Effectiveness of Brassica oleracea L. Var. Italica on Liver Repair of Mice Exposed to the Heavy Metal Pb

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ABSTRACT
Lead toxicity has a broad impact on human health, specifically on the liver. Lead can neutralize antioxidants so there is a risk of increasing the number of free radicals and can damage the liver. Therefore, the amount of antioxidants in the body can be a factor in preventing damage to the body due to lead. This research was carried out to determine the effectiveness of Broccoli extract in repairing the livers of mice exposed to the heavy metal Pb Acetate. This research is experimental research with a Posttest-only Control Group Design. The research object was mice (Mus musculus) which were divided into 5 groups. Data was collected by observing liver tissue objects using histology techniques. The research results showed the effectiveness of broccoli extract (Brassica oleracea L. Var. Italica) in improving the liver of mice. In the 50% dose group, hepatocytes turned normal. Fatty degeneration occurred in groups with a dose of 75% and a dose of 100%. From these results, it can be said that broccoli extract can be an alternative for treating liver damage due to exposure to heavy metals. However, you need to pay attention to the dosage used.

KEYWORDS
Heavy metals; lead; liver; Brassica oleracea

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Introduction
Lead (Pb) is currently the main pollutant in the environment. Lead can be found in organic and inorganic forms. Organic lead is a type of compound that is quite volatile (more volatile than inorganic lead). When organic lead is introduced into gasoline as a raw additive, the gasoline will allow the lead to reach the air. On the other hand, most of the inorganic materials found in the surrounding environment are usually found in cat faeces, soil, dust, cosmetics, baby clothes and other consumer goods. Lead (Pb) is the only type of heavy metal that is experiencing a decline in its use in industry today (Batool et al., 2018; Khotijah et al., 2017). The colour of organic lead depends on its chemical form. White lead (lead carbonate compound), yellow lead (lead chromate, lead monoxide), or red lead (lead tetroxide) are the three most common taps (Adhani & Husaini, 2017; Fernanda, 2012; Palar, 2012).

Because air and water processes produce lead’s ability to be incorporated into living systems, because lead comes from the earth’s crust (Flora et al., 2012; Rabinowitz, 1991). Motor vehicle fumes, drinks and food are the main sources of lead pollution (Baehaki et al., 2020a; Gunawan, 2015; Muliyadi et al., 2015; Purnomo & Muchyiddin, 2007; UNICEF, 2020). Lead can also enter the body through the respiratory and digestive systems, such as inhaling polluted air or vomiting food (Mardani et al., 2005; Nawrot & Staessen, 2006; Palar, 2012; Tamayo y Ortiz et al., 2016). Lead that enters the human body can inhibit the activity of enzymes found in hemoglobin (Hb), and some of it can be excreted through urine and faeces because some of it is bound to protein and some of it will be further developed by the body in several organs. Lead can be absorbed in the human body between 20% and 50% during inhalation and between 15% and 25% when swallowed (Baehaki et al., 2020a; Flora et al., 2012; Palar, 2012). Lead has the ability to cause oxidative tissue damage, increase lipid oxidation, damage DNA, and has the potential to increase the production of ROS (Reactive Oxygen Species).

Lead toxicity has a severe impact on human health, ranging from neurological metabolic problems to kidney and liver function problems (Baehaki et al., 2020a; Kosnett et al., 2007; Nawrot & Staessen, 2006). Especially for young people, lead compounds are very effective in damaging the liver. The human heart is the main organ for toxins and has a strong relationship with the chemical conditions of substances. As a result, body organs are susceptible to damage. This happens because the brain is an important organ that functions to classify and store materials used in the digestive process, and plays an important role in the metabolism and transformation of materials originating from the environment (Flora et al., 2012; Malekirad et al., 2013; Rabinowitz, 1991). Therefore, the liver is a common organ that contains a lot of metallothionein protein, which can be used to detect heavy metals (Palar, 2012). Metallothionein protein is a small protein that is specific to the liver. Not only that, inflammation which is mainly caused by viral
This happens because some of the toxins or toxic substances that enter the body after being cut by the cells will be carried to the head via the liver portal vein so that the liver has the potential to suffer damage. The reaction in the liver to heavy metals (Pb) is caused by the activity of the metal concerned in enzymatic work (Ali et al., 2019; Batool et al., 2018). In addition, liver necrosis can begin with inflammation with symptoms such as swelling of hepatocytes and tissue death. There is a third type of liver damage, namely mild liver damage, which is characterized by fatty cells-swelling. Moderate-level damage is congestion and haemorrhage. Hemorrhage is a type of blood coming out of blood vessels that can occur in body cavities or even tissue (Rabinowitz, 1991). However, severity is associated with necrosis.

