



Protective Effects of *Nothopanax scutellarium* on Hepatotoxicity of Copper(II) Induced to Experimental Rats

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Abstract

Edited by: Sinisa Stojanoski
Citation: Nasution SW, Yerizel E, Chaidir Z, Zein R. Protective Effects of *Nothopanax scutellarium* on Hepatotoxicity of Copper(II) Induced to Experimental Rats. Open Access Maced J Med Sci. 2020 Apr 28; 8(A):283-286. <https://doi.org/10.3889/oamjms.2020.4238>
Keywords: Copper(II); Hepatotoxicity; *Nothopanax scutellarium*; Serum glutamic pyruvic transaminase; Serum glutamic oxaloacetic transaminase
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Received: 27-Dec-2019
Revised: 29-Mar-2020
Accepted: 23-Jan-2020
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Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist
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BACKGROUND: *Nothopanax scutellarium* is a plant containing substance such as flavonoids, saponins, amygdalin, peroxidase, calcium oxalate, polyphenols, proteins, fats, calcium, phosphorus, iron, and Vitamins A, B1, and C.

AIM: The purpose of this study was to investigate the hepatoprotective effect of *N. scutellarium* leaves against rats induced with Cu(II).

MATERIALS AND METHODS: Three groups of female Wistar rats (*Rattus norvegicus*) within the range of age 2.5–3 months were used. Each group was composed of three female rats. Group I was a control given a normal diet and distilled water. Group II was exposed to 1000 mg/L of Cu(II) solution with a dosage of 1 mL × bw/200 g. Group III was given 5 mL of antidote *N. scutellarium* extract for 7 days then exposed to 1000 mg/L of Cu(II) solution with a dosage of 1 mL × bw/200 g. The blood test (malondialdehyde [MDA], serum glutamic oxaloacetic transaminase [SGOT], and serum glutamic pyruvic transaminase [SGPT]) and histopathology of liver were examined after 5 h of exposure.

RESULTS: The administration of *N. scutellarium* as hepatoprotective in experimental rats resulted in significant reductions in MDA, SGOT, and SGPT as much as 33.87%, 51.14 %, and 12.57 %, respectively.

CONCLUSION: This study concluded that indicates the increase of the parameters of liver function and stress oxidative parameters including MDA, SGOT, and SGPT.

Introduction

Copper (Cu(II)) is a mineral that is essential for physical and mental health. In the case of an increase or overuse of Cu(II), it can cause Cu(II) poisoning. Cu(II) commonly present in foods, hot water pipes, malnutrition tablets, birth control pills, etc. [1]. Excessive copper exposure (>2 mg/L) in drinking water causes poisoning to humans and damages the environment [2]. Many methods are used to remove toxic metal ions from wastewater such as adsorption, precipitation, evaporation, electroplating, ion exchange, and membrane processes. Exposure to Cu(II) in experimental rats caused changes in the level of biochemical parameters of malondialdehyde (MDA), serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT). Biosorption is one of the most effective and low-cost biotechnology innovation processes to remove heavy metals from aqueous solutions. The use of microorganisms (especially bacteria, algae, yeast, and fungi) as biosorbents can remove heavy metals widely used [3-6].

Nothopanax scutellarium has the potential to utilize as an efficient material adsorbent for removing Cu(II) metal ions [7]. *N. scutellarium* contains compounds such as alkaloids, tannins, saponins, and flavonoids [8]. Lemang leaves are also used as vegetables to enhance the delicacy of the taste of cooking. The aim of the research was to investigate the effect of the hepatoprotective of *N. scutellarium* leaves against rats induced with Cu(II).

Materials and Methods

Preparation for experimental rats

Three groups of female Wistar rats (*Rattus norvegicus*) within the range of age 2.5–3 months were used. Each group was composed of three female rats. Group I was a control given a normal diet and distilled water. Group II was exposed to 1000 mg/L of Cu(II) solution with a dosage of 1 mL × bw/200 g.

Group III was given 5 mL of antidote *N. scutellarium* extract for 7 days then exposed to 1000 mg/L of Cu(II) solution with a dosage of 1 mL × bw/200 g. After 5 h, the blood test was drawn and analyzed for stress oxidative parameters and parameter function of the liver (MDA, SGOT, and SGPT).

