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EFFECTS OF ROBUSTA COFFEE (*Coffea canephora*) EXTRACT WITH DIFFERENT ROASTING LEVELS ON LIVER HISTOPATHOLOGY OF MALE SPRAGUE DAWLEY STRAIN WHITE RATS (*Rattusnorvegicus*) INDUCED BY ASPIRIN

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ABSTRACT

Background: Coffee is a plant that widely consumed by the community. It contains antioxidant-rich compounds such as caffeine, chlorogenic acid, trigonelin, kafestol, and kahweol. Coffee quality related to the roasting process. This study aimed to determine the effect of different roasting level of robusta coffee extract (*Coffea canephora*) on liver histopathology of male Sprague Dawley strain white rats (*Rattus norvegicus*) induced by aspirin. **Methods:** This research is an experimental study with Post-Test Only Control Group design for 15 days. This research used 30 rats in 5 groups, K(-) (aquadest); K(+) (aspirin 90mg/day); P1 (aspirin and robusta coffee extract Light roast 25 mg/kgBW/day); P2 (aspirin and robusta coffee extract Medium roast 25mg/kgBW/day); and P3 (aspirin and dark roast robusta coffee extract 25mg/kgBW/day). Assessment of liver cell damage using modified Suzuki scoring. **Results:** Mean score of hepatocyte damage on the histopathological findings was K(-)=0,44; K(+)=1,92; P1=1,28; P2=1,20; and P3=1,68. Data analysis performed using One Way ANOVA test followed by Post-Hoc LSD and obtained significant results between all groups. **Conclusion:** There is an effect of roasting level of robusta coffee extract (*Coffea canephora*) with the roasting level is Medium Roast on liver histopathology of male Sprague Dawley strain white rats (*Rattus norvegicus*) induced by aspirin.
Keywords : aspirin, coffee roasting, liver histopathology, robusta coffee extract.

INTRODUCTION

Coffee is a type of plantation crop that has been cultivated for a long time and has high economic value. Arabica and robusta are types of coffee that are often consumed by Indonesian people. Before it can be consumed, coffee beans will go through a roasting process after going through the harvesting process. This roasting is the stage of forming the distinctive aroma and taste of coffee which is naturally found in coffee beans.[1]

Besides distinctive taste and aroma, coffee also contains high antioxidants and is beneficial for health. Antioxidants are substances that can neutralize free radicals resulting from excessive oxidation which are detrimental to the body.[2] Robusta green coffee beans have higher antioxidant activity than Arabica coffee, but after roasting this is no longer significant.[3] Roasting is one of the post-harvest activities to improve quality of coffee beans. This roasting aims to obtain the appropriate water content and acidity level. It is necessary to roast the coffee correctly regarding the temperature and duration of roasting so that the quality and benefits can be improved.[4]

Besides high in antioxidants, coffee also has antifibrogenic chemoprotective mechanisms. According to various clinical data, this antioxidant activity and mechanism shows a protective effect on the liver and makes coffee a promising hepatoprotector.[5] The liver is the largest metabolic organ which is very important for maintaining life functions and plays a role in almost every metabolism in the body. However, in carrying out its function the liver can experience limitations in its detoxification function as a result of an overdose of toxic substances. If hepatotoxic substances exceed physiological limits, damage to liver cells will occur.[6] Damage to the liver can be caused by various factors, including drugs, infection, alcohol, autoimmune disease, or hepatitis.[7]

Aspirin or known as salicylic acid is a class of NSAIDs, which have analgesic, antipyretic and anti-inflammatory effects which are very widely used by the public and are classified as over-the-counter drugs. When using aspirin, toxicity is often reported.[8] Aspirin can cause liver damage, usually occurring in patients who are given high doses of aspirin which causes the concentration of aspirin in plasma to be above 150 µg/ml. These side effects

usually appear in the first weeks of therapy, because the use of high doses causes too much reactive metabolite from aspirin in the liver. Glutathione (GSH) cannot detoxify excess metabolites, causing liver toxicity and ultimately liver problems.[9]

