride is transformed into a highly toxic free radical which can lead to an oxidative stress condition that causes a great damage in cells, especially hepatocytes. CCl_4 is widely used to induce the

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experimental animals to become hepatitis model because of its hepatotoxicity effect that are similar to classic presentations of viral hepatitis, such as necrosis, fatty liver, and toxic hepatitis.[9]

The study aims to determine the protective effect of *Solanum nigrum* L. in CCl_4-induced hepatic damage in rats using fruit infusion preparation. This infusion is simple to prepare, and easily applicable.

Methods

A complete randomize expremintal study was conducted using 25 healthy male Wistar strain-white rats (180–200 g) with 12 weeks of age procured from Laboratorium Ilmu Hayati Pusat Antar Universitas (PAU), Instituteof Technology Bandung (ITB) on 24 September–10 October 2012.

The experimental protocol was approved by Health Research Ethics Committee, Faculty of Medicine Universitas Padjadjaran as follows: 1) All the rats were fed by a standard animal food and water ad libitum in Animal Laboratory of Department of Pharmacology and Therapy in Faculty of Medicine Universitas Padjadjaran, 2) After acclimatization of one week period, the animals were randomly divided into five groups which each group consists of five rats. 3) The plant materials were obtained from Manoko farm, Lembang, Bandung and were botanically identified at the Herbarium Jatinangor, Universitas Padjadjaran. 4) The *Solanum nigrum* L. used were green colored and washed thoroughly, the stalks and leaf petals were removed. Each infusion preparation was respectively prepared using 22.5 g, 45 g, and 90 g of *Solanum nigrum* L. fruit in 100mL of distillated water and was boiled in an infusion pan at 900 C for 15 minutes. After that, the herbal infusion was filtered and extra water was added to obtain the required volume. 5) Group I served as negative control; group II served as positive control; group III, IV, V served as treated groups which were respectively given 3 mL of SNFI by 22.5g/100mL, 45g/100mL and 90g/100mL concentrations daily for eight days. On the eighth day, CCl_4 10% paraffin (8 mL/kg body weight) was intraperitoneally (IP) administered to all rats except rats in group I. After 48 hours, all the rats were sacrificed under ketamine hydrochloride anesthesia. The liver of each rat was promptly removed and washed with normal saline for further histopathological study. 6) Liver samples were taken from medial one-third of the largest lobes and immediately fixed in 10% buffered formaldehyde solution until it is harden. Samples were then embedded in paraffin wax, sectioned (5µm) with a microtome, stained with Hematoxilin-Eosin (H.E.), and mounted on glass slides with Canada balsam. 7) One section from each rat was randomly chosen,then ten fields were observed. Degrees of liver damages were estimated by counting the number of necrotic cells in each field under Olympus light microscope at magnification of 10 x 40. Images were captured by a digital camera. The grades of liver damage in different groups were assigned in percentage of necrosis (%).

Data were expressed as means ± standard deviation (SD). Statistical analysis was performed using SPSS for Windows version 17.0. The study data was analyzed using Kruskal-Wallis non-parametric test followed by Mann-Whitney post-hoc test for multiple comparison groups. Statistical significance was set at the $p < 0.05$ level.

Results

The hepatocytes of normal rats (group I) were intact and observed in fairly radial position in relation to central vein. CCl_4-induced rats (group II) revealed the big areas of centrolobular hepatocytes necrosis, enlargement of sinusoids, reaction of inflammatory cells, vacuolization of cytoplasm,

Table 1 Percentage of Necrosis (%)

	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean	(SD)*
Group I	1.29	1.33	1.18	1.22	1.33	1.27	(0.07)
Group II	39.08	37.79	42.55	33.56	33.35	38.95	(3.84)
Group III	14.00	13.53	15.24	12.02	11.94	13.35	(1.39)
Group IV	16.48	16.30	22.49	17.34	14.22	17.35	(3.09)
Group V	21.45	22.82	19.38	24.68	23.57	22.38	(2.05)

*SD: Standard Deviation

pycnotic and basophilic nuclear, and disarrangement of hepatocyte architecture. Microscopic examination of rats treated with SNFI (group III, IV, V) showed slight alteration in hepatocytes with less necrotic areas in comparison to the positive control rats, the liver also showed some evidences of regeneration of hepatic cells which is manifested by increased number of binuclated cells.

