RESEARCH ARTICLE

Protective Role of Kelakai (*Stenochlaena Palustris*) Extract on Malathion-induced Genotoxic: FTIR Spectroscopy Study

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ABSTRACT

Malathion is a genotoxic pesticide that destructs DNA. Many research have proven it, yet the mechanism has not been clear. That is the reason why this research needs to be conducted. This research used two groups, control, and two treatment group. The control group (P0) was 100 µL of Human Genomic DNA Female solution, while the treatment group (P1) was 100 µL of Human Genomic DNA Female solution which was added with 100 µL of 6 mM malathion solution. The other treatment group (P2) was 100 µL of Human Genomic DNA Female solution which was added with 100 µL of 6 mM malathion solution. Each group of solutions was incubated at 37^{o}C for 48 hours. Then the absorbance was determined by FTIR. The content of the kelakai extract was determined by GC-MS. The results of the GC-MS analysis showed that the ethanolic extract of kelakai contained 6.1% hexadecanoic acid and 6.54% Neophytadiene. the percentage changes in the absorbance of guanine, thymine, cytocin, and adenine were significantly different in each group. So as deformation of the NH-groups in DNA bases. This means that kelakai extract can inhibit DNA damage.

Keywords: Genotoxic, Hexadecenoic acid, Kelakai, Neophytadiene, Malathion.

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INTRODUCTION

Malathion is an organophosphate pesticide that is widely used by farmers as pest control. On the other hand, malathion is also a cancer-causing toxic substance. Previous research stated that malathion is a type of pesticide that triggers ovarian cancer and breast cancer.[1] Research by El Baz *et al*.[2] also revealed that malathion can trigger leukemia in the children of farmers who use the malathion pesticide.

The mechanism of malathion as a carcinogen is not fully known, but many studies have linked it to the DNA methylation process. DNA methylation can interfere with the transcription process so that gene expression is disrupted.[3] In addition, the nucleophilic group in malathion can cause the breaking of phosphodiester bonds in DNA.[4,5]

Several studies have revealed that hexadecanoic acid compounds can prevent proliferation by inhibiting DNA-Topoisemerase-1.[6] Research by Bharath *et al*.[7] also stated that hexadecanoic acid derived from the algae *Turbinaria ornata* has the potential as an anticancer of the colon in vitro. Nurmalatina's research[8] states that hexadecanoic acid compounds can also be found in kelakai (*Stenochlaena palustris*).

Kelakai is a type of ferns, belonging to the pteridaceae family that grows in areas of high humidity such as peatlands in

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Kalimantan. Kelakai is consumed by the people in Kalimantan as a traditional food and is empirically useful in preventing anemia, stimulating breast milk production, and treating wounds.[9,10] In addition, at a dose of 100 mg/kg, Kelakai acts as immunomodulators, which modulate the production of IL-10 cytokines in *Plasmodium berghei* infection and suppress the production of IFN-γ, IL-6, TNF-α by T cells.[11] Kelakai also has a cytotoxic effect on T47D breast cancer cells by increasing the expression of the p53 gene, but the mechanism is still unclear.[12] Therefore, in this study, it is necessary to study the Malathion-DNA interaction and the role of malathion extract in inhibiting DNA damage due to malathion.

MATERIAL AND METHODS

Material

The Kelakai were taken from a peat swamp in Banjarbaru, South Kalimantan, and the young leaves were selected. The leaves are dried for maceration. Calf Thymus DNA was obtained from SigmaAldrich, St. Louis, MO, USA to examine its interaction with malathion (SigmaAldrich, St. Louis, MO, USA) and Kelakai extract.

Measurement by GC-MS

The leaves are washed and then dried under the sun to air dry for 2–3 days. After drying, 250 g were ground and then extracted by maceration with ethanol as a solvent. Maceration was carried out for 3 days. The extraction results were filtered and evaporated using a rotary evaporator and then measured by GC-MS at the Banjarbaru Industrial and Trade Research Institute, Indonesia.

Experiment

This research used two groups, control, and two treatment group. The control group (P0) was 100 µL of Human Genomic DNA Female solution, while the treatment group (P1) was 100 µL of Human Genomic DNA Female solution which was added with 100 µL of 6 mM malathion solution. The other treatment group (P2) was 100 µL of Human Genomic DNA Female solution which was added with 100 µL of 6 mM malathion solution. Each group of solutions was incubated at 37°C for 48 hours.