Necrosis is the death of liver cells. Cell death occurs simultaneously with the rupture of the plasma membrane (Flora et al., 2012). However, recent research has revealed that the three organs that can contain lead, including the liver, kidneys and brain, are sensitive to carcinogenicity and can cause cancer in humans (Baehaki et al., 2020a; Batool et al., 2018; Flora et al., 2012; Palar, 2012). Because lead can reduce antioxidant levels and increase the production of free radicals, a lack of balance between the toxicity of oxidants and antioxidants in tissues and cells can result in organ damage. Health effects may occur without clinical symptoms at low levels of lead exposure. Lead has also made progress in increasing the number of deaths in heart disease patients. Treatment for lead poisoning, for example, is an antidote to free radicals, in this case, an antioxidant.

Antioxidant compounds are substances the body needs to neutralize harmful radiation and prevent the buildup of damage caused by harmful radiation to cells, proteins and fats (Ali et al., 2019). There are two types of antioxidants, namely endogenous antioxidants which are produced by the body itself, and exogenous antioxidants which are the expulsion of antioxidants from outside the body. Apart from that, there are also signs of antioxidants that behave like endogenous antioxidants in neutralizing free radicals that are inhibited by exogenous antioxidants (Kebede & Admassu, 2019; Rebaya et al., 2015). These antioxidants can be found in various tissues, including roots, stems, bark, twigs, leaves, flowers and seeds. Isoflavones which also function as metal chelators allow antioxidants to break down metal levels in the blood, similar to antioxidants in the broccoli plant. From a chemical perspective, substances commonly found in plants are phenolic compounds or polyphenols. Antioxidants, provitamin A, vitamin C, and folic acid are all contained in broccoli. The Sulforaphane content can destroy multidrug-resistant H. pylori bacteria, which is known to be the main cause of very aggressive stomach cancer. Isothiocyanates are active ingredients used as anti-cancer substances in sulfur breakdown products which contain sulfur.

We have reviewed research related to different aspects. We review aspects of using broccoli as a source of antioxidants and aspects of using antioxidants in healing therapy for liver cell damage. Many types of plants have been studied as potential sources of antioxidants, such as Phytolacca dioica (Ashafa et al., 2010), Asteraceae family (Asres et al., 2007), Moringa oleifera (Younis et al., 2022), Aloe barbadensis miller (Sharma et al., 2022), Silybum marianum (Levy C et al., 2004), Foeniculum vulgare and Artemisia annua (He et al., 2022), and Brassica oleracea L. Var. Italica (Castelão-Baptista et al., 2023; Kusuma et al., 2017; Le et al., 2019). The potential of broccoli as a source of antioxidants has also been widely studied, especially its potential characteristics as an herbal medicine. Castelão-Baptista et al. have studied the potential of broccoli as an antioxidative agent that can prevent lipid accumulation and cell death in the liver (Castelão-Baptista et al., 2023). The results show quite a positive impact. Le et al. also conducted research related to the use of broccoli as an anti-bacterial (Le et al., 2019). Another study by Kusuma et al. was conducted regarding the hepatoprotective effect of broccoli ethanol extract from DMBA induction in liver cells which showed a positive impact on liver healing (Kusuma et al., 2017). DMBA is a compound produced by traffic and industrial activities which enter the air and are dangerous for liver health. Research conducted by Sim et al. regarding the use of broccoli extract was also studied as an anti-inflammatory in RAW 264.7 macrophages and liver damage by lipopolysaccharide (Sim et al., 2023). We found more uses of the broccoli plant as an herbal medicine in a literature search. The results of studies on references that have been collected show a trend in the use of broccoli extract in the prevention and treatment of damage to the liver and other organs caused by toxicants originating from the surrounding environment, food or drink. However, we have not found much research that focuses on the use of broccoli extract in healing the liver due to lead exposure. Even research on lead itself tends to focus more on the effects of exposure rather than treatment. Even though research results from (Baehaki et al., 2020), Flora et al. (2012), Malekirad et al. (2013), Mulyadi et al. (2015), and Nasir (2018) show worrying lead exposure in several areas. Therefore, this research was conducted to determine the potential of broccoli to reduce the impact of lead exposure, especially on the liver as one of the target organs. The results of this research can be used as empirical data in treating patients with lead exposure.

