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Chronic Cadmium Hepatooxidative in Rats: Treatment with
Haruan Fish (*Channa striata*) Extract

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Abstract

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Cadmium is an extremely toxic metal commonly found in industrial workplaces. Cd is unable to generate reactive oxygen species (ROS) directly, but Cd-induced oxidative stress is a common phenomenon observed in multiple studies. Haruan or Snakehead (*Channa Striata*), is a fresh water snakehead fish indigenous to Kalimantan of Indonesian. The extract of haruan fillet also known have a protective effect. The aim of this study was to investigate a hepatooxidative in rat exposed to Cd and protective effect of haruan extract. The present study was undertaken to examine the inhibitory effect of the haruan extract on cadmium induced oxidative stress in liver. The levels of MDA and peroxide were assessed by colorimetric method. Significant increases was observed in the levels of MDA and peroxide in Cd treated rats. These levels were significantly decreased in Cd and haruan extract treated rats. It can be concluded from presented results that Cd induced oxidative damage in liver by increased MDA and peroxide level. Our results show that haruan extract expressed protective role against toxic influence of Cd in liver.

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Keywords: Chronic Cadmium Hepatooxidative; Treatment; Haruan Fish; *Channa striata*

1. Introduction

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Cadmium (Cd), a common toxic heavy metal, is widely distributed in the environment due to its use in industry [1,2]. Acute exposure to Cd causes dysuria, polyuria, chest pain, fatigue and headache [3]. Chronic intake of Cd in contaminated food or air produces organ dysfunction as a result of cell death, resulting in pulmonary, hepatic and renal tubular diseases [4]. The liver is the most important target organ when considering Cd-induced toxicity because Cd primarily accumulates in the liver [5-7].

Cadmium generates ROS which depletes endogenous ROS scavengers. ROS also damage a variety of transport proteins, including Na^+/K^+ , ATPase, which are subsequently degraded by the endolysosomal proteases. Long term exposure to Cd increased lipid peroxidation and caused inhibition of SOD activity indicating oxidative damage in liver. The mechanisms of chronic Cd hepatotoxicity are not completely understood [8].

Haruan or Snakehead (*Channa Striata*), is a fresh water snakehead fish indigenous to Kalimantan of Indonesian. The aqueous extract of haruan fillet produced wound healing effect in rodents antinociceptive activity in rodents and protective effect against experimentally induced osteoarthritis in rabbits. The mucus extract of haruan showed antibacterial activity. Haruan was analysed for amino acid and fatty acid compositions and found to contain glutamic acid, aspartic acid and lysine as major amino acids and palmitic acid, stearic acid, arachidonic acid (omega-6 fatty acid) and docosahexanoic acid (omega-3 fatty acid) as major fatty acids [9-23]. The major fraction of haruan extract protein is albumin (64.61% of total protein) [24]. The aim of this study was to investigate a hepatooxidative in rat exposed to Cd and protective effect of haruan extract.

2. Material and methods

2.1 Chemical

100 μl TCA 100%, 100 μl Na-Tiobarbiturat 1%, 250 μl HCl 1 N, 1mM FeCl_3 , 2 mM FeCl_2 , 1mM 1,10-phenanthroline, 0.2 M phosphate buffer (pH 7.8), and 0.17 M H_2O_2 , were from Sigma. All other reagents were of the highest quality grade available

2.2 Animal experiment

The experimental animals used in this study were 2-3 months' old male albino rats (*rattus novergicus*) weighing approximately 180-200 g, obtained from the Veterinary Research and Investigation Station (Balai Penelitian dan Penyidikan Veteriner, BPPV) at Banjarbaru. The animals were kept for one week prior to the experimental treatment to bring them to approximately equal physical and psychological conditions. In the adaptation period, the animals received animal feed and water ad libitum, the water being tap water supplied by the provincial water purification plant. Complete random sampling was used to assign the animals into groups, each consisting of 4 animals. Each group was kept in one small cage for adaptation. Group 1 was used as controls. The rats of the experimental groups were exposed to: Group 2 to 1 ml cadmium 6 mg/L Cd/day per oral for 4 weeks), Group 3 to haruan extract (2 mg extract/kg body mass/day per oral for 4 weeks).

The animals were sacrificed by decapitation and their livers surgically removed. Subsequently the livers were washed in phosphate buffer at pH 7, then minced into a liquid, of which 5 ml was centrifuged at 3500 rpm for 10 minutes, and 200 μL of the supernatant was taken for determination of MDA and peroxide concentration.

