Sungkai Leaf Extract (Peronema canescens Jack) Reduces MDA Levels and Increases IL-10 Levels in MSG-Induced Wistar Rats

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ABSTRACT

The addition of Monosodium Glutamate (MSG) to ready-to-eat food can change eating habits, coupled with a lack of activity which can have an impact on health. MSG as a trigger for increased reactive oxygen species (ROS), causing systemic damage, the impact of excessive MSG consumption can be reduced by modifying the diet to contain high sources of antioxidants. Sungkai leaf extract can be an alternative as a natural antioxidant. Sungkai leaf extract antioxidants are expected to neutralize ROS thereby repairing cell damage. Objective: This study aims to determine how giving sungkai leaf extract affects MDA and IL-10 levels in MSG-induced mice. Method: Experimental research with a Randomized Post test only control group design. The total sample was 24 male Wistar rats divided into 4 groups. KN healthy mice, K(-) were given 1g MSG/rat, P1 was given 1g MSG/rat and 28mg sungkai leaf extract/rat, P2 was given 1g MSG/rat and 56mg sungkai leaf extract/rat. Results: The average results showed a decrease in MDA levels and an increase in IL-10 levels after treatment for 21 days, the One way Anova test followed by Post hoc LSD showed that each treatment group was significantly different from the control group where the MDA level in group P2 was 0.07mg/ml ± 0.01 experienced a significant decrease compared to the KN group 0.07mg/ml ± 0.01 while IL-10 levels experienced a significant increase in the P1 group 130.10pg/ml ± 13.29 when compared to the KN group 60.43pg/ml ±17.40. Conclusion: Sungkai leaf extract (Penonema Canescens Jack) was able to reduce MDA levels at a dose of 56 mg/mouse in mice injected with 1gr MSG/rat and experienced a significant increase in IL-10 levels with a dose of 28 mg sungkai leaf extract/rat injected with 1gr MSG/rat.

Keywords: Sungkai leaf extract, MDA levels, IL-10 levels, MSG

https://doi.org/10.33860/jik.v17i3.3015

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INTRODUCTION

The habit of consuming fast food and lack of physical activity causes changes in eating patterns, the majority of these foods have harmful effects on human health. Food manufacturers use Monosodium Glutamate (MSG) to improve the taste of food¹. MSG is a commonly used food additive and can pose a threat to health². MSG is added to many processed foods with an estimated average daily human intake of around 0.3-1.0 g in industrialized European countries³.
In Germany, 10.0 g of MSG is consumed daily on average, compared to 0.58 g in the UK. The typical daily consumption in Nigeria is 0.56–1.00 g, however in Asia it is greater, with intakes of 1.1–1.6 g in Japan, 1.5–3 g in Taiwan, and 1.6–2.3 g in South Korea documented. Although the FDA claims that MSG is harmless, animal studies have shown that prolonged MSG use has harmful consequences. Report on the use of MSG in Indonesia in 2013. RISKESDAS 2013 reported MSG consumption at 77.3%. Other intakes that are considered dangerous, such as sweet foods (53.1%), fatty foods (40.7%), and coffee (29.3%).

MSG can trigger an increase in reactive oxygen species (ROS), changing redox homeostasis and causing systemic damage. Induces male reproductive toxicity through oxidative damage mechanisms in the form of increased lipid peroxidation and decreased antioxidant enzyme activity, hormonal dysfunction, and decreased sperm quality.

The product of lipid peroxidation produced when oxidative stress increases is Malondialdehyde (MDA). High MDA levels are influenced by lipid peroxidation, indirectly indicating a high number of free radicals and indicating that the cell membrane is undergoing oxidation. Potential damage to tissue activates Interleukin 10 (IL-10) to reduce the effects of inflammation and infectious conditions. IL-10 is a potent anti-inflammatory cytokine, critical for maintaining immune system homeostasis and preventing chronic inflammatory disorders.

IL-10 increases antibody synthesis and B cell proliferation and can increase the release of TNF receptors and decrease the production of reactive oxygen species (ROS), which can counteract the effects of TNF-α.

