Pharmacogn J. 2023; 15(5): 750-755
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Supplementation of *Caesalpinia Sappan L.* Extract to Increase Superoxide Dismutase Activity and Suppress Malondialdehyde Levels in *Sprague Dawley* Exposed to Inhaled Formaldehyde

Ulfa Nurullita[1,2,*], Neni Susilaningsih[1], Ari Suwondo[1], Suhartono[3], Kisdjamiatun Retna Mustika Djati[1]

[1]*Faculty of Medicine, Diponegoro University, INDONESIA.*

[2]*Faculty of Public Health, Universitas Muhammadiyah Semarang, INDONESIA.*

[3]*Faculty of Public Health, Diponegoro University, INDONESIA*

Correspondence

Ulfa Nurullita

Faculty of Medicine, Diponegoro University, INDONESIA; Faculty of Public Health, Universitas Muhammadiyah Semarang, INDONESIA.

E-mail: ulfa@unimus.ac.id

History

- Submission Date: 29-06-2023;
- Review completed: 16-07-2023;
- Accepted Date: 29-07-2023.

DOI : 10.5530/pj.2023.15.147

Article Available online

http://www.phcogj.com/v15/i5

ABSTRACT

Introduction: It is not yet known how *Caesalpinia sappan* L. extract (CSE) affects the activity of SOD and MDA levels in rats exposed to inhaled formaldehyde. **Method**: This true experimental study *in vivo* uses a randomized post-test-only control group design. The subjects were male Sprague Dawley, 2-4 months old, weight 200-300 grams, a total of 30 heads divided into 6 experimental groups. The treatment group was given CSE at doses of 0, 100, 400, and 1000 g/kg BW for 28 days, and exposed to inhaled formaldehyde for 2 weeks, and 8 hours/day. SOD and MDA were measured using the ELISA kit. Statistical analysis used Kruskall Wallis, Mann Whitney, and rank spearman tests with $\alpha = 5\%$. **Results**: The highest average SOD was in the CSE 1000 group and the lowest was in the negative control group. The highest average MDA levels were in the negative control group and the lowest was in the positive control group. The difference in SOD levels between groups with a p-value = 0.016, while the difference in MDA between groups has a p-value of 0.915. the p-value of the relationship between SOD levels and MDA levels is 0.02 (correlation coefficient = -0.422). **Conclusion**: *Caesalpinia sappan* L. extract can increase the activity of superoxide dismutase enzymes but has not been able to suppress malondialdehyde levels. There was an association of increased superoxide dismutase enzyme activity with decreased malondialdehyde levels in Sprague Dawley exposed to inhaled formaldehyde

Key words: *Caesalpinia Sappan L.* Extract, Superoxide Dismutase Activity, Suppress Malondialdehyde Levels, inhaled formaldehyde, *Sprague Dawley.*

INTRODUCTION

The mortality rate due to occupational accidents and occupational diseases is quite high. Occupational diseases can be any health condition that mainly occurs due to risk factors from work activities. WHO states that efforts to diagnose, record, and report occupational diseases are very important.[1] Occupational diseases are an important concern because they can be viewed from 2 aspects, namely medical and legal aspects.[2] According to the deed, occupational diseases are so difficult to legally recognize as a consequence of employment.[3] This circumstance is certainly not favorable to workers. A very important indicator of the quality of the condition and work environment, of which is an occupational disease.[4]

The causes of occupational diseases can come from physical, chemical, biological, ergonomic, and psychological factors. During work, workers will be constantly exposed to physical and chemical risk factors of their work environment.[5] Gas is a chemical risk factor that has great potential to threaten human health.[6] One of the chemicals widely used in industry today is formaldehyde which is used for raw materials and accompanying materials of various products and other chemicals such as textiles, plywood, paints, food, carpets, insecticides, and plastics. Exposure to formaldehyde in the work environment mainly occurs through the respiratory tract. Formaldehyde (HCHO) is a reactive ingredient and is an environmental pollutant that is genotoxic and mutagenic.[7,8]