Liver dysfunction is a problem in both developed and developing countries, especially in Indonesia. The prevalence of liver disease sufferers in Indonesia, both from viral and non-infectious infections, tends to increase rapidly.[10] The high cost of treating liver disorders has resulted in people switching to using medicinal plants as an alternative for prevention and treatment.[11] Riskesdas 2014 data shows the percentage of the Indonesian population who 59.12% have ever consumed traditional medicine in all age groups, men and women, both in rural and urban areas.[10,12]

Given the problem of hepatotoxicity caused by aspirin, researchers see the potential of robusta coffee beans as a promising hepatoprotective substance with one process to maximize its benefits, namely through the roasting process. Therefore, the aim of this study was to determine the effect of administering robusta coffee extract and the effect of different levels of robusta coffee roasting on the histopathological features of male white rats (*Rattus norvegicus*) of the Sprague Dawley strain that had been induced by aspirin.

METHODS

This research type is experimental with a post-test only control group design which uses experimental animals as research objects. This research group consisted of negative control, positive control, treatment 1, treatment 2, and treatment 3 groups.

This research was carried out in September – December 2021. Treatment and testing on male white mice was carried out in the animal house of the Faculty of Medicine, University of Lampung. Robusta coffee bean extract (*Coffea canephora*) was made in the organic chemistry laboratory of the Faculty of Mathematics and Natural Sciences at the University of Lampung, and preparation and reading of the preparations were carried out at the Histology and Anatomical Pathology Laboratory at the University of Lampung.

The population of this study was male white rats (*Rattus norvegicus*) of the Sprague Dawley strain aged 8 - 12 weeks, with a body weight ranging from 100 - 200 grams. This research consisted of 5 treatment groups. The number of mice in each group was determined using Federer's formula for experimental tests $(t-1)(n-1) \geq 15$, where t is the number of experimental groups and n is the number of repetitions or number of samples for each group. Obtain a sample size of 25 mice and correct it using an anticipated drop out of 10% to obtain a reserve number of 5 mice. The total number of mice used in this research was 30 white mice.

The inclusion criteria for mice used as samples for this study were healthy mice, with indications of clear eyes, clean fur, and active movement and free from disease, gender of male mice, to avoid the influence of reproductive hormones on the immune system, menstrual cycle and pregnancy. as well as mice aged 8 - 12 weeks with a body weight of 100 - 200 grams. Meanwhile, the sample exclusion criteria include mice that died during the treatment, mice that experienced weight loss of >10% after the adaptation period in the laboratory, and illness (appearance of hair loss, dullness and inactivity, abnormal exudate discharge from the mouth, anus, genital or eye).

After acclimatization for 7 days, randomization was carried out for each group consisting of 5 mice. In the negative control group (K-) mice were only given food and drink ad libitum, the positive control group (K+) were given aspirin induction at a dose of 90 mg/day, treatment 1 (P1), treatment 2 (P2) and treatment 3 (P3) Aspirin will be induced at a dose of 90 mg/day, and coffee extract will be given at 25 mg/kg/day with light, medium and dark roasting degrees. Mice were kept in cages covered with woven wire and placed in a room with sufficient light and ventilation and not exposed to direct sunlight. The cage is cleaned 3 times a week so that the mice are protected from dirt that can cause infection.

Data were analyzed with SPSS version 27.0 statistical software (IBM Corp). Data analysis was carried out using the One Way ANOVA statistical test followed by the Post-hoc LSD test because the data was normally distributed and the variance between groups was the same.

RESULTS

Liver cell damage was assessed from observations in the liver parenchymal area, between the central vein and the portal triad, which included sinusoidal dilatation, congestion, and also necrosis in one field of view using the modified Suzuki Scoring which can be seen in table 1.

Table 1. Modified Suzuki Scoring

Score	Congestion	Vacuolization	Necrosis
0	*None*	*None*	*None*
1	*Minimal*	*Minimal*	*Single cell necrosis*
2	*Mild*	*Mild*	*Mild*
3	*Moderate*	*Moderate*	*Moderate*
4	*Severe*	*Severe*	*Severe*

In each field of view, the level of severity experienced in one field of view is seen, then the average is calculated. The score used is 0 - 4, where a score of 0 is given to the visual field which is not damaged by congestion, vacuolization and necrosis. A score of 1 is given to visual fields that experience slight (minimal) damage, including congestion, vacuolization and necrosis. A score of 2 is given to visual fields that experience some (mild)

damage, including congestion, vacuolization and necrosis. A score of 3 is given to the visual field which has experienced moderate damage, including congestion, vacuolization and necrosis, and a score of 4 is given to the visual field which has experienced severe damage, including congestion, vacuolization and necrosis, so that quite extensive necrosis cells are found. .After observing and reading the preparations, the results of the analysis in the form of liver damage scores in each group were obtained as follows.