Repeated MSG consumption has been linked in pre-clinical trials to the development of asthma, cancer-related obesity, diabetes, and oxidative stress. MSG use has also been linked to neurotoxic side effects as well as hepatotoxic, genotoxic, reproductive, renal, and other toxicities. MSG use has also been associated with epilepsy, Parkinson's disease, Alzheimer's disease, addiction, brain injury, anxiety, stroke, and depression.

The impact of excessive MSG consumption can be reduced by modifying your diet to contain high sources of antioxidants. The use of natural ingredients is becoming relevant as an option to reduce foodborne toxins and is a promising alternative. High free radical content can be reduced with antioxidant compounds. Antioxidants are compounds that have the ability to release hydrogen to reduce levels of free radicals. Based on this antioxidant mechanism, sungkai leaf extract can be an alternative as a natural antioxidant. Using sungkai leaf extract which is known to reduce free radicals in the body. However, the relationship with MDA and IL-10 levels still needs further investigation.

Sungkai leaf extract antioxidants can neutralize ROS thereby repairing cell damage and balancing hormone secretion.

**METHOD**

The type of research carried out was laboratory experimental with a Post Test Only Control Group Design research design. The research was carried out at the Integrated Biomedical Laboratory (IBL) Faculty of Medicine, Sultan Agung University, Semarang.

Sungkai leaves are obtained in the Jambi city area, Jambi Province because many sungkai plants grow well and the type of sungkai leaves used is the perfect sungkai leaf type because it can be obtained throughout the year. 1000 grams of sungkai leaf powder is then put in a dark colored container, stir until homogeneous, cover immediately then stored in a room protected from sunlight for 5 days and shaken frequently. The soak was filtered with flannel cloth, the dregs were washed with solvent to a volume of 750 mL. The results were concentrated using a vacuum evaporator until a thick extract was obtained.

The research population was male Wistar rats (Rattus norvegicus) aged 3-4 months, weighing 200-250 grams, acclimatized for 7 days and kept in a room with good ventilation, room temperature 28-32°C. Wistar rats were given standard food and enough water to drink. Determining the subjects consisted of 24 mice. Subjects were taken randomly from mice that had been acclimatized for 7 days. Randomization was used to determine control and treatment group mice.

The treatment group was divided into 4 groups, namely the normal group (KN) without MSG induction and sungkai leaf extract, the negative group (K-) induced by MSG at a dose
of 1g/mouse without being given sungkai leaf extract, the treatment group 1 (P1) induced by MSG at a dose of 1g/rat and given a dose of 28 mg of sungkai leaf extract/rat, and treatment group 2 (P2) was induced by MSG at a dose of 1g/rat and given a dose of 56 mg of sungkai leaf extract/rat. On the 22nd day, blood was taken from all mice via the orbital sinus of the eye, then processed to obtain serum and MDA levels were measured using the Thiobarbituric acid assay (TBARS) method with spectrophotometry at a wavelength of 532-535 nm and IL-10 using the Enzyme-Linked method. Immunosorbent Assay (ELISA) was analyzed using the ELISA Reader. The data obtained from the research was tested for normality of the data using the Shapiro Wilk test and homogeneity test was carried out with the Levene test (p˃0.05). To determine the differences between each group, the one way ANOVA test was used (p˂0.05) and continued with the Posit hoc/LSD test to determine significant differences between groups (p<0.05).