Formaldehyde is a source of Reactive Oxygen Species (ROS) and exogenous free radicals. Free radicals that cause oxidative stress have been reported in various diseases such as diabetes mellitus, neurodegenerative disorders, cardiovascular diseases, respiratory diseases, cataract development, rheumatoid arthritis, and in various cancers.[9] Personal air formaldehyde (air-FA) is a risk factor for airway inflammation and induces oxidative stress.[10] Oxidative stress is a disturbance in the balance between free radicals and antioxidants present in biological systems.[11] Several studies show malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione sulfhydryl (GSH) are parameters of oxidative stress.[12,13]

Formaldehyde, which is a free radical, is certainly also able to reduce antioxidant activity and increase cell damage. Several research proved formaldehyde significantly decreased superoxide dismutase and glutathione peroxidase, increased lipid peroxidation with malondialdehyde formation, increased isoprostane levels, and TNF-α, as well as there was a dosing response relationship between lipid peroxidation index and formaldehyde concentration.[8,14] Rat exposed to formaldehyde inhalation showed higher MDA values than the control group, while Superoxide Dismutase (SOD) values were lower than those of the control group. There is a dose-response relationship between formaldehyde concentration and lipid peroxidation index.[15]

Cite this article: Nurullita U, Susilaningsih N, Suwondo A, Suhartono, Djati KRM. Supplementation of *Caesalpinia Sappan L.* Extract to Increase Superoxide Dismutase Activity and Suppress Malondialdehyde Levels in *Sprague Dawley* Exposed to Inhaled Formaldehyde. Pharmacogn J. 2023;15(5): 750-755.

Free radicals and oxidative stress can be inhibited and prevented with antioxidant compounds. Antioxidants work by adding a hydrogen atomic group to electrons that do not have a pair so that they are stable.[10] The use of antioxidants in preventing oxidative stress in workers in the industry is necessary considering the continuous exposure to harmful materials in the workplace. One type of natural antioxidant is Sappan wood (*Caesalpinia sappan* L). Natural homoisoflavanone derived from Caesalpinia sappan L. extract has anti-inflammatory and antioxidative properties.[16] *Caesalpinia sappan* L. contains Brazilin and Brazilein as a component of flavonoids which are antioxidants. Brazilin is a compound isolated from *Caesalpinia sappan* L., has pharmacological abilities in the form of cardiotonic, and immunosuppressive effects, and gives effects on nerves and antitumor activity.[17]

Research on the impact caused by formaldehyde has been widely carried out. Formaldehyde may induce lung injury,[18] reproductive injuries,[7] semen quality,[19] and induced lipid peroxidation.[8] Research on *Caesalpinia sappan* L. has also been widely carried out, which shows that nano-soursop leaves and nano-sappan wood induce apoptosis and necrosis in HeLa cells.[20] Other studies have shown sappanone from *Caesalpinia sappan* L. reduces endoplasmic reticulum stress, relieves inflammation, oxidative stress, and apoptosis, and thus serves as a therapeutic potential for protection against cerebral ischemia-reperfusion injury in ischemic stroke.[16] *Caesalpinia sappan* L. is also antiviral against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) as a feed additive or veterinary drug in the pig industry.[21] It is not yet known how *Caesalpinia sappan* L. affects subjects exposed to inhaled formaldehyde.

Brazilin can reduce oxidative stress by reducing the accumulation of Reactive oxygen species and malondialdehyde, and increasing the activity of superoxide dismutase and glutathione peroxidase (GXH-Px).[22] It is not yet known how *Caesalpinia sappan* L. extract affects the activity of superoxide dismutase and malondialdehyde levels in rats exposed to inhaled formaldehyde. In addition, it is also known that the dose of *Caesalpinia sappan* L. extract can increase superoxide dismutase activity and suppress malondialdehyde levels, thus this research needs to be done.

Research analyzing the relationship between superoxide dismutase and malondialdehyde has been widely conducted. However, the study that analyze the relationship between the two, was not caused by exposure to inhaled formaldehyde. Existing research is largely due to exposure to formaldehyde in solid/liquid form that enters through the digestive tract. The purpose of this study was to analyze the effect of *Caesalpinia sappan* L. ethanol extract on increased superoxide dismutase activity and suppression of malondialdehyde levels and analyze the relationship between superoxide dismutase levels and malondialdehyde levels in rats (*Sprague Dawley*) exposed to inhaled formaldehyde. It is hoped that the results of this study can be applied to human interests, especially to workers who are exposed to inhaled formaldehyde from their work environment.

