Conference Paper

Liver Histopathological Measurement Due to Maximum Dosage for Acute Oral Toxicity Test Using Ethanol Extract Of Coffee Pulp In Female Mice

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Abstract

Coffee processing into fast food ingredients that must be done in order to get maximum results. Behind the processing there are various problems that arise, one of them are the presence of solid waste in the form of pulp coffee. Based on several studies, coffee pulp turned out to have high antioxidants.This research was conducted an acute oral toxicity test of the maximum dose (2000 mg/kg BW) of ethanol extract of coffee pulp was tested on mice for 24 hours. Acute oral toxicity studies of coffee pulp based on guidelines for National Drug and Food Control in female mice. The study was conducted in 2 groups of test animals consisting of a negative control group and an acute oral treatment group. Each group of 5 mice were treated for 24 hours. After treatment, the object was analyzed by histological observations descriptively. Descriptive histological analysis (qualitative analysis) of the liver of female mice showed no difference between the study groups. The treatment group that was given ethanol extract of coffee pulp showed a normal nucleus which was shown by the appearance of scattered chromatin beads. This is in line with the description of the normal cytoplasm like a group of normal mice. The maximum dose of 2,000 mg / kg for acute oral testing for 24 hours does not show microscopic changes in female mice.Recommendation for further research is to conduct oral subchronic and chronic toxicity testing in the normal group and in groups given free radical-producing compounds with various examinations of other organs.

Keywords: health drinks, antioxidant, acute toxicity, maximum dose.

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1. Introduction

Coffee is the result of plantations that exist throughout the world, one of which is in Indonesia. Coffee is currently a relatively high export commodity in the world. Coffee has an important role in the national economy. The contribution of the coffee commodity to the national economy include as a source of foreign exchange, local farmers' income, job creation, regional development, agribusiness and agroindustry. Indonesia is the third

largest coffee producer in the world after Brazil and Vietnam. Around 96% of majority of existing plantations of coffee in Indonesia produces in small holders coffee, while the rest are large plantation coffee (Martauli, 2018).

Coffee are included in the Genus Coffea which consists of several types. Types that exist include Arabica coffee, Robusta coffee and Liberika Coffee(Danarti; & Najiyanti., 1999; Martauli, 2018).One of the coffee producers in West Java is in the south Bandung area, especially in the Ciwidey area. The condition of the region which is supported by the physical condition of nature supports the coffee cultivators to grow Arabica coffee types. This type of coffee grows in areas with an altitude of 700-1700 meters above sea level. The temperature is 16-20 ° C (Suhendi; & Akliyah., 2017).

Currently there are two ways of processing coffee bean from fresh fruit to being ready to be enjoyed. This method is the wet method (fully wet process) and the dry method (dry process). Stripping coffee peel (pulping) is one of the stages of the coffee processing process. The difference between the processing of wet and dry coffee is the initial stage when the coffee is finished. One of the wet stripping methods is using a coffee peeler in a wet condition (pulper). This machine is used to remove the rind component from the part of the coffee bean. In the processing of dry method, the harvested coffee fruit is first dried either by drying or using a drying machine to obtain a moisture content of approximately 12%. The dried coffee fruit is then peeled using a huller to separate the skin and seeds(Widyotomo, 2011, 2013).

One of the main problems posed in the coffee processing is the handling of waste in both solid and liquid forms. Solid waste produced during coffee processing is the peel of dried coffee fruit (coffee pulp), where in every ton of wet coffee will produce approximately 200 kg of dry coffee pulp. The large amount of solid waste has triggered many researchers and other efforts to treat the waste produced. Coffee pulp contains nutritious compounds and has the potential to be converted into a valuable product. Several studies have been conducted to produce a product from coffee processing waste into food and non-food products. Forms of product diversification include particle boards, growing media, organic compost, to food and beverages(Widyotomo, 2013).

Fresh coffee fruit that has been processed will produce pulp around 43-50% of the total weight of fresh coffee fruit(Rangarajan, 2019; Roussos et al., 1995). Coffee pulp contains various chemical components where the carbohydrate content is up to 50%, 10% protein, 20% fiber, 2.5% fat, caffeine 1.3% and other phenolic compounds around 1%, tannins around 8-10%(Bhoite & Murthy, 2014). Phenolic compounds of coffee pulp mainly consist 42.2%, of chlorogenic acid, 21.6% of epicatechin, 29.4% of isochlorogenic acid, 2% of catechins and other compounds. The group of phenolic compounds found in

coffee pulp consists of monomers and procyanidin groups, hydroxycinamic acid groups, flavonol groups, and anthocyanidin groups(Ramirez-Martinez, 1988).