RESULTS

This research quantitatively analyzed the flavonoid content of sungkai leaf extract in simplicia powder using 70% ethanol solvent, resulting in a result of 53.3 mg/ml. This result is higher when compared with the flavonoid content of sungkai bark extract of 29.41 ± 0.64 mg quercetin equivalent/g extract. After giving treatment to the 4 test groups for 21 days, blood samples were taken and centrifuged to obtain serum on the 22nd day. The results of measuring MDA levels in the various groups are depicted in the following descriptive table:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA levels (mg/ml)</td>
<td>KN N=5</td>
<td>K- N=5</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.07±0.01</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td>Shapiro Wilk</td>
<td>0.32*</td>
<td>0.27*</td>
</tr>
<tr>
<td>Levene test</td>
<td>0.13**</td>
<td>0.000***</td>
</tr>
</tbody>
</table>

Table 1, it shows that the average MDA level of the normal group (KN) was 0.070 mg/ml ± 0.007, the negative group (K-) 0.142 mg/ml ± 0.217, the average of treatment group 1 (P1) 0.146 mg/ml ± 0.029 and the treatment 2 (P2) 0.070 mg/ml ± 0.010. The MDA level data for the four groups were all normally distributed (p>0.05) and also had a homogeneous data variance with a value of 0.132 (p>0.05). Based on the results of the One Way ANOVA test, a value of 0.000 (p<0.05) was obtained, indicating that there was a significant difference in average MDA levels between the four groups. Figure 1. Graph of the average value of MDA levels for each group.

The lowest average MDA levels were in the normal group (KN). In the negative group (K-) with the administration of 1gr MSG/rat there was an increase in MDA levels, in treatment group 1 (K1) with the administration of 1gr MSG/rat and 28 mg dose of sungkai leaf extract/mice MDA levels did not decrease, in fact tended to increase, whereas in treatment group 2 (K2) with 1gr MSG/rat and 56 mg sungkai leaf extract/rat experienced a decrease in MDA levels or the same as the normal group. Table 2. Differences in average MDA levels for each group

<table>
<thead>
<tr>
<th>Group</th>
<th>KN</th>
<th>K-</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>KN</td>
<td>- 0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>K-</td>
<td>0.000*</td>
<td>- 0.744</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>0.000*</td>
<td>0.744</td>
<td>- 0.000*</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>1.000</td>
<td>0.000*</td>
<td>0.000*</td>
<td>-</td>
</tr>
</tbody>
</table>

LSD post hoc test to see differences between groups. The differences in MDA levels are shown by pairs of groups, with the results of the LSD post hoc test. The MDA level of the normal group (KN) using the LSD post hoc test was significantly different from the negative group.
(K-), KN was significantly different from the P1 group and KN was not significant from P2. K- is significantly different from the KN group, K- is not significantly different from the P1 group and KN is significantly different from the P2 group. P1 is significantly different from KN, P1 is not significant from K-, and P1 is significantly different from P2. P2 is not significant with KN, P2 is significantly different from K-, and P2 is significantly different from P1.

Table 3. Description of treatment MDA levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>KN</th>
<th>K-</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>60.43± 17.40</td>
<td>76.02± 5.71</td>
<td>130.10± 13.287</td>
<td>71.841± 9.233</td>
</tr>
<tr>
<td>Shapiro wilk</td>
<td>0.603*</td>
<td>0.425*</td>
<td>0.693*</td>
<td>0.428*</td>
</tr>
<tr>
<td>Levene test</td>
<td>-</td>
<td>0.28**</td>
<td>0.000***</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3, it shows that the average level of IL-10 in the normal group (KN) was 60.427 pg/ml ± 17.40, the negative group (K-) 76.02 pg/ml ± 5.71, treatment group 1 (P1) 130.096 pg /ml ± 13.287 and treatment group 2 (P2) 71.841 pg/ml ± 9.233.

Data on IL-10 levels for the four groups were all normally distributed (p>0.05), and had a homogeneous data variance with a value of 0.28 (p>0.05). Based on the results of the one way ANOVA test, a value of 0.000 (p<0.05) was obtained, indicating that there was a significant difference in average IL-10 levels between the four groups.