Examination of liver damage scores in this study showed that the average score in the 5 visual fields of the K(-) group was 0.44; in the K(+) group it was 1.92; group P1 is 1.28; group P2 is 1.20; and the P3 group is 1.68. The highest mean liver damage score was found in the K(+) group which was induced by aspirin 90 mg/day without being given coffee extract. Data from the analysis of liver damage scores from each group can be seen in table 2.

Table 2. Scoring Results of Average Liver Cell Damage Scores for Each Group

Sample	Treatment Group				
	K(-)	K(+)	P1	P2	P3
1	0.4	1.4	1	1.8	2
2	0	3	1.6	1.4	1.4
3	1	2	1,2	1.6	1.6
4	0.8	1.8	1.6	1	1.6
5	0	1.4	1	0.2	1.8
Average	0.44	1.92	1.28	1.20	1.68

Information:

K- = Group giveneating and drinking ad libitium.

K+ = Group that was given inductionaspirin 90 mg/day but no coffee.

P1 =The group was given aspirin induction 90 mg/day and light roast coffee extract at a dose of 25 mg/kgBW/day.

P2 =The group was given aspirin induction 90 mg/day and medium roast coffee extract at a dose of 25 mg/kgBW/day.

P3 =The group given aspirin induction 90 mg/day and dark roast coffee extract at a dose of 25 mg/kgBW/day

Based on the results of calculating the average hepatocyte cell damage score in the five research groups, the liver cell damage observed included sinusoidal dilatation, congestion in the form of sinusoidal dilation and bleeding, vacuolization and also necrosis in each group in each visual field. The highest average damage score was obtained in the K(+) group, while in the treatment group, the lowest average cell damage score was obtained, namely in the P2 group, then P1 and P3. The results of the observation of liver cell damage that occurred by observing under a light microscope using 400 times magnification in each research group can be seen in Figure 1.

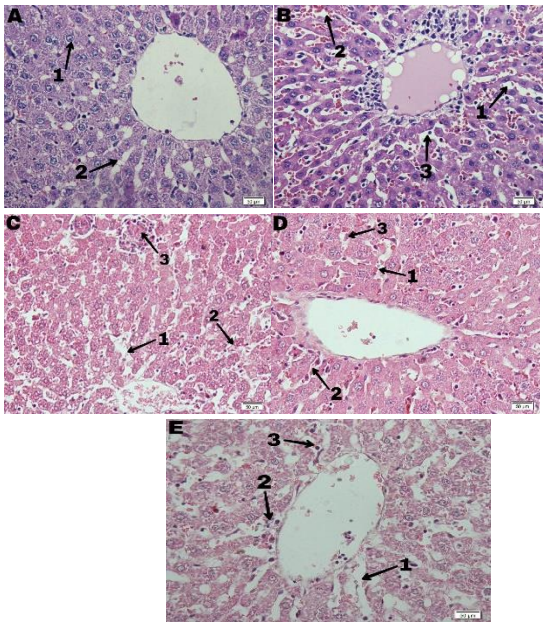

Figure 1. Histopathological image of liver cells

Description: a) group K(-) with 1=normal hepatocyte cells, 2=hepatic sinusoids, b) group K(+), c) group P1, d) group P2, e) group P3 with 1=dilated sinusoids, 2 =congestion, 3=necrosis

The normality test in this study uses a test*Shapiro-Wilk*because the number of samples used is less than 50. Interpretation of the normality test is if the significance value (*p*)>0.05 then the data is normally distributed. In this research, values were obtained*p*>0.05 means that all groups in the data for both variables are normally distributed. Normality test results can be seen in table 3.