Method

This research is an experimental study with a Posttest-only Control Group Design, where the experimental group and control group are not chosen randomly. In this study, the experimental groups (50% dose, 70% dose, and 100% dose) will compare the results of their histological observations with the control group. The data taken in this study is in the form of primary data obtained from histological observations of the livers of mice exposed to Pb acetate. The data obtained is in the form of tables and pictures showing the percentage of liver cell damage in mice. Criteria for assessing the quality of the preparations and results of the level of hepatocyte damage and scores for evaluating the histopathological picture of liver cells after treatment using distilled water, Pb acetate 0.52 mg/g bw/day, and variant doses of broccoli extract in various treatment groups. The treatment given to the mice can be seen in Table 1.
Table 1. Treatment for each group of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Days to</th>
<th>Pb acetate induction</th>
<th>Liver organ harvesting</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-</td>
<td>1-7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K+</td>
<td>8-17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KP1</td>
<td>Adaptation</td>
<td>Pb acetate Induction</td>
<td>Giving broccoli extract at dose 1</td>
</tr>
<tr>
<td>KP2</td>
<td>-</td>
<td>-</td>
<td>Giving broccoli extract at dose 1</td>
</tr>
<tr>
<td>KP3</td>
<td>-</td>
<td>-</td>
<td>Giving broccoli extract at dose 1</td>
</tr>
</tbody>
</table>

**Sample**

The samples in this study were mice which were divided into five groups, namely Negative Control Group, Positive Control Group, 50% Dose Group, 75% Dose Group, and 100% Dose Group. The Negative Control Group (K-) is a group of mice that were only given distilled water, without any Pb acetate. The Positive Control Group (K+) is a group of mice that were injected with Pb acetate without being given broccoli extract. The mice sampled must meet several criteria, such as 1) they are male white mice; 2) weigh 20-30 grams; 3) the visual impact does not cause dull hair and hair loss; and 4) the mice are healthy and move actively.

**Histology Procedures**

**Mouse liver preparation**

The collection of mouse livers is carried out in several stages, namely 1) preparing a medium-sized glass bottle; 2) putting cotton wool that has been treated with chloroform into the bottle; 3) euthanise the mice by placing the mice in the bottle and closing it tightly; and 4) after the mice faint, surgery is performed. The mouse livers obtained were then washed with 0.9% NaCl solution until clean.

**Histology preparation**

Histological preparation was carried out using the procedure, namely 1) timming, carried out by cutting the liver with a size of 1x1x1 cm and inserting it into a tissue cassette; 2) fixation, carried out by preparing a 10% NBF fixative solution to soak the sample for 24 hours; 3) dehydration, carried out by washing the sample from the fixative solution using running water for at least 2 hours and placing it in alcohol with varying concentrations (70% - 80% - 90% - 100%), each for 30 minutes; 4) clearing, carried out by gradually placing the sample in xylol, namely xylol 1 for 15 minutes and xylol 2 for one night; 5) infiltration, carried out by placing the sample in a 1:1 mixture of xylol: paraffin for 30 minutes and placing it in three stages of paraffin for one hour each; 6) blocking, carried out by making a cube-shaped paraffin mold from paper with a smooth surface (2x2x2 cm) and leaving it overnight after pouring in liquid paraffin; and 7) cutting, carried out by making slices using a microtome with a thickness of 4-5 microns (Hew) and 10-15 microns (Tumb).

**Histological observations**

Observations were made using a microscope to see the shape of the cells in the liver tissue after staining. Observations focused on damage to hepatocyte cells by observing fatty degeneration and hydrophic degeneration.

**Determination of the degree of liver cell damage in mice**

To determine the level of cell damage in the liver of mice, the researchers did so by referring to the criteria for the level of damage by Setyaningsih et al. (2006) which can be seen in Table 2.

Table 2. Criteria for the degree of cell damage

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Quality</th>
<th>Percentage (%)</th>
<th>Skor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal hep</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cloudy swelling +</td>
<td>0,15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cloudy swelling ++, hydroptic degeneration +, necrosis +</td>
<td>6-25</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hydropic degeneration ++++, fatty degeneration ++, necrosis +++</td>
<td>26-50</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hydropic degeneration ++++, fatty degeneration ++, necrosis occurs ++++, unclear lumen, hyperemia</td>
<td>&gt;50</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**Results**

