

## Green robusta coffee and its hepatoprotective effect on ALT levels of white mice induced with Monosodium Glutamate

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### Abstract

**Background of the study:** Liver injury or damage to liver function, also known as hepatotoxicity, can occur due to various reasons, including chemical substances. Monosodium Glutamate (MSG) is one such chemical that can cause liver damage if consumed in large amounts for a prolonged period. This study aimed to determine the effect of green Robusta Coffee extract on the alanine aminotransferase (ALT) levels of white mice induced with MSG.

**Material and methods:** This research study used 24 male white mice (*Rattus norvegicus*) divided into three groups. The negative control group (C-) was given nothing, the positive control group (C+) was given MSG at a dosage of 5 mg/g/day, and the experimental group (E) was given MSG at a dosage of 5 mg/g/day and green Robusta Coffee extract at a dosage of 250 mg/kg/day for 14 days.

**Results:** On the 15th day, blood was drawn from all three groups to measure ALT levels. The ALT levels were then analyzed using SPSS statistics. The study found a significant increase in ALT levels in the C+ group compared to the C- group ( $P < 0.05$ ). However, there was no significant increase or decrease in ALT levels between the experimental group (E) and the C- and C+ groups ( $P > 0.05$ ).

**Conclusion:** The study concluded that MSG induction of 5 mg/g/day for 14 days significantly increased the ALT levels of white mice. However, giving green Robusta Coffee (*Coffea canephora*) extract at a dosage of 250 mg/kg/day did not have a significant effect on the increased ALT enzyme levels of white mice induced with MSG at a dosage of 5 mg/g/day for 14 days.

**Keywords:** Hepatotoxicity; Monosodium Glutamate; Green Robusta Coffee; ALT levels

### 1. Introduction

Coffee has become one of the must-have components for some people to start the day with more energy as it is known for its caffeine that makes you feel energized when consumed. Meanwhile, only a few knew that coffee contains other chemicals that give health benefits to the body. Some of them are chlorogenic acid, magnesium, potassium, niacin, and vitamin C [1]. The one that works as the main hepatoprotective agent is chlorogenic acid. Chlorogenic acid is a conjugated hydroxycinnamate that exists in Robusta coffee which has hepatoprotective effects by inhibiting de novo lipogenesis, inducing autophagy, and reducing hepatocyte steatosis thereby reducing ALT enzyme levels [2].

The chemicals in coffee can change depending on geographical factors, species, and preparation processes such as roasting. The geographical factor is the soil where the coffee plants grow, which means that coffee that grows at high altitudes will have a different chemical composition compared with coffee that grows at low altitudes [11]. Meanwhile,

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each species' chemical composition differs such two most consumed coffee types (species), Robusta and Arabica, have different compositions of chlorogenic acid and other chemicals as Robusta has a higher amount of chlorogenic acid. And then, the roasting process is an important process in which the chlorogenic acid will lower after being roasted [8].

The liver is an organ that works in the metabolism and excretion of substances that can become poison or toxins if left in the body. Hepatotoxicity is injury or damage to the structure or function of the liver that can occur due to pathological damage and chemical induction from lipid peroxidation and ROS production [3]. Some examples of chemicals that can induce hepatotoxicity in experimental mice are drugs such as paracetamol and food flavorings such as Monosodium Glutamate (MSG).

While it is not commonly used in western cooking, MSG is widely used in Asian cuisines especially Chinese, in which makes a group of symptoms tightly associated MSG like headaches, swellings, and abdominal pain, called Chinese Restaurant Syndrome. MSG, when consumed, will dissolve into salt, glutamate, and water that if glutamate exists in large quantities in the body will cause damage to liver cell and therefore increase ALT levels [4].

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## 2. Material and methods

### 2.1. Materials

The tools used for this experiment are cages, scales, foods and drinks for the mice then syringes, test tubes, centrifuge tubes, and micropipettes. A feeding tube is used for intra-gastric feeding through the mice's mouth until it reaches stomachs.

The materials that consumed are standard feed, tap water, Robusta coffee extract with ethanol 95%, MSG dissolved in distilled water, and ketamine chloride.

The mice used were male Wistar mice (*Rattus norvegicus*), aged 9-12 weeks and weights 150-200 grams.

### 2.2. Methods

Mice acclimatization; the mice that met the criteria are divided into 3 groups of with each 8 mice with food and water for 7 days.

Making the Robusta coffee extract; green coffee beans are grinded into powder then went through maceration with ethanol 96% and evaporation to get the concentrated Robusta Coffee extract which then mixed with the suspending agent CMC-Na 0,1%.

Administration of MSG and Robusta Coffee; after 7 days of adaptation in the experimental environment in each group, the C- continued to be given only feeds, C+ were given MSG intra-gastric for 14 days, and EG were given MSG and Robusta Coffee extract also intra-gastric with feeding tube for 14 days.