Measurement of MDA

Provided three test tubes containing blanks (distilled water), standard, serum (sample) 0.5 mL. Add 2.5 ml of 5% trichloroacetic acid each. Mix using a vortex mixer, centrifuge for 10 min at 2000 rpm. Each pipette 1.5 mL filtrate, put into a tube in accordance with the label. Add 1.5 mL Na each. Thiobarbituric acid mix with a vortex mixer, heat it in a water bath for 30 min. Cool, read the adsorbent with spectrophotometers at a wavelength of 530 nm.

Measurement of SGPT levels in serum

Arranged tubes on shelves that have been provided, then labeled, starting samples 1, 2, 3, etc. Pipette 100 µl serum 1 input into the S1 tube, added 1000 µl reagent 1 (Tris pH 7.15 100 mmol/L, L-alanine 500 mmol/L, lactate dehydrogenase [(LDH) ≥ 1700 U/L]), mixed, incubated at room temperature for 5 min, added reagent 2 of 250 µl (2-oxo-glutaric 15 mmol/L, NADH 0.18 mmol/L, pyridoxal-5-phosphate 0.09 mmol/L, buffer pH 9.6 0.7 mmol/L), mixed, and read the absorbance after 1, 2, and 3 min, continued for the tube (sample 2, 3, etc.).

Measurement of SGOT levels in serum

The tubes are arranged in a rack that has been provided, then labeled, starting samples 1, 2, 3, etc. Then, a 100 µl serum 1 pipette is input into the S1 tube, 1000 µl Tris reagent is added pH 7.65 80 mmol/L, L-aspartate 240 mmol/L, malate dehydrogenase a600 U/L, LDH ≥900 U/L mixed, incubated at room temperature for 5 min, added reagent 2 (2-oxoglutaric 12 mmol/L, NADH 0.18 mmol/L pyridoxal-5-phosphate 0.09 mmol/L, buffer pH 9.60, 7 mmol/L), as much as 250 µl, mixed, and read the absorbance after 1, 2, and 3 min.

Results

Examination of MDA, SGOT, and SGPT levels in serum

Effect of *N. scutellarium* leaf as an antidote to liver function parameters and pre-treatment effects with *N. scutellarium* leaf antidote on MDA, SGPT, and SGOT levels is shown in Table 1.

Histological analysis

Table 1: The mean level of MDA, SGOT, and SGPT

No.	Parameters	Group 1 (Control)	Group 2 (Cu (II)) treatment)	Group 3 (Antidote+Cu (II))
1.	MDA (mg/dl)	3.61	8.06	5.33*
2.	SGOT (U/L)	111.987	123.97	60.57*
3.	SGPT (U/L)	25.88	92.5	80.87*

MDA: Malondialdehyde, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase.

The protective effect of *N. scutellarium* leaf powder on liver damage caused by the toxicity of Cu(II) ions is histopathologically shown in Figure 1.

Figure 1 indicates the effect of Cu(II) and *N. scutellarium* extract induction on the liver of

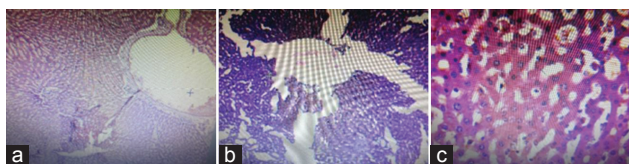


Figure 1: Histopathology of liver on experimental rats, (a) control; (b) Cu(II) ion induction without an antidote, (c) *Nothopanax scutellarium* extract with Cu(II) induction, ×40

experimental rats. It showed that the Cu(II) induction gave significant damage to the liver marked by broadening of central vein and some of the hepatocytes experiencing cloudy swelling (Figure 1b). Whereas, the antidote seems to have a protective effect toward the liver indicated by mild fatty formation although it has been induced by Cu(II) ion. The same result also has been reported by the previous research and stated that particular plant extract was able to reduce live damage due to Cu(II) poisoning [9].

Discussion

Examination of MDA, SGOT, and SGPT levels in serum

MDA level listed in Table 1 of Group 1 as control was 3.61 mg/dl. After Cu(II) exposure, MDA increased to 8.06 mg/dl (Group II). The MDA level decreased after the experimental rats were induced by antidote from *N. scutellarium* leaves extract from 8.06 mg/dl to 5.33 mg/dl. MDA is one of the most famous secondary products of lipid peroxidation that functions in biomaterials as an indicator of cell membrane damage. *N. scutellarium* contained flavonoids, saponins, alkaloids, proteins, fats, calcium, phosphorus, iron, Vitamins A, B, and C, calcium oxalate, peroxidase, amygdalin, and tannin [10,11]. Exposure to free radicals can cause oxidative stress. In the liver, this process causes impaired liver function. Oxidative stress compresses antioxidants [12]. Oxidative stress is a pathological condition, can cause abnormalities in the body one of the developments of liver damage. Many

risk factors, including environmental pollution, alcohol, drugs, and irradiation, can cause oxidative stress in the liver. Antioxidants as a rational curative strategy to prevent and cure liver diseases involving oxidative stress. Natural antioxidants contained in edible plants or medicines have antioxidant benefits and free radical flushing and anti-inflammatory action, the basis for bioactivity and other health benefits [13].