METHOD

This research is a true experimental study in vivo. The design of this study is a Randomized Post Test Only Control Group Design. The subjects of the study were male Sprague Dawley, 2-4 months old, weight 200-300 grams, and in healthy condition (active, not deformed). The calculation of the number of samples with the Federer formula, where the number of treatment groups was 6 so that 5 tests were obtained for each treatment so that the total study subjects were 30.

Research materials include standard feed, *Caesalpinia sappan* L. extract, and 10% liquid formalin. The research equipment includes experimental animal cages, a Petri dish, sonde needles, anesthesia equipment (Ketamine dose:0.2 mg/kg BB and Xylazine dose: 0.1 mg/kg BB), a 3-cc syringe, an ELISA kit.

The experimental animals are placed in a cage that is given a hole and connected by a plastic pipe to a box containing liquid formaldehyde. The formaldehyde gas presented comes from 10% liquid formaldehyde. Formaldehyde is presented for 8 hours/day following the daily pattern of labor hours. The design of the experiment is as follows:

Negative control group	:	Exposure to inhaled formaldehyde for 5 days, then without exposure for 2 days, followed by exposure for 5 days, and without exposure again for 2 days
Positive control group	:	14 days given vitamin C 0,075 mg/g BW/day, then given to inhaled formaldehyde exposure for 5 days, then without exposure 2 days, followed by 5 days exposure, and without exposure again 2 days
Normal control group	:	Standard feed only for 28 days
CSE 1000 group	:	14 days given *Caesalpinia sappan L.* extract as much as 1000 mg/kg body weight, then given to inhaled formaldehyde exposure for 5 days, then without exposure for 2 days, followed by 5 days exposure, and without exposure again for 2 days
CSE 400 group	:	14 days given *Caesalpinia sappan L.* extract as much 400 mg/kg body weight, then given to inhaled formaldehyde exposure for 5 days, then without exposure for 2 days, followed by 5 days exposure, and without exposure again for 2 days
CSE 100 group	:	14 days given *Caesalpinia sappan L.* extract 100 mg/kg body weight, then given to inhaled formaldehyde exposure for 5 days, then without exposure for 2 days, followed by 5 days exposure, and without exposure again for 2 days

*CSE: *Caesalpinia sappan L.* Ethanol Extract

After the intervention, blood serum is taken and measurements of endogenous antioxidant enzyme status (superoxide dismutase levels) and lipid peroxidation status (malondialdehyde levels) are carried out. superoxide dismutase levels and malondialdehyde levels were analyzed by the ELISA method. Data analysis was carried out descriptively and analytically with the Kruskall Wallis, Mann Whitney, and Spearman Rank tests with a meaningfulness rate of 95%.

RESULTS AND DISCUSSION

Superoxide dismutase levels in various treatment groups

The results of measuring the superoxide dismutase level of the entire sample are at least 9,317, a maximum of 15,863, and an average of 12,921 ± 2,007 nano mol/ml.

Based on table 1, the highest average superoxide dismutase was in the CSE 1000 group (which was given a 1000 dose of CSE) and the lowest was in the negative control group. When compared to the positive control group, the superoxide dismutase levels of the CSE group were higher. Among the groups that received *Caesalpinia sappan* L. ethanol extract, the CSE 400 group had the lowest superoxide dismutase levels.

In addition, the percentage value of antioxidant inhibition in rat blood serum samples was also calculated with the results as shown in table 2 below:

The percentage of inhibition is the percentage of free radicals that can be counteracted by endogenous antioxidants. The highest percentage of inhibition was in the CSE 1000 group which means that endogenous antioxidants in the group given 1000 mg/kg BW *Caesalpinia sappan* L. extract were able to ward off 72.46% of free radicals derived from

Table 1: Rat blood serum SOD (nano mol/ml) levels in various treatments.