The percentage of necrosis (Table 1) was determined by comparing the number of necrotic hepatocytes of each rat by the mean of total number of normal hepatocytes of negative control rats. All treated groups showed smaller percentage of necrosis in comparison to the positive control group. The smallest percentage of necrosis of treated groups could be seen in group III (22.5g/100mL).

The study showed that there was a significant difference between Group I and Group II ($p=0.008$; $p<0.05$) indicating successful induction of CCl4. The SNFI in concentrations of 22.5g/100mL (group III), 45 g/100mL (group IV), and 90g/100mL(group V) was proven to inhibit liver cell damage significantly ($p=0.008$; $p<0.05$).

Discussion

The present study investigates the protective effect of SNFI on CCl_4-induced liver damage of rats. Carbon tetrachloride (CCl_4) is widely used

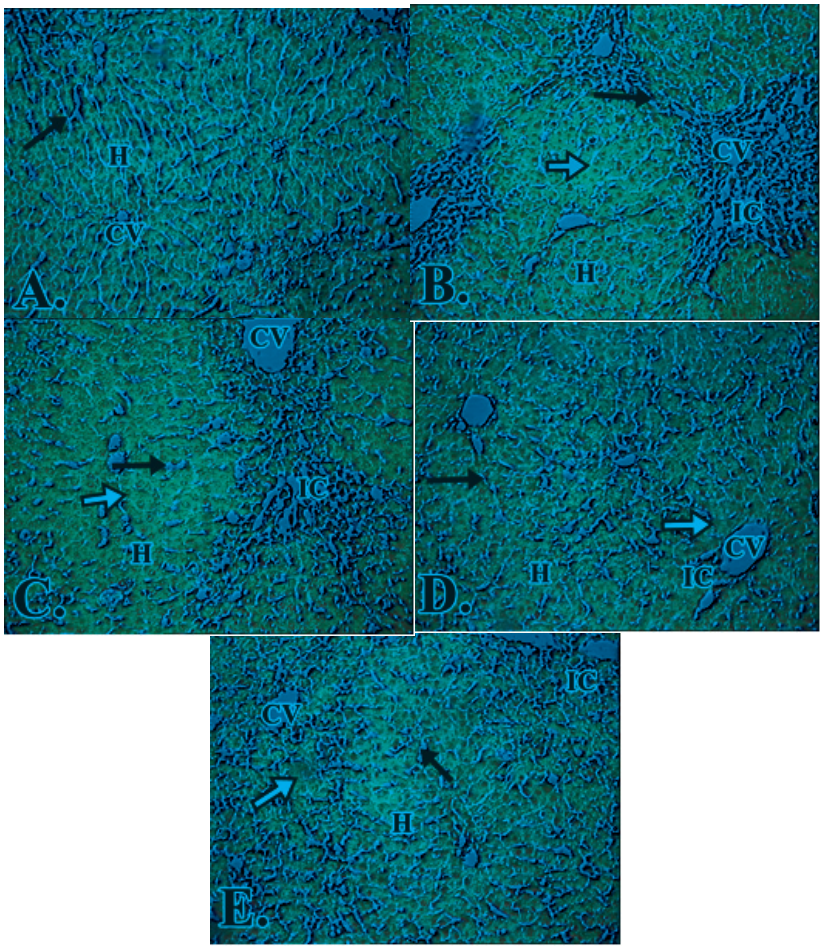

Figure 1 Liver sections of rats.

A. Liver section of normal rats; B. Liver section of control rat induced with CCl4 only; C. Liver section of CCl_4-induced rat pre-treated with SNFI (22.5g/100mL); D. Liver section of CCl4-induced rat pre-treated with SNFI (45g/100mL); E. Liver section of CCl4-induced rat pre-treated with SNFI (90g/100mL). (H&E stain, x100). H= hepatocytes; CV= central vein; IC= inflammatory cells; black arrow= sinusoid; white arrow= necrosis.