Table 3. Test S*aphiro-wilk*

Variable	Treatment Group	Significance (p)
Liver Damage Score	K-	0.329
	K+	0.179
	P1	0.086
	P2	0.482
	P3	0.814

The homogeneity test in this study was used*Levene test*.Data is said to be homogeneous if it has variance value *p*>0.05. In this study, the proportion value obtained for the liver damage score variable was 0.309, which means that the data for the two variables were homogeneous. The homogeneity test results can be seen in table 4.

Table 4. Homogeneity Test - *Levene test*

	df1	df2	Sig.
Liver Damage Score	4	20	0.309

To be able to see whether there is an influence on the level of roasting of robusta coffee beans (*Coffea canephora*) on the histopathological picture of the liver of male white rats (*Rattus norvegicus*) aspirin-induced Sparague dawley strain can use the test One Way ANOVA. This test can be carried out if the data obtained is normally distributed and homogeneous. In this study, data were obtained that were normally distributed and homogeneous, so the univariate analysis used the mean which will later be followed by a test. One Way ANOVA. Test One Way ANOVA in this study uses a significance levelα5%. In this research, values were obtained$p$namely 0.001 (p<0.05) which indicates there is a significant difference between groups. Results One Way ANOVA can be seen in table 5.

Table 5 Test One Way ANOVA

	Mean Square	F	Significance
Liver damage score	1,598	6,750	0.001

Based on test results One Way ANOVA results were obtained p<0.05 which means there is a significant difference between groups. Determinate the level of Robusta coffee bean (*Coffea canephora*) roasting that is effective in repairing liver cell damage in male white rats (*Rattus norvegicus*) of the Sparague Dawley strain that is induced by aspirin can be seen using the Post-Hoc LSD test.

Based on the Post Hoc LSD test for the mean score of liver cell damage, a significant difference (p <0.005) was found between K- and K+, P1, P2, and P3; between K+ and P2; while in the other groups the differences were not significant (p > 0.005). It was found that the level of roasting of robusta coffee beans (*Coffea canephora*) that was effective in repairing aspirin-induced liver cell damage in mice was coffee with a medium roast level. The results of the LSD post hoc test can be seen in table 6.

Table 6. LSD Post Hoc Test Mean Liver Damage Score

Group	K(-)	K(+)	P1	P2	P3
K(-)	-	0,000*	0.013*	0.023*	0.001*
K(+)	0,000*	-	0.051	0.030*	0.445
P1	0.013*	0.051	-	0.798	0.208
P2	0.023*	0.030*	0.798	-	0.135
P3	0.001*	0.445	0.208	0.135	-

Information:
* = p-value < 0.05 (significant)

DISCUSSION

Based on the results of microscopic observations of the histopathological picture of the K(-) group which was only given food and distilled water ad libitum, the average liver cell damage score was 0.44 with a normal hepatocyte cell structure with a few cells experiencing necrosis (single-cell necrosis). Hepatocyte cells are arranged in radial cell plates with the central vein as the center. The liver sinusoids looked normal, there was slight dilation, there was no bleeding in the sinusoids but there were some inflammatory cells.

In theory, the negative control group should not have experienced any damage, namely without sinusoid dilation, bleeding, or necrosis accompanied by some inflammatory cells, however in this study in several fields of view we found sinusoid dilation, and also single-cell necrosis in the centrilobular area. This can be caused by stress factors in mice. Under stressful conditions, immune tolerance is impaired, resulting in inflammation in the liver. Contributors to this process can be categorized as follows: hypoxia-reoxygenation, over-activation of Kupffer cells and oxidative stress, influx of gut-derived lipopolysaccharide and norepinephrine, and excessive production of stress hormones and activation of sympathetic nerves.[13]

In the K(+) group which received treatment in the form of aspirin induction at a dose of 90 mg/day for 14 days, liver histopathological results were obtained in the form of hepatocyte cell damage in all mouse samples. Damage in the K(+) group includes changes in the structure of the hepatocyte cells which appear not arranged in radials around the central vein, the boundaries between hepatocytes are not clear, and the shape of the sinusoids is widened with bleeding. Hepatocyte cells also appear to experience swelling and necrosis. To see significant mean differences between groups, it can be seen using the Post-Hoc LSD test statistical assessment. Results with Post-Hoc LSD showed significant results (p<0.05) on the mean hepatocyte cell damage score between the K(-) group and the K(+) group (p=0.000). This value shows that mice given aspirin induction were able to show damage to liver cells.