The research was carried out in May-June 2023 at the Rajawali Bandung Health Institute Laboratory. The samples used in this study were mouse livers that had been induced with Lead Acetate and Broccoli (Brassica oleracea L. Var. Italica) extract with different concentration variants based on the treatment group. Consisting of 5 treatment groups, each group consisted of 5 mice. Then the samples were made into histological preparations using the paraffin method and HE (Hematoxylin Eosin) staining. This preparation was made at the Bandung Health Polytechnic Integrated Laboratory in June 2023. The results of observations on the histology of the livers of mice induced by lead acetate and given broccoli extract can be seen in Figure 1.
Figure 1. Results of histological observations on mouse livers

Information:
K-: Normal hepatocyte cells
KP1: Normal hepatocyte cells
KP2: Fatty degeneration occurs
KP3: Fatty degeneration occurs
K+: There is hydrophic degeneration where the cytoplasm begins to become clear and sparse swelling and fatty degeneration occurs

(a) normal hepatocyte cells
(b) hydrophic degeneration
(c) fatty degeneration

Treatment group 1 (50% dose)

Treatment group 2 (75% dose)

Treatment group 3 (100% dose)
Based on the image above, the results of histological microscope observations of mouse liver were observed using a light microscope at 400 times magnification. Changes were found in the cells in the form of hydrophic degeneration, slight swelling of the cell cytoplasm and fatty degeneration. There was also treatment group 1, namely administering a dose of 50% broccoli extract, and cell recovery occurred and hepatocytes became normal. Fat degeneration was found in treatment group 2 with a dose of 75% and treatment group 3 with a dose of 100% broccoli extract. Fat degeneration occurred in hepatocyte cells with normal hepatocyte cell nuclei. The assessment scores for the criteria for assessing the quality of preparations and assessing the quality of preparations for liver cells from mice induced by the heavy metal Pb acetate and broccoli extract can be seen in Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Preparation 1</th>
<th>Preparation 2</th>
<th>Preparation 3</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>Normal Hepatocytes</td>
</tr>
<tr>
<td>Positive Control</td>
<td>6%</td>
<td>6%</td>
<td>6%</td>
<td>Hydrophic Degeneration</td>
</tr>
<tr>
<td>KP1 (Broccoli Extract 50%)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>Normal Hepatocytes</td>
</tr>
<tr>
<td>KP2 (Broccoli Extract 75%)</td>
<td>6%</td>
<td>5%</td>
<td>6%</td>
<td>Fatty Degeneration</td>
</tr>
<tr>
<td>KP3 (Broccoli Extract 100%)</td>
<td>6%</td>
<td>5%</td>
<td>5%</td>
<td>Fatty Degeneration</td>
</tr>
</tbody>
</table>

There is also an assessment score for changes in liver cells of mice (Mus musculus) induced by heavy metal Pb acetate and broccoli extract, which can be seen in Table 4.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mice</th>
<th>Negative</th>
<th>Positive</th>
<th>KP1</th>
<th>KP2</th>
<th>KP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Summary</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1.6</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

Information:
0 : No liver cell damage occurs
1 : There is damage to liver cells reaching 0.1-5%
2 : Liver cell damage reaches 6-25%
3 : Liver cell damage reaches 26-50%
4 : More than 50% liver cell damage occurs

Discussion

Observations were carried out microscopically with a magnification of 40 x 10 to see the histological changes in the mice’s livers using histology preparations using lead poisoning HE (Hematoxylin Eosin) staining from each treatment group. If the toxic compound enters too much so that it is toxic to the liver, it will cause degeneration of the liver tissue. Then necrosis will occur which can damage liver tissue. This is caused by Pb's ability to form free radicals in the body, thereby reducing antioxidant capacity and causing oxidative stress. Apart from that, from various studies, it is known that Pb directly causes disturbances in normal biochemical processes (Flora et al., 2012).