2.3 Preparation of haruan extract

The extract was prepared from haruan fish according to the previously method [12]. Briefly fillet free of bones was prepared from fresh fish. The fish was cut into small pieces, placed on a stainless steel wire mesh mounted on a stainless steel tripod in the pressure cooker sets at 100°C for thirty minutes. Fresh distilled water was then added in volume ratios of 1:2. Water level was below the wire mesh so that the fillet was not submerged in the water. Extract was then obtained through steaming. At the end of extraction procedure, the fillet was discarded while the liquid extract was collected, filtered, centrifuged and stored at 4°C until use.

This extract was diluted with physiological saline to the required concentration (20%) and administered orally in a volume of 2 mg extract/kg body mass.

2.4. Determination MDA concentration

MDA was measured by the method of Buege and Aust [25]. The colour was measured spectrophotometrically at $\lambda=532$ nm.

2.5 Determination H₂O₂ concentration

Determination of peroxide concentration by the modified FOX2 method. Solutions measured spectrophotometrically at $\lambda=505$ nm

2.5. Statistical analysis

Data are presented as means \pm SEM. The determinations were performed from 12 animals per group and the differences were examined by the one-way analysis of variance (ANOVA) followed by the Fisher test and the significance was accepted at $p<0.05$.

3. Results and discussion

MDA and peroxide levels in the liver tissue were used as a measure of lipid peroxidation. Figures 1 showed the changes of MDA and peroxide levels respectively in all groups. Administration of Cd caused significantly increase of MDA and peroxide levels as compared to the control group ($p<0.05$). The administrations of snakehead extract caused significantly decrease of MDA and peroxide levels as compared to the control group ($p <0.05$).

MDA is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of Cd. TBARS in Cd intoxicated rats could be possibly due to excessive formation of free radicals which leads to the deterioration of biological macromolecules.

Enhanced levels of MDA in liver of Cd treated rats indicated the increased levels of lipid peroxidation. Reports have shown that cadmium promotes the formation of ROS by Fenton transition equation, such as hydrogen peroxides and enhances the subsequent iron and copper-induced production of lipid peroxidations and the highly reactive hydroxyl radical. Simultaneously administration of haruan extract decreased the formation of lipid peroxidation products, and it possesses antioxidant activity.

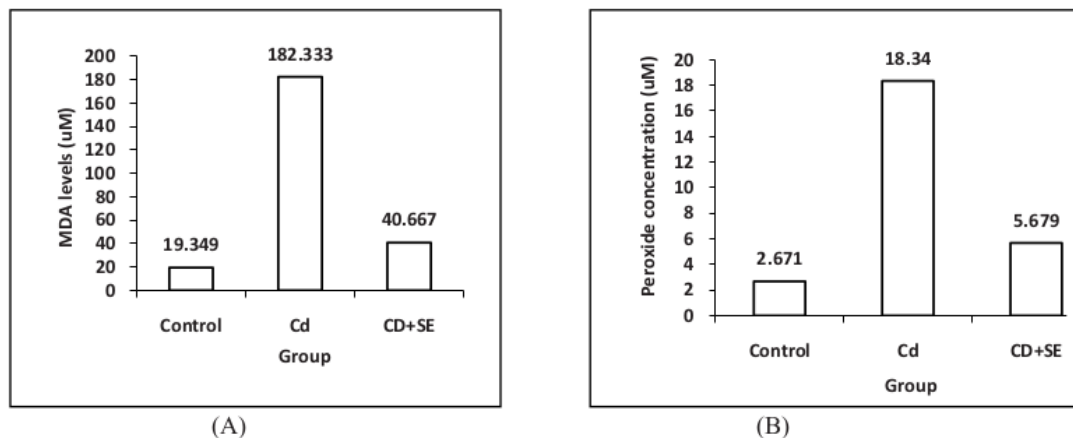


Fig. 1. Effects of haruan extract on MDA (A) and peroxide concentration (B) in rat liver homogenate induced by Cd.

Thus, this agent might provide more medical benefit because the use of this agent could simultaneously

alleviate oxidative damage. The ability of haruan extract, consumed within a balanced controlled diet, to improve overall the antioxidants status and to protect against oxidative damage in humans [26-28].

The effect of haruan extract is attributable its radical scavenging activity. Further, the albumin of defense against free radical induced damage and the restoration of this enzyme activity by haruan extract may account for its protective effect. Lipid peroxidation is one of the main manifestation of oxidative damage, which plays an important role in the toxicity of many xenobiotics. It has been reported that haruan extract possess strong antioxidant and free radical scavenging properties [8].

Albumin contains many sulfhydryl groups (-SH) that can serve as radical binder and plays an important role in sepsis. Physiological solution of human serum albumin has been shown to inhibit free radical production by polymorphnuclear leukocytes. This binding capacity is related to the abundance of sulfhydryl groups (-SH) in the albumin [24].

4. Conclusion

It can be concluded from presented results that Cd induced oxidative damage in liver by increased MDA and peroxide level. Our results show that haruan extract expressed protective role against toxic influence of Cd in liver.

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