Treatment groups	Minimum	Maximum	Average
Negative control	9.317	11.044	9.992 (SD ± 0.698)
Positive control	13.052	15.141	13.494 (SD ± 1.359)
Normal Control	11.325	15.060	13.542 (SD ± 1.363)
CSE 1000	13.635	15.321	14.490 (SD ± 0.725)
CSE 400	9.93	15.863	12.271 (SD ± 2.453)
CSE 100	11.265	15.422	13.739 (SD ± 1.544)

Table 2: Percentage of antioxidant inhibition in various treatments.

Treatment groups	Minimum	Maximum	Average
Negative control	46.7	55.2	49.96 (SD ± 0.035)
Positive control	57.3	75.7	67.48 (SD ± 0.068)
Normal control	56.6	75.3	67.7 (SD ± 0.068)
CSE 1000	68.2	76.6	72.46 (SD ± 0.036)
CSE 400	49.6	79.3	61.36 (SD ± 0.122)
CSE 100	56.3	77.1	68.7 (SD±0.077)

Table 3: Rat blood serum malondialdehyde levels (nano mol/ml) in various treatments.

Treatment groups	Minimum	Maximum	Average
Negative control	0.667	189.2	71.107 (SD±78,846)
Positive control	12.133	60.667	28.173 (SD±19.657)
Normal control	1.467	173.333	47.493 (SD±71.418)
CSE 1000	12.933	70.333	38.347 (SD±25.042)
CSE 400	16.333	48.267	33.120 (SD±11.898)
CSE 100	8.6	86.6	41.227 (SD±32.743)

Table 4: Differences in superoxide dismutase levels between treatments.

Spouse Treatment	p- value	Meaning
Negative control- positive control	0.008	significant differences
Negative control- normal control	0.008	significant differences
Negative control- CSE 1000	0.008	significant differences
Negative control- CSE 400	0.095	No significant differences
Negative control- CSE 100	0.008	significant differences
Positive control- normal control	0.841	No significant differences
Positive control- CSE 1000	0.310	No significant differences
Positive control- CSE 400	0.310	No significant differences
Positive control- CSE 100	0.841	No significant differences
Normal control- CSE 1000	0.222	No significant differences
Normal control- CSE 400	0.310	No significant differences
Normal control- CSE 100	0.841	No significant differences
CSE 1000 - CSE 400	0.151	No significant differences
CSE 1000 - CSE 100	0.690	No significant differences
CSE 400 - CSE 100	0.310	No significant differences

exposure to inhaled formaldehyde. The lowest percentage of inhibition in the negative control group was 49.96%. This indicates the percentage of inhibition in line with superoxide dismutase levels.

Malondialdehyde levels in various treatment groups

Malondialdehyde levels are at least 0.667, maximum 189.2, and average 43,244 ± 44.52 nano mol/ml. The distribution of malondialdehyde patterns by treatment group is as follows in table 3:

Based on table 3, the highest average malondialdehyde levels were in the negative control group and the lowest was in the positive control group (vitamin C administration). The malondialdehyde levels of the negative control group were more than 2.5 times the malondialdehyde levels of the positive controls. When compared to the CSE 1000 group, the malondialdehyde level of the negative control group was more than 2.5 times the value. Among the groups that received *Caesalpinia sappan L.* extract, the CSE 400 group had the best malondialdehyde levels. The malondialdehyde CSE 1000 level was almost 1.5 times the CSE 400 group, while the CSE 100 group was 1.8 times the value compared to the CSE 400 group.

The normality test result of superoxide dismutase content data is 0.017, while the malondialdehyde level is 0.000, thus all data are abnormally distributed. The analysis for differences in superoxide dismutase levels between groups with the Kruskall Wallis test obtained a p-value = 0.016, and a malondialdehyde level with a p-value = 0.915. This showing there was a significant difference in superoxide dismutase levels between groups, but there was no significant difference in malondialdehyde levels between groups.

Analysis of differences in superoxide dismutase levels in different treatment groups with the Mann-Whitney test is shown in table 4 below:

Based on table 4, it can be concluded that CSE administration produced better superoxide dismutase levels in rats that obtained formaldehyde exposure and those who did not get formaldehyde exposure than those who did not get CSE. However, at a dose of CSE 400, superoxide dismutase levels were the same as those of the group that did not get CSE.