As presented in Table 1, *N. scutellarium* extract decreased the level of SGPT and SGOT after the administration of Cu(II) in experimental rats. The increased levels of SGPT and SGOT levels in Group II were a sign of liver damage due to the exposure to Cu(II). The provision of *N. scutellarium* leaf protection can significantly lower SGOT and SGPT levels. *N. scutellarium* has a protective effect of flavonoids on copper toxicity and may be due to the chelating effect of Cu(II).

The previous study investigated the effect of *Annona muricata* leaf protection against Cu(II) toxicity induced in experimental mice. Rats were induced with Cu(II) as much as 1000 mg/L intraperitoneally lead to the increase of liver and kidney parameter function (SGOT, SGPT, urea, creatinine, and MDA). Histologic analysis of liver tissue after induced by Cu(II) showed central venous dilation, hepatocyte swelling, and necrosis. After being given *A. muricata*, the antidote can reduce the level of liver and kidney function parameters and oxidative stress in mice. Histologically, pre-treatment with *A. muricata* leaf antidote can reduce the effects of liver structure damage due to Cu(II) toxicity [9].

Liver profile for detecting liver disease, SGOT and SGPT, is sensitive markers of hepatocellular injury. Serum SGOT is found in various tissues, including heart, skeletal muscle, kidney, brain, pancreas, lung, leukocytes, and erythrocytes. SGPT is a metabolic enzyme released when damage occurs in hepatocytes [14]. The protective effect of *A. muricata* leaf against toxic Cu(II) induced in experimental mice intraperitoneally could increase in liver and kidney function parameters and oxidative stress parameters including SGOT, SGPT, urea, creatinine, and MDA. After being given *A. muricata*, the antidote can reduce the level of liver and kidney function parameters and oxidative stress in mice [15].

Cu(II) exposure caused changes in the level of biochemical parameters of MDA, SGPT, and SGOT. Pre-treatment with *N. scutellarium* leaf antidote was able to return the parameters of MDA, SGPT, and SGOT to normal. This means that *N. scutellarium* leaves antidote could reduce the toxic effects of Cu(II) toxicity in experimental rats. Pre-treatment with *N. scutellarium* antidote can reduce liver damage which may be due to the high content of antioxidants and free radical activity.

Histological analysis

Rats liver photomicrographs in the control group are shown in Figure 1a. Group II treatment with

the addition of Cu, in the picture above, shows that in this treatment, liver damage has occurred marked by lysis of venous central cells. Another damage is hepatocyte cell necrosis in the form of picnics, i.e., the cell nucleus (nucleus) looks more rounded, smaller and rounder size and karyolysis, i.e., the nucleus lysis is not visible, only the empty cavity is aborted by the membrane of the nucleus in Figure 2 and c. Group III treatment with the addition of the leaf tread after the administration of leman tread leaves resulted in histopathological improvement in liver cells in Figure 3.

Polyphenols and flavonoids have been known to have antioxidant, anti-inflammatory, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities [16]. Copper is an essential nutrient that reacts with a number of metalloenzymes involved in the formation of hemoglobin, carbohydrate metabolism, catecholamine biosynthesis, and cross-linking of collagen, elastin, and hair keratin. The ability of copper to cycle between the oxidation state of Cu(II) and the oxidation level of Cu(I) used by cuproenzyme is involved in redox reactions, this copper property is also potentially toxic because it can produce superoxide and hydroxyl radicals that can cause damage to various organs [17].

Copper can also accumulate in water and soil even to a higher degree and can stick to clothing and skin. Circulation or presence of copper in the body requires good functioning of the liver, bladder, and adrenal glands. If one of these organs is disrupted, the body is unable to excrete and utilize copper properly. Copper will accumulate in the liver, the effect can further cause the inability to excrete copper. Increased copper concentrations will cause accumulation in the brain, joints, and lungs, which will affect the structure and function of tissues [15].

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