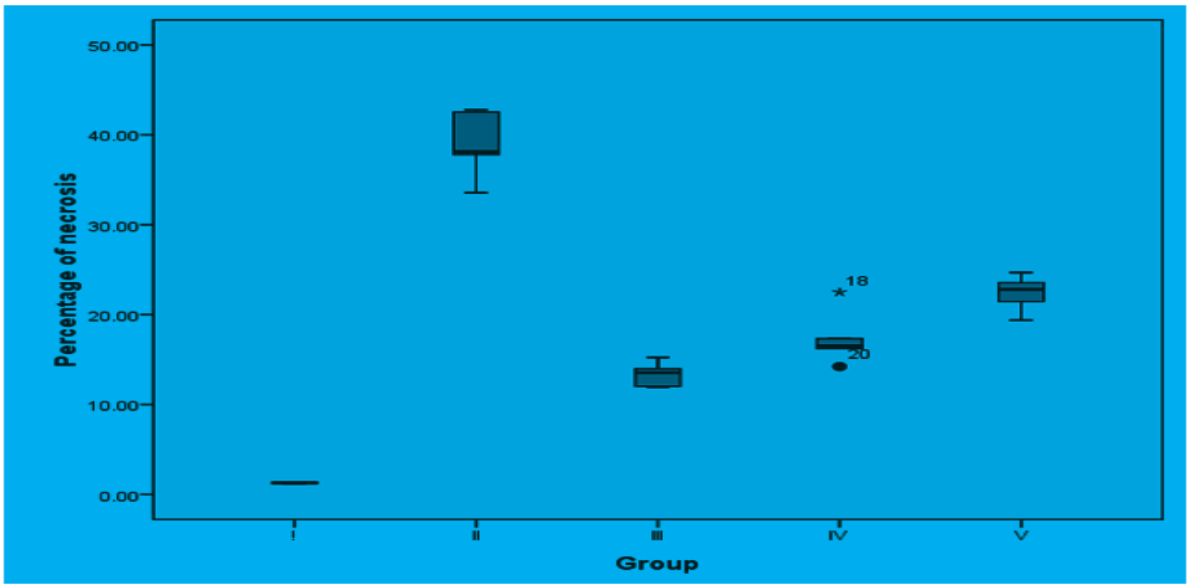

Figure 2 Box-Plot Graphic of Percentage of necrosis based on groups

to induce liver damage because it is metabolized by cytochrome p450 in the hepatocytes, forming a highly reactive trichlormethyl radical, leading to peroxidation of the lipid and further damage of cellular structures.[2] In an agreement with Giffen *et al.*[10], CCl_4 injection to rats in the present study resulted in significant increase of necrotic hepatocytes and also generated the appearance of sinusoidal enlargement, vacuolization of cytoplasm, and infiltration of inflammatory cells.

Antioxidant is a substance which can inhibit the oxidative processes and neutralize the free radicals.[7] The protective effect of SNFI against CCl_4-induced hepatotoxicity may be related to its ability to elevate the antioxidant agent in the body. Flavonoid is one of the most important antioxidant contained in *Solanum nigrum* L. fruit. 6It was stated that many activities of flavonoid against free radicals that cause hepatocellular damage can be explained by its ability to act as the chelator and the anion superoxide scavenger that scavenges reactive oxygen species. Moreover, flavonoid can lower the metabolic activation of CCl_4 by cytochrome p450 by means of further inhibition of free radical generations.[11]

The study by histopathological examination of livers shows that the protective effect of Solanumnigrum L.fruitproved that rats administered with SNFI prior to CCl_4 induction shows only slight alteration in the hepatocytes with less necrotic areas in comparison to the untreated group. This protection may be due to the effective blockage of oxidative stress and enhancement of the natural antioxidant in the liver.[12]

It is noted that the percentage of hepatocyt necrosis of rats in group III, IV, and V were smaller when it is compared to the positive control group. The percentage of necrosis is then statistically analyzed using Kruskal-Walis non-parametrical test followed by Mann-Whitney post-hoc test. Three different concentrations of SNFI has given the same significant hepatoprotective effect ($p=0.008$; $p<0.05$) against CCl_4-induced necrotic liver cells. This is in accordance with a previous study by Raju *et al.*[8] that dried fruit of *Solanum nigrum* L. can counteract with hepatic injury induced by CCl_4 injection due to its ability to maintain the structural integrity of hepatocytic cell membrane and regenerates the damaged liver cells. In conclusion, the present study shows that SNFI at concentration of 22.5g/100mL, 45g/100mL, dan 90g/100mL demonstrates hepatoprotective effect against CCl_4-induced the hepatic cell damage in rats.

Further investigation is required to characterize the active ingredients of this plant as well as its mechanism of action to confirm the hepatoprotective and antioxidant qualities. This study should also contribute to the evidence-based traditional medicines for the hepatoprotective effect of *Solanum nigrum* L. fruit infusion.

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