Blood sampling; on the 15<sup>th</sup> day, the mice were given ketamine and blood samples were taken through cardiac puncture. ALT level then analyzed through blood samples.

Data analysis; data obtained are analyzed with SPSS statistics and, as it is normally distributed and homogenous, the test used is one-way ANOVA.

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## 3. Results and discussion

From the data given above, we could see that the C- group mice that were only given standard feed and water had the lowest ALT levels, C+ group mice that were given MSG 5 mg/g/day had the highest ALT levels, and the EG group mice that were given both MSG 5 mg/g/day and Robusta Coffee extract 250 mg/kg/day had lower ALT levels than C+ group but not as low as the C- group.

Comparison of the C- and C+ groups showed us that MSG (Monosodium Glutamate) could increase ALT levels as a hepatotoxic agent. These results are in accordance with the result of research conducted previously using MSG 5 mg/g/day for 14 days can cause histological damage to the liver of mice which will increase ALT levels [5]. Other research with smaller doses but for a longer time, using MSG 0.005 mg/g/day and 0.015 mg/g/day for 4 weeks can also cause an increase in enzyme levels, both ALT, AST and ALP [6].

**Table 1** ALT levels on the 15<sup>th</sup> day in U/L

SN.	C- group	C+ group	E group
1	40.6	51.2	65.3
2	45.2	55.1	51.9
3	50.8	91.6	46.3
4	48.4	60.7	44.1
5	36.2	70.4	62.9
6	56.8	54.9	56.9
7	41.8	60.2	48.5
8	53.6	64.6	64.5
mean	46.67	63.59	55.05

Meanwhile, even though the EG group has lower ALT levels than the C+ group, it couldn't bring the levels back to normal (C-group). This could mean that green Robusta Coffee could help lower ALT levels increased by hepatotoxicity but not significantly.

### 3.1. MSG mechanism of actions to increase ALT levels

MSG works with decreasing in the enzymes Glutathione Peroxidase (GPX) and Glutathione-S-Peroxidase (GST) which will cause an increase in ROS (Reactive Oxygen Species) production above the normal limits [6]. Increased ROS will increase vacuolization of cytoplasmic lipids and liver cells necrosis which will trigger the production of liver damage biomarkers such as ALT. An increase of ALT levels can also occur due to the influence of free glutamate on the release of ammonium ions which will cause injury to liver cells and trigger the release of ALT biomarker [4].

### 3.2. Green Robusta Coffee beans as hepatoprotective agent

Inhibition of the increase in SGPT levels with green Robusta coffee extract can occur due to the hepatoprotective content in Robusta coffee, namely chlorogenic acid. Chlorogenic acid works to reduce lesions such as edema in hepatocytes, oxidative damage, and helps the expression of Nrf2 which increases the action of antioxidants [7]. Even though the coffee used for research is the species with the highest chlorogenic acid content compared to other coffee species, *Coffea canephora*, coffee has other ingredients that work in the opposite way in reducing SGPT levels, namely cafestol and kahweol [8]. Cafestol and kahweol, which work to increase SGPT levels, are found in coffee in much smaller amounts than chlorogenic acid [9].

A research found that there's a decrease in ALT enzyme levels in mice with induced liver injury was seen after being given green Arabica coffee fruit skin extract, or without going through the roasting process, at a dose of 250 mg/kgBW/day and 500 mg/kgBW/day [10]. On the other hand, an increase in ALT levels was seen in the SGPT results before and after administration of Robusta coffee and a small mixture of roasted Arabica coffee at doses of 0.54 g/kgBW/day and 0.9 g/kgBW/day [8]. These two studies had opposite results due to the reduction in chlorogenic acid during the roasting process [9]. Apart from the roasting process, the condition of the soil also influences the coffee content, where the content of coffee grown in mountainous areas (Banyuwangi) will be different from the content of coffee grown in lowland areas [11].

A better way to see whether the chlorogenic acid content in coffee can inhibit the increase in SGPT levels caused by MSG can be done by isolating the chlorogenic acid content from coffee so that there are no other effects from other coffee contents. Research with the isolation and identification of chlorogenic acid using Nuclear Magnetic Resonance (NMR) from coffee shows that chlorogenic acid as a single substance can have anti-inflammatory, anti-oxidant, hepatoprotective and neuroprotective effects [9].

## 4. Conclusion

Based on this research, the green Robusta Coffee may help to lower ALT levels that increased due to hepatotoxicity

cause by Monosodium Glutamate (MSG) but further researches and studies are needed with more advanced and diverse way to isolate the substance that work as hepatoprotective agents in coffee (chlorogenic acid).

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## Compliance with ethical standards

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### *Disclosure of Conflict of interest*

The authors declare that there is no conflict of interest in this research.

### *Statement of ethical approval*

As the research involved animal experiments, the ethic has already been obtained from Hang Tuah University' Research Ethic Team (Komisi Etik Penelitian Kesehatan Fakultas Kedokteran Hang Tuah Surabaya).

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