In this study, liver cell damage that occurred in the K(+) group and other treatment groups was mainly caused by the induction of toxic doses of aspirin. Hepatotoxicity occurs with the use of high-dose, short-latency aspirin, and is associated with high plasma salicylate levels. This suggests that aspirin is a direct intrinsic hepatotoxin. The mechanism by which aspirin affects the liver occurs through the mechanism, accumulation of aspirin in the liver which will inhibit the oxidative phosphorylation process similar to the effect caused by 2,4-dinitrophenol. In toxic doses, aspirin can inhibit the aerobic metabolism of several dehydrogenase enzymes in the liver and other tissues by competing with the pyridine nucleotide coenzyme and inhibiting several oxidase enzymes that require nucleotides as coenzymes, such as xanthin oxidase which is useful for defense in the liver.[14]

When large amounts of toxic substances reach the liver, necrosis occurs due to dramatic intracellular changes, or as a result of the formation of large amounts of free radicals (ROS and NO-) as well as TNF-α and IL-6 from Kupffer cells and endothelial cells that are activated after stimulation by lipopolysaccharide (LPS) and small intestine-derived cytokines delivered via the portal vein. In this state, the drug is oxidized by the enzyme cytochrome P-450

(CYP-450), with the release of large amounts of reactive metabolites, triggering lipid and protein peroxidation and reduced GSH. The result of this process is that oxidized proteins and additional proteins may have immunogenic properties that can activate Kupffer cells and PMN, resulting in the subsequent release of ROS.[15] Concentrations of free radicals whose levels cannot be neutralized by antioxidants can cause oxidative stress, apoptosis and necrosis in body. Lipid peroxidation and the decrease in antioxidants that occur are indicators of oxidative stress. This oxidative stress can then cause lipid peroxidation, causing damage to cells, tissues and organs such as the liver.[16]

Microscopically, liver cell damage observed is inflammation, widening of the sinusoids, congestion or bleeding in the sinusoids and also necrosis in the hepatic parenchyma. In this study, it was found that there was a difference in the mean liver cell damage scores in the K(+) group with P1, P2, and P3 in the form of a decrease in the mean cell damage scores in the P1, P2, and P3 groups compared to the K(+) group. This is in line with previous research conducted by Bahcecioglu, regarding the protective effect of Pistachia terebinthus coffee extract on thioacetamide-induced liver cell damage in rat models, stating that there was a significant reduction in inflammation, necrosis and fibrosis scores and the results of ALT and AST enzyme examinations did not increase. which showed that there was no toxic injury in liver biopsies in the group of mice induced by Thiocetamide (TAA) plus Pistachia terebinthus coffee extract (PTC), compared to the group that was only induced by TAA.[17] These results may be related to the protective effect of PTC coffee on liver cells. Due to the high content of phenols and flavonoid components, the membrane stabilizing effect of alpha tocopherol with the content is rich in alpha tocopherol, so it can show hepatoprotective activity.[18]

Research conducted by Moreno (2011) has shown that coffee has compounds that can provide a protective effect against histological fibrosis and clinical fibrosis by suppressing factors such as the fibrogenic cytokine collagen I, changing TGF-β, and stellate cell activation. In the liver, coffee can reduce lipid peroxidation, collagen levels (4-fold), TGF-β levels, glycogen mRNA and protein, and glutathione. The results of this study indicate that coffee's role in preventing cirrhosis appears to be related to its antioxidant properties and ability to reduce the profibrogenic cytokine TGF-β.[19]

Research by Savira (2022) has shown that coffee is a source of antioxidants. Coffee contains phenolic compounds (such as chlorogenic acid, caffeic acid, tryptophan, caffeoyl) and melanoidin which have been tested in vitro to have antioxidant and antimutagenic effects. The antioxidant activity of these compounds is known to inhibit lipid peroxidation in vitro.[20]