To assess the relationship between superoxide dismutase and malondialdehyde levels, the Spearman Rank test obtained a value of p = 0.02, with a correlation value (r) = -0.422. Thus, it was concluded that there was a relationship between superoxide dismutase and malondialdehyde levels in Sprague Dawley exposed to inhaled formaldehyde and supplemented *Caesalpinia sappan L.* ethanol extract with a fairly strong correlation and a negative correlation direction, which means that an increase in superoxide dismutase levels will lower the malondialdehyde levels of blood plasma Sprague Dawley exposed to inhaled formaldehyde and supplemented *Caesalpinia sappan L.* ethanol extract.

Descriptive analysis of superoxide dismutase levels based on doses of *Caesalpinia sappan L.* Ethanol extract

A descriptive analysis of superoxide dismutase levels based on the dose of *Caesalpinia sappan L.* ethanol extract appears in the following figure 1:

Based on figure 1, the best superoxide dismutase levels were in the CSE 1000 group (dose 1000 mg/kg BB), and the lowest was in the negative control group (without the administration of *Caesalpinia sappan L.* ethanol extract). Based on the graph, it appears that at a dose of 100 mg/kg BW, superoxide dismutase increased compared to the group without the administration of *Caesalpinia sappan L.* extract, but with an increase in dose to 400 mg/kg BW superoxide dismutase levels decreased. Superoxide dismutase levels increased again at a dose of 1000 mg/kg WB.

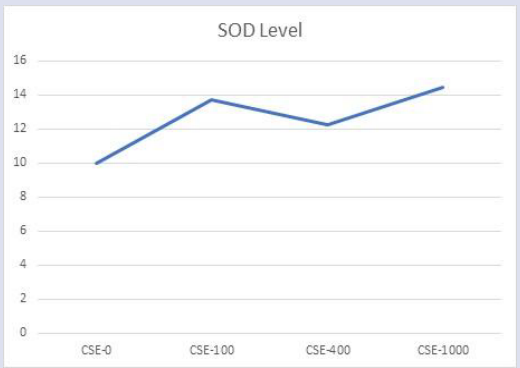

Figure 1: Superoxide dismutase levels based on *Caesalpinia sappan L.* ethanol extract dosage.

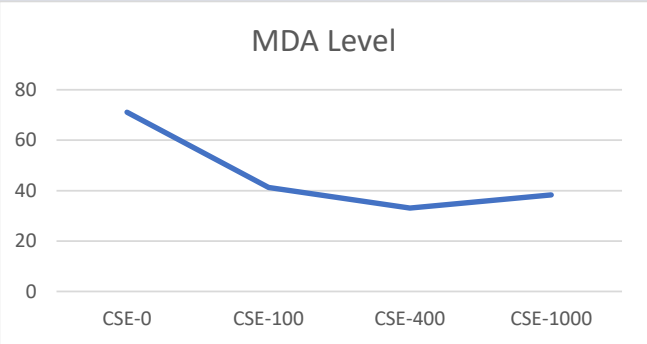

Figure 2: Malondialdehyde levels based on dosage of *Caesalpinia sappan L.* ethanol extract.

Descriptive analysis of malondialdehyde levels based on doses of *Caesalpinia sappan L* ethanol extract

Malondialdehyde levels based on the dosage of *Caesalpinia sappan L.* ethanol extract are depicted in the following figure 2:

Based on chart 4, shows a pattern that is the opposite of superoxide dismutase levels, where malondialdehyde levels decrease further with an increase in the dose of *Caesalpinia sappan* L. extract up to a dose of 400 mg/kg BW. However, malondialdehyde levels increased with an increase in dose to 1000 mg/kg BW. In general, the results showed that the lowest superoxide dismutase levels and the highest malondialdehyde levels were in the negative control group.

Effect of *Caesalpinia sappan L.* ethanol extract on blood serum superoxide dismutase levels of *Sprague Dawley* exposed to inhaled formaldehyde

Measurement of superoxide dismutase enzyme activity was used to determine the ability of *Caesalpinia sappan L.* ethanol extract in scavenging superoxide radicals in the *Sprague Dawley* exposed to inhaled formaldehyde. The results showed a significant difference in superoxide dismutase levels in each treatment group. The lowest superoxide dismutase levels were in the negative control group who received exposure to inhaled formaldehyde but did not get the supplementation of *Caesalpinia sappan L.* extract which can trigger an increase in free radicals in the body,[23] which in this study came from inhaled formaldehyde. Formaldehyde has been shown to inhibit superoxide dismutase activity.[24] The administration of *Caesalpinia sappan L.* ethanol extract was able to significantly increase the activity of the superoxide dismutase enzyme in Sprague Dawley.