Figure 1(K-), the negative control shows the presence of normal hepatocytes, with the characteristics of cells arranged radially towards the central vein in the form of round and oval cells and there are plates of hepatocytes. Cells appear to have one nucleus, but some have more than one nucleus in the middle of the cell (Baehaki et al., 2020a). This is because the mice (Mus musculus) were not given any special treatment. The mice were only given normal food and drink. Based on Table 4, mice 1, 2, and 3 show a degree of damage to mice of 0% or no liver cell damage. Figure 1 (K+), the positive control shows changes in the cells in the form of hydrogen degeneration as indicated by the following characteristics: the cytoplasm experiences vacuolization, the vacuoles appear clear and occur due to increased water entry into the cells and then the water enters the vacuoles. There is also fatty degeneration characterized by the characteristic that in the hepatocyte cytoplasm a large vacuole filled with fat is formed so that the nucleus is pushed to the edge of the cell (see Table 3). Fat in the form of triglycerides cannot be removed from hepatocytes and is stored in vacuoles in the middle of hepatocytes (Standard for Examination of Lead Levels in Human Biomarker Specimens, 2002). Based on Table 4, the degree of damage in the positive control was found to be liver cells experiencing hydrophic degeneration and fatty degeneration with a liver cell damage degree score reaching 6% for each mouse in the entire field of view. Administration of Pb acetate at a dose of 0.52 ml/ 20 grams BW/ day orally showed the same results as previous research in the form of hydrophic degeneration and swelling of the cell nuclei. This proves that Pb acetate can cause histological damage to the liver of mice (Mus musculus). However, necrosis or tissue damage to the hepatocyte cell nucleus does not occur. The causes of necrosis include damaging the enzyme structure in the cell because lead can inhibit the activity of enzymes such as those in the endoplasmic reticulum and mitochondria, which then inhibits them and over time the cell will die. This is due to the long duration of lead treatment in mice because lead that is absorbed in the body will be distributed into blood cells, soft tissue and bones. In blood, lead is excreted after 25 days, lead in tissue is excreted after 40 days and lead in Ardin bones is excreted after 25 years (Ardillah, 2016). So the livers of mice only experience hepatocyte degeneration and fatty degeneration.
Figure 1(KP1) shows that the liver cell repair process is taking place and there are normal hepatocyte cells. Based on Table 4, the degree of damage showed that the average hepatocyte cell damage score was lower compared to the positive control treatment group, which was obtained with a score of liver cell damage reaching 0% or no liver cell damage. This shows that there is a significant reduction in liver cell repair in mice. Treatment group 1 showed changes in hepatocyte cells to normal. Administering a 50% dose of broccoli extract provides a hepatoprotective effect on liver cell damage in mice.

Figure 1(KP2), treatment group 2 with a dose of 75% broccoli extract, shows the occurrence of fatty degeneration in mouse liver hepatocyte cells. Based on Table 3, liver cell damage reached 6% in mice one and three, and 5% damage in mouse two. Likewise in Figure 1(KP3), treatment group 3 with a dose of 100% broccoli extract, shows the occurrence of fatty degeneration in the liver hepatocyte cells of mice. Based on Table 4, the degree of damage to liver cells reached 6% in mouse one, and damage of 5% in mice two and three. This is due to the active compounds contained in broccoli such as flavonoids, which if there are excessive levels of these compounds can induce the formation of free radicals by reducing metal ions such as Cu²⁺ to Cu¹⁺ and Fe³⁺ to Fe²⁺ (Batari, 2012; Rebaya et al., 2015; Sembiring et al., 2017). According to Palar (2012), fatty degeneration can be reversible, namely the beginning of necrosis. Necrosis is an irreversible change in cell morphology or structure. It is suspected that the causal factor is the toxic antiproliferative effect that broccoli has when given high doses. Apart from having a toxic effect in high doses, broccoli can induce the apoptosis process in liver cells that have mutations due to Pb acetate. Liver cells that are experiencing degeneration are indicated by refraction of the liver cell cytoplasm and swelling of the cell nucleus.

Of all types of doses, it turns out that the 50% dose is better than the 75% and 100% doses because of the occurrence of fat degeneration in liver cells. Fat degeneration can occur due to fat metabolism disorders, such as disturbances in mitochondrial function, hypoxia which can inhibit fat oxidation and then enter into cells, and can also be caused by protein nutrition. Fatty degeneration in the liver of mice can be shown in empty vacuoles of various sizes in the cytoplasm. Other factors can cause fatty degeneration in the treatment group because an error occurred when sampling the mice's liver so the liver experienced damage beforehand. This could also be due to inadequate feeding and drinking, stress factors in the mice and the influence of substances or disease. others as well as other internal factors such as resistance and susceptibility in mice.

Conclusion

Based on research results, broccoli extract was administered with a volume of 0.52 ml/kg BW/day with different extract doses, namely 50%, 75% and 100%. The effectiveness of broccoli extract in repairing the livers of mice exposed to metal Pb acetate has the potential to repair liver damage in mice exposed to metal Pb acetate in the histology of mice livers there has been a significant change in treatment group 1 with 50% dose, this occurred because the dose level was normal and fatty degeneration occurred in treatment group 2 with a 75% dose and treatment group 3 with a 100% dose.

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References