Administration of robusta coffee (*Coffea canephora*) extract with various levels of roasting was proven to be able to influence the histopathological picture of liver cells which was observed in the form of congestion accompanied by bleeding in the sinusoids in the area around the central vein and cell necrosis in the hepatic parenchyma. This was proven through hypothesis testing where the K(+) group who were given a toxic dose of 90 mg/kgBW/day of aspirin had a higher mean liver damage score compared to the treatment groups, both groups P1, P2, and P3 who were given robusta coffee extract (*Coffea canephora*) with light roast, medium roast, and dark roast levels at a dose of 25 mg/kgBB/day. Based on the One Way ANOVA analysis test, the p value = 0.001, namely p value <0.05, the results of this statistical test show that there is a difference in the average liver damage score for groups K(-), K(+), P1, P2 and P3 in white rats (*Rattus norvegicus*) of the Sprague Dawley strain subjected to toxic doses of aspirin.

To see significant mean differences between groups, you can see the Post Hoc LSD test. Results with Post Hoc LSD showed significant results (p<0.05) on the mean hepatocyte cell damage score between the K(+) group and the P2 group (p=0.030). However, the differences that occurred between K(+) and groups P1 (p=0.051) and P3 (p=0.445) did not show significant results. This value shows that group P2 can provide a picture of significant improvement in liver cells, whereas in groups P1 and P3 there was no significant improvement in liver cells. It can be concluded that there is an effect of giving robusta coffee (*Coffea canephora*) extract with a medium roasting degree or P2 group on the average liver cell damage score of white rats (*Rattus norvegicus*) induced by aspirin.

This is in line with research conducted by Cammerer, in coffee with increasing degrees of roasting, optimal antioxidant action was found for coffee with medium roast degrees. When coffee is roasted, the chlorogenic acid content decreases but at the same time, the content of melanoidin compounds which also have antiradical and antioxidant activity in coffee increases, the formation of melanoidin seems to compensate for the possible reduction in the level of antioxidant activity.[21,22,23] In other studies it was found that melanoidin can reduce colon inflammation through improving the balance of the microbiota (prebiotic effect).[24,25]

Coffee quality is significantly related to the roasting process.[26] In addition, during the roasting process there are many changes in the coffee beans related to the compound profile of the coffee bean composition and increased aroma. Major changes in coffee bean composition occur during the roasting process as a result of the Maillard reaction.[27] The roasting process greatly influences the chlorogenic acid content, leading to hydrolysis of chlorogenic acid as temperature and roasting time increase. There are also new compounds that are formed during the roasting process, one of which is melanoidin. The resulting melanoidin is the nitrogen-containing final product of the Maillard reaction, and is formed through the reaction of reducing sugars with lysine residues of proteins and peptides. Its formation can change the overall antioxidant capacity of coffee beans after roasting.[28,29]

In research conducted by Jung was carried out to look at the antioxidant properties of coffee extracts with different roasting levels, this research was carried out in vitro to look at the compound content in coffee. The results showed that the best antioxidant activity was proven in light roast coffee extract and the lowest in dark roast coffee. These results indicate that the antioxidant properties of coffee extracts are related to the roasting process and duration, with negative effects occurring as roasting increases. It was also found that this anti-inflammatory effect showed the same pattern as the antioxidant properties, which were negatively correlated with the level of roasting. This means that coffee extract has an anti-inflammatory effect in the biological system and this effect decreases as the coffee is further roasted. It seems that the differences in the anti-inflammatory effects of coffee extracts with different levels of roasting may be caused by differences in the caffeine and chlorogenic acid content in the coffee extracts.[26]

Meanwhile, research conducted by Choi (2018) conducted in vivo, showed results that the level of coffee roasting did not dramatically affect the physiological antioxidant system in LPS-induced mice. Hepatic portal vein invasion and liver necrosis were reduced when mice were pretreated with Medium (204° for 9 minutes) or City (209° for 10.33 minutes) roasted coffee extract. However, when mice were pretreated with French roast coffee extract (212° for 11.33 minutes), hepatic necrosis and inflammatory cell infiltration increased. Meanwhile, mice that were pretreated with light roasted coffee extract (204° for 9 minutes) showed insignificant results.[30]