An increase in the activity of the superoxide dismutase enzyme begins to occur at a dose of 100 mg/kg BW/day. However, at a dose of 400 mg/kg BW per day superoxide dismutase decreased and those again at a dose of 1000 mg/kg BW. Compared to the control negative group, all CSE doses different significantly except for doses of 400 mg/kg BW. SOD levels at CSE doses of 100 and 1000 mg/kg BB SOD levels are better than vitamin C administration.

An increase in superoxide dismutase activity in Sprague Dawley given *Caesalpinia sappan L.* ethanol extract was possible due to flavonoids in it. Flavonoids have antioxidant properties that decrease superoxide activity.[25] The ability of flavonoids in scavenging free radicals depends on their structure.[26] Flavonoids have a double action in ROS homeostasis. The first under normal circumstances plays the role of an antioxidant, and the second trigger the apoptosis pathway and lowers the pro-inflammatory signaling pathway so that it is a potent pro-oxidant for cancer cells.[27] A special active substance in *Caesalpinia sappan L.* that belongs to the flavonoid group is brazilin. Brazilin exhibits strong 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and cooling of superoxide anions. Brazilin can increase the activity of superoxide dismutase and lower intracellular reactive oxygen species.[28]

The ability of flavonoids to capture free radicals caused by hydroxyl groups through the following reactions: F-OH + R * → F-O * + RH. This study is in line with other studies that have shown an increase in superoxide dismutase activity in formalin-induced and apigenin-given mice, which is one of the flavonoid components found in celery, as a natural antioxidant.[24]

Effect of Caesalpinia sappan L. ethanol extract on blood serum malondialdehyde levels of *Sprague Dawley* exposed to inhaled formaldehyde

Measurement of malondialdehyde levels was carried out to determine the ability of *Caesalpinia sappan L.* ethanol extract in preventing lipid oxidation due to oxidative stress. The lowest average malondialdehyde levels were in the positive control group and the highest was in the negative control group. Formaldehyde causes lipid peroxidation by reducing the activity of superoxide dismutase and glutathione peroxidase so that the concentration of malondialdehyde increases.[29-31] Research in formaldehyde-induced rats was shown to increase malondialdehyde levels.[24]

The absence of a significant difference in malondialdehyde levels in this study showed that the administration of *Caesalpinia sappan L.* extract during the 28 days of intervention has not been able to suppress the lipid peroxidation process caused by inhaled formaldehyde. Although there was no significant difference in malondialdehyde levels between groups, in the negative control and normal control groups malondialdehyde was much higher than in other groups that received antioxidants. This shows that although not statistically significant, lipid peroxidation can be suppressed in the presence of antioxidants from *Caesalpinia sappan L.* by lowering malondialdehyde levels. There is a failure of endogenous antioxidant defense mechanisms to prevent excessive reactive oxygen species formation due to inhaled formaldehyde exposure, where the biggest failure is in the negative control group.

The lowest amount of *Caesalpinia sappan L.* extract in the CSE 100 group, showed better activity in the management of malondialdehyde levels compared to the negative control group. The lowest malondialdehyde levels of the three doses administered were in the CSE 400 group, which indicated a dose of 400 mg/kg BW had the best positive effect in suppressing malondialdehyde levels. Based on malondialdehyde levels in the group that received vitamin C intake, had lower malondialdehyde levels than the CSE group, thus vitamin C was more effective in resisting oxidative damage caused by inhaled formaldehyde than Caesalpinia sappan L. ethanol extract.