![Figure 2. Graph of the average value of IL-10 levels for each group](image)

Table 4. Differences in average IL-10 levels for each group

<table>
<thead>
<tr>
<th>Group</th>
<th>KN</th>
<th>K-</th>
<th>P1</th>
<th>P2</th>
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<td>0.28**</td>
<td>0.000***</td>
<td>-</td>
</tr>
</tbody>
</table>

The lowest average level of IL-10 in the normal group (KN) without administration of MSG and sungkai leaf extract was 60.427 pg/ml ± 17.40 pg/mL, IL-10 levels increased in the negative group (K-) with The average value was 76.02 pg/ml ± 5.71, while the highest value was in treatment group 1 (P1) with sungkai extract administered at a dose of 28 mg/rat, the result was 130.096 pg/ml ± 13.287. IL-10 levels decreased in Treatment group 2 (P2) by administering 56 mg sungkai leaf extract/rat obtained an average value of 71.841 pg/ml ± 9.233.

DISCUSSION

MDA levels in mice induced by MSG were 1g/rat for 21 days in group 2 with a dose of 56 mg/rat, namely an average of 0.070±0.010 mg/ml. In line with previous research by Kassab et al. (2022) and Elmas et al (2023) showed that administration of excess MSG increased MDA levels as indicated by a decrease in glutathione levels and a decrease in the activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase

MDA as an inflammatory mediator (MDA epitope) can be found as a biomarker for oxidative stress in various inflammatory diseases, due to excessive or inadequate production of lipid peroxidation in situations of oxidative stress.
The MDA epitope as an indicator of damaged tissue or oxidized biomolecules and its exposure to autologous structures to alert the immune system to danger because non-physiological accumulation can trigger the activation of proinflammatory responses. In addition, IL-10 increases antibody synthesis and B cell proliferation and can increase receptor shedding\(^7\), TNF and reduces the production of reactive oxygen species (ROS), which can counteract the effects of TNF-\(\alpha\)\(^11\).

Sungkai extract stimulates the immune system by increasing the number of leukocytes, proportion of lymphocytes, activity and phagocytic ability of macrophage cells, number of leukocytes, number of segmental neutrophil cells, and levels of proinflammatory cytokines (TNF- and IL-6)\(^17\).

Research by Lestari et al. (2021) who reported a microscopic histology of the liver when administered MSG at a dose of 5 g/kg BW showed a number of cell abnormalities of 40.5 (40-43) % with moderate damage, fatty degeneration and sinusoidal congestion were found\(^18\). Administration of sungkai leaf extract can improve (reversible) cell inflammation in the mitochondria and endoplasmic reticulum due to oxidation disorders. The active components of sungkai leaves increase the total strength of antioxidants in the blood and reduce peroxidation levels.

The results showed a significant increase in IL-10 levels in treatment group 1 (P1) with an average value of 130,096 pg/ml ±13,287, endogenous glutamate mechanisms play a role in physiological and pathological processes, glutamate produces energy in erythrocytes, an intermediate substance in protein metabolism, precursors of important metabolites such as GSH, oxidative stress modulators and central nervous system (CNS) neurotransmitters\(^3\).

This effect is mediated by intake factors of sungkai leaf extract which modulate signaling pathways and influence various mechanisms involved in inflammation\(^17\). Inflammation is characterized by interactions between pro- and anti-inflammatory cytokines associated with immune cell infiltration which facilitates the further development of tissue damage\(^19\). Defense against oxidative stress can occur through several mechanisms, the most effective of which is the antioxidant defense system\(^20\).

**CONCLUSION**

Administration of sungkai leaf extract (*Peronema Canescens* Jack) reduced Malondialdehyde (MDA) levels at a dose of 56 mg/head (200gr rat) in male Wistar rats induced by MSG and administration of sungkai leaf extract (*Peronema Canescens* Jack) increased levels of interleukin 10 (IL-10) at a dose of 28 mg/head (200gr rat) in male Wistar rats induced by Monosodium Glutamate (MSG). The suggestion further research is needed on other molecular parameters such as levels of superoxide dismutase (SOD) and glutathione (GSH), as well as pro-inflammatory cytokines (TNF-\(\alpha\) and IL-6) and examine the effect of sungkai leaves on the histopathological appearance of liver, kidney and testicular tissue.

**REFERENCE**


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