These results are not in accordance with the results of research that has been carried out previously using in vitro models. However, the complexity of the physiological system in animal models may produce different results in the form of a reduction in liver cell damage as an anti-inflammatory effect that is not in line with increasing the degree of coffee roasting. The anti-inflammatory effect of coffee extract seen in the histopathology of animal models was found to differ depending on the level of roasting. This may occur due to the fact that the proportion of constituents in the coffee extract is changed by the roasting process as well as the influence of physiological factors in experimental animals.[31]

Caffeine is the main component in coffee and has antioxidant properties. The carbohydrates, proteins, and chlorogenic acids present in coffee all decrease during processing.[32] The caffeine content shows no difference between roasting levels, but the chlorogenic acid content decreases as the degree of roasting increases. Because caffeine is thermostable, it is understood that the degree of roasting has no effect.[33] In addition, the antioxidant capacity of coffee is also associated with the presence of polyphenolic compounds, and it is well understood that roasting affects the antioxidant properties of coffee.[34]

When coffee is roasted, the trigoneline content decreases and niacin increases. Niacin (nicotinic acid) is a compound that is very important for specific oxidation-reduction reactions in the body and has also been proven to be able to reduce several neurological disorders, while trigonelline is also known to have antioxidant activity. 35,36 Dark roast coffee has higher levels of N-methylpyridinium, which This compound is a content in coffee which has the opposite effect to caffeine and cafestol, so it can reduce the risk of a significant increase in stomach acid.[37] In another study conducted by Safe gastric acid secretion was lower after consuming dark roast blend coffee compared to medium roast blend38.Subsequent research stated that roasted coffee is the main source of norharman and harman carbolines, which are monoamine oxidase (MAO) inhibitors. The amount of this substance is very low in green coffee and increases significantly during roasting.[39]

A comparison of the effectiveness of the ingredients in coffee in its antioxidant activity is known as follows, caffeine has antioxidant properties, which are indicated by its affinity for reducing hydroxyl radicals with biomolecular rate constants ranging from 2.6×10^9 $M^{-1}s^{-1}$ to $5.9 \times 10^9 M^{-1}s^{-1}$.[40] Chlorogenic acid (CGAs) are polyphenolic compounds which are known to have antioxidant activity as strong hydroxyl radical reducers with a hydroxyl reduction rate constant of 7.73×10^9 $M^{-1}s^{-1}$.[41] Meanwhile, the antioxidant activity of melanoidin after digestion shows the highest antioxidant activity.[42] Each compound in coffee contributes to each free radical reducing activity and has different reducing power. Coffee roasting is a complex system, and further studies are needed to elucidate the contribution of each compound to the antioxidant activity of coffee.[43,44]

Based on the results of the research and studies in the discussion, Ha in this study which states that there is an influence of the level of roasting of robusta coffee beans (*Coffea canephora*) on the liver histopathology of male white rats (*Rattus norvegicus*) of the Sprague Dawley strain that has been induced by aspirin is acceptable.

Based on hypothesis testing and follow-up in this research, meaningful and significant results were also obtained between the research groups. This shows that there is an effect of giving Robusta coffee extract with medium roasting degree on the histopathological picture of the liver of white rats induced by aspirin. However, an increase in the degree of roasting is not directly proportional to a decrease in the picture or average score of liver cell damage that occurs. So the optimal roasting level recommended for reducing liver damage based on this research is robusta coffee with a medium roast degree.

CONCLUSION AND SUGGESTION

Based on the research that has been carried out, it can be concluded that there is an influence on the level of roasting of robusta coffee beans (*Coffea canephora*) on the liver histopathology of male white rats (*Rattus norvegicus*) of the Sprague Dawley strain which have been induced by aspirin with a level of roasting that can significantly reduce hepatocyte cell damage, namely robusta coffee with medium roast degree.

It is suggest that further research be carried out examining liver enzyme levels as a marker for liver damage or repair so that the research results are more comprehensive and accurate. It can also be refined by using more variables such as comparing various types of coffee and further researching the amount of coffee consumption that is effective in providing benefits in improving liver disease.

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CONFLICT OF INTEREST

There are no conflict of interest.

ETHICAL ASPECT

This research has been approved by the Health Research Ethics Committee, Faculty of Medicine, University of Lampung in accordance with ethical approval letter number 2808/UN26.18/PP.05.02.00/2021.

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