Although coffee pulp are said to be waste, many farmers and creative entrepreneurs try to process them in the form of food because of their unique taste. One of the foods produced from coffee pulp is coffee tea or commonly called *cascara*. *Cascara* processing is generally used as animal feed, fertilizer and is often directly disposed of. *Cascara* can be reused as a useful product. The benefits of *cascara* can be used as an antidote to free radicals, gastric protection and as a cosmetic to lighten the skin (Pirdan Garis, Atika Romalasari, & Purwasih, 2019; Putri, Risa Andriana, Anggraeini, & Elisa, 2017). *Cascara* allegedly has the ability to ward off free radicals that are very good and is very suitable to prevent the growth of cancer cells and increase endurance. The active compound contained in *cascara* is tannin 1.8-8.56%, pectin 6.5%, chlorogenic acid 2.6%, caffeine 1.3%, caffeic acid 1.6% and total anthocyanin 43% (cyanidine, delpinidine, cyanidine 3-glycoside, delpinidin 3-glycoside, and pelargonidin 3-glycoside(M. Sumihati, Isroli, & Widiyanto, 2011).

The appearance of a creative business of food derived from coffee waste issue the question of the extent to which the level of safety of the drink with a certain dose. The purpose of this study was to see the extent of the effect of extract ethanol of coffee pulp using the maximum dose in the liver histology.

2. Methods

2.1. Study Design

A total of 12 the Balb/C female mice (6 mice/group (1 mice for drop out reserve if one mice was died), were randomly selected and marked for individual identification. The test groups included a control group (aquades) and the other treatment group with dosages at 2000 mg/kg body weight of ethanol extract of coffee pulp. The test substance is administered in a single dose by gavage using oral feeding needle.

2.2. Preparation of extract

Arabica coffee pulp was purchased from coffee farmer at CiwideyIndonesia. The Arabica coffee pulp was dried using oven for 2 days with 80°C. The Arabica coffee pulp soaked in 80% of ethanol for 3 days (100 gram in 500 ml of ethanol). Pure sample was collected by separating the sample and ethanol via vacuum rotary evaporator.

2.3. Animal material

These the Balb/C female mice come from Biofarma Corp., and are aged 8-10 weeks. They weight between 30 and 40 g. These mice are raised in wire cages airy at 20 ± 2 °C, with a photoperiod of 12 hours and a humidity of 30% for 5 days prior to experimentation. The mice have ad libitum access aquades for drinking and food twice a day.

2.4. Single-Dose Oral Toxicity Study

An acute oral toxicity test was done within 24 hours after oral administration of extract ethanol of Arabica coffee pulp.

2.5. Data Collection for Histological study

Liver tissue from mice was excised and fixed in 10% neutral buffered formalin. After 24 hours, the liver was processed to obtain5µm thin paraffin sections. The Sections stainedwith hematoxylin and eosin stain. The thin slice was observed by microscopic analysis at 400x magnification.

2.6. Data analysis

Data analysis was performed by comparing the liver morphology of the control group with the treatment group.

3. Results

3.1. Histopathology Analysis

Macroscopic examination of liver from mice given ethanol extract of coffee pulp showed no change compare control group. This was shown with relatively similar color and no visible lysis morphological. Microscopic examination of liver with 400x magnification of mice given ethanol extract of coffee pulp in general showed no morphological differences in both the nucleus and cytoplasm when compared to the control, but some thin section of the trearmentgroup of pulp extract ethanol were seen to be denser compared to the control group.

The control group (left) showed good nucleus and cytoplasm, as well as the treatment group (right). The nucleus shows scattered chromatin beads (black arrow) and cytoplasm that looks good (red arrow). In the treatment group showed a cytoplasm which was stronger in color eosin which showed a higher cytoplasmic density than the control group.