The decrease in malondialdehyde levels is mainly due to flavonoid compounds in *Caesalpinia sappan L.* ethanol extract to capture hydroxyl radicals. Two homoisoflavonoids derived from *Caesalpinia sappan L.* are sappanol and brazilin. Both of these substances are significantly able to withstand the deposition of intracellular reactive oxygen species. Sappanol inhibits lipid peroxidation and depends on its concentration.[32]

Hydroxyl radicals can initiate lipid peroxidation reactions. Lipid peroxidation can be prevented in the presence of flavonoids that can also reduce free radicals, which indicates that flavonoids react with peroxyl radicals (ROO*) and proceed to the termination stage of the autoxidation reaction. Flavonoids may have an additive effect on endogenous scavenger compounds. Flavonoids can prevent damage caused by free radicals in various ways and one of them is the direct scavenging of free radicals. Flavonoids are oxidized by radicals, resulting in more stable and less reactive radicals.[25] This study is not

in line with *in vitro* studies that concluded that extracts of *Caesalpinia sappan* L, protosappanin A, and protosappanin B are more abundant in malondialdehyde and hydrogenperoxide.[33]

Relationship of superoxide dismutase and malondialdehyde levels of *Sprague Dawley* Exposed to Formaldehyde inhalation

The results of statistical analysis showed a significant relationship between superoxide dismutase levels and malondialdehyde levels (p=0.02) with sufficient relationship strength (r= -0.422). Naturally, the body already has antioxidants that can inhibit the formation of free radicals. However, if there is too much exposure to free radicals, these natural antioxidants are not able to overcome it, so superoxide dismutase can decrease. However, if exposure to free radicals can be overcome by antioxidants by resting the body to repair damaged body tissues, the body will get used to it and continue to develop to produce enough antioxidants to neutralize free radicals.

Exposure to free radicals from inhaled formaldehyde will activate endogenous antioxidant mechanisms such as superoxide dismutase. Increased production of superoxide anion radicals (O2*) can directly increase the production of more harmful ROS such as hydrogen peroxide and hydroxyl radicals. Hydroxyl radicals will initiate the lipid peroxidation process directly in the Polyunsaturated fatty acid (PUFA) structure found on the cell membranes and mitochondria. The end product of lipid peroxidation such as malondialdehyde is an indicator of oxidative stress.

In general, there was a relationship between superoxide dismutase levels and malondialdehyde levels in this study. This suggests that the presence of superoxide dismutase as an endogenous antioxidant has been used to fight oxidative damage due to exposure to formaldehyde gas so that the amount of malondialdehyde decreases. The higher the superoxide dismutase levels, the malondialdehyde levels will decrease. In this study, the negative control group had the lowest superoxide dismutase levels and the highest malondialdehyde levels compared to the other groups, which showed a clear negative relationship between superoxide dismutase and MDA malondialdehyde levels due to exposure to inhaled formaldehyde.

In biological systems, the body can usually produce its antioxidants in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (endogenous antioxidant). When oxidative stress occurs due to excess ROS production, these endogenous antioxidants must get additional antioxidants from outside the body (exogenous antioxidants). In this study, *Caesalpinia sappan L.* ethanol extract was given as an exogenous antioxidant. Based on the *Caesalpinia sappan L.* ethanol extract dose given, it shows a decrease in malondialdehyde levels with an increasing dose of *Caesalpinia sappan L.* ethanol extract up to a dose of 400 mg/kg WB. At *Caesalpinia sappan L.* ethanol extract doses of more than 400 (i.e. doses of 1000 mg/kg WB), malondialdehyde increased again. This study is in line with studies in rats exposed to noise,[34] studies in rats that have peptic ulcers,[35] and coroner heart disease patients[35] proved the presence of oxidative stress marker changes seen with decreased superoxide dismutase expression and increased malondialdehyde expression.

CONCLUSION

Caesalpinia sappan L. extract can increase the activity of superoxide dismutase enzymes but has not been able to suppress malondialdehyde levels. There was an association of increased superoxide dismutase enzyme activity with decreased malondialdehyde levels in *Sprague Dawley* exposed to inhaled formaldehyde.

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Cite this article: Nurullita U, Susilaningsih N, Suwondo A, Suhartono, Djati KRM. Supplementation of *Caesalpinia Sappan L.* Extract to Increase Superoxide Dismutase Activity and Suppress Malondialdehyde Levels in *Sprague Dawley* Exposed to Inhaled Formaldehyde. Pharmacogn J. 2023;15(5): 750-755.