4. Discussion

Phytochemical products from plants have become popular for health, especially in developing countries. Phytochemical products are considered safe because they come from natural sources. Phytochemical products from plants are considered safe without harm to health, and thus are widely used as self-medication(Vaghasiya, Shukla, & Chanda, 2011). Some secondary metabolite compounds are sometimes toxic. This is because metabolite compounds are part of the defense mechanism(de Castilhos, Gómez-Alonso, García-Romero, Del Bianchi, & Hermosín-Gutiérrez, 2017). Therefore, further acute oral toxicity studies are needed to identify the range of doses that can be used next. This test is also a useful parameter for investigating the therapeutic index of drugs and xenobiotics. Testing acute oral toxicity in mice can be used to evaluate natural medicines for different pharmacological activities, taking into account the basic premise that pharmacology is only the lowest dose toxicology (Niwano & Beppu, 2011).

Histological analysis was carried out to further confirm changes in the cell structure of organs. Histological examination is the gold standard for evaluating treatments related to pathological changes in tissues and organs. In this study, histopathological evaluation of oral consumption of acute ethanol extract did not contain morphology of mice organs especially liver (Wilhelm & Maibach, 2012). The ethanol extract of coffee pulp does not cause toxicity to organs because there is no damage to the liver. The liver is the main target organ of acute toxicity where it releases foreign substances that are absorbed in the intestine and metabolized into other compounds which may or may not be hepatotoxic compatible (Atsamo, 2018). In addition, acute toxicity studies carried out on pulp coffee ethanol extract and histological examination of mice organs given a dose of 2000 mg/kg revealed no potential toxicity or damage to the cell structure of the liver. There was also no necrosis, inflammatory reaction, fibrosis or local fat degeneration considered in the liver and structure arrangement similar to mice organs in the control group (Akanmu, Iwalewa, Elujoba, & Adelusola, 2010). The histological picture of the liver from this study was showed in Figure 1. The morphology of the liver cells in the

control group and those given permission were normal and there was no significant structural damage.

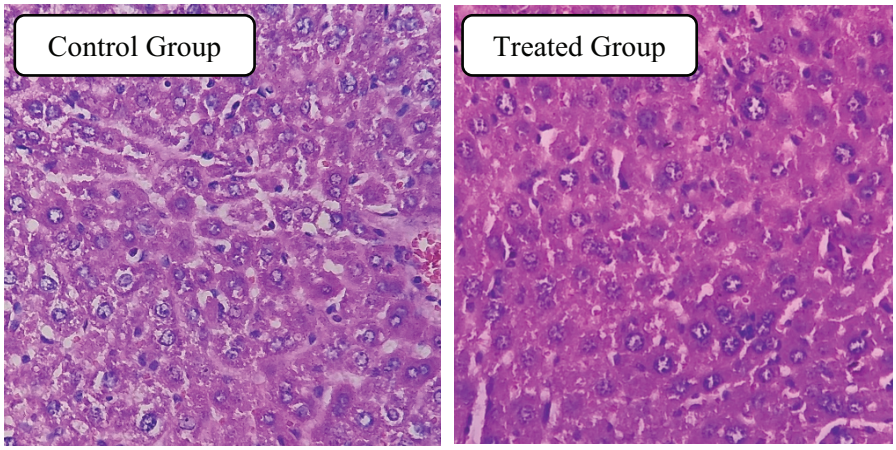

Figure 1: Histological results of liver after oral administration of ethanol extract of coffee pulp.

Coffee pulp which is used as a by-product in the form of food contains polyphenols, caffeine, chlorogenic acid, protocatechuic acid and gallic acid. Caffeic acid, gallic acid, and chlorogenic acid have been shown in various studies to have anticarcinogenic, anti-inflammatory and antioxidant properties when evaluating in vitro and in vivo research (Ameca et al., 2018; Sato et al., 2011). Antioxidant compounds can work synergistically so that they have a strong ability to protect biological systems and reduce damage caused by reactive oxygen species (ROS). This is allegedly able to protect liver cells from damage both by internal and external influences seen in the results of this study(Safrina Dyah Hardiningtyas, Sri Purwaningsih, & Handharyani, 2014).

5. Conslusion

Based on the research results, it can be concluded that the ethanol extract of coffee pulp is safe up to a dose of 2000 mg/kg given once in 24 hours an acute oral dose test. The histopathological picture clearly shows that oral administration of a dose of 2000 mg/kg in an acute oral toxicity test does not cause damage to the liver. This study provides significant data on the use of coffee pulp as waste which can be used as a reference for processing in food and beverages.

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