Measurement Absorbance by ATR-FTIR

The solutions contained in the control and treatment groups were each taken by 20 µL and their absorbance measured by ATR-FTIR spectroscopy using ATR crystalline silicon (top plate 45°C) from BioATR Cell II.[13,14]

Data Analysis

Observation, that was focussed to DNA vibration ribbon at 1710, 1662, 1613, and 1492 cm^{-1}, showed the existence of guanine (G), thymine (T), adenine (A), dan cytosine nitrogen (C) for each. In addition, in the ribbon 1228 and 1087 cm^{-1} each showed asymmetrical and symmetrical phosphate vibrations.[15] In addition, DNA oxidation was observed at wave number 1050 cm^{-1}, while DNA methylation was observed at 1596 cm^{-1}. NH deformation was observed at wave numbers 1288, 1294 and 1684 cm^{-1}.[15] Data obtained were then compared between the control and treatment groups using the Kruskal Wallis test at $\alpha = 0.05$.

RESULT

The results of the GC-MS analysis showed that the ethanolic extract of kelakai contained 6.1% hexadecanoic acid and 6.54% Neophytadiene (Figure 1).

Spectroscopy is the study of the interaction of light with atoms and molecules. This interaction can be used to assess the strength of the bonds between molecules by calculating the value of the binding constant. The results of the interaction between DNA-Malathion and DNA-Malathion-extract kelakai are presented in Figure 2.

Based on Figure 1, there is a change in the absorbance spectra between DNA and DNA+Malathion. The presence of an extract of malachite showed that there was DNA protection from Malathion. The percentage change in absorbance of each group can be seen in Table 1.

DISCUSSION

Based on data from the World Health Organization (WHO) in 2014, pesticide toxication reached one million cases in the world, with 20,000 death per year. Malathion toxication as pesticide started when malathion enters the body and inhibits the metabolism of acetylcholinesterase (AchE), cytochrome

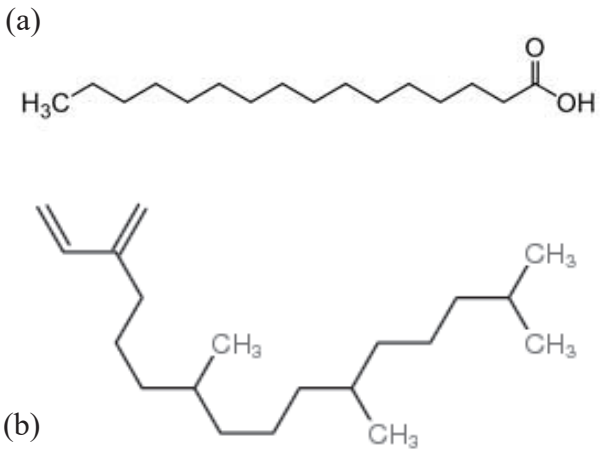

Figure 1: Hexadecanoic Acid (a) dan Neophytadiene (b)

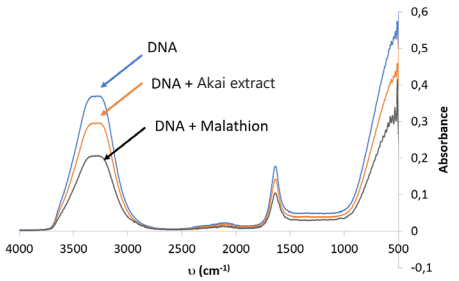

Figure 2:. Spectra of FTIR DNA

Table 1: The percentage change in DNA base

		Absorbance change in FTIR (%)		
Base	*Wavenumbers*	P0	P1	P2
Guanine	1710	100	61,75 ± 1,21	79,99 ± 2,39*
Thymine	1992	100	56,96 ± 1,19	79,99 ± 1,97*
Adenine	1613	100	58,71 ± 1,44	79,99 ± 2,04*
Cytocin,	1492	100	60,74 ± 1,38	80,00 ± 2,72*

Note: *) significantly different ($p < 0,05$)

Changes in DNA bases can be caused by deformation of the NH-groups in DNA. This can be seen in table 2.

Table 2: Deformation of the NH-groups in DNA bases

		Absorbance change in FTIR (%)		
Base	*Wavenumbers*	*P0*	*P1*	*P2*
Guanine	1684	100	59,43 ± 3,02	80,00 ± 2,87*
Thymine	1288	100	62,31 ± 2,66	80,00 ± 2,91*
Adenine	-	-	-	-
Cytosine	1294	100	62,04 ± 2,98	80,00 ± 2,07*

Note: *) significantly different ($p < 0,05$)

P450, and glutathione S-transferases (GSTs).[16,17] Next, the inhibition caused the increase of reactive oxygen species (ROS) production, the decrease of enzymatic antioxidant activity, induce apoptosis (cytotoxicity), and DNA damage.[18,19]

FT-IR is one of the many spectroscopic methods used in the study of DNA with pollutants, drugs, and other molecules in solution. This method is ideal for systematic DNA studies because it requires small samples. The region of 1800–800 cm^{-1} FT-IR spectroscopy is a characteristic peak of infrared absorption from the free located B-form DNA. The ribbon at 1710 cm^{-1} shows stretching carbonyl (C=O). This is associated with guanine (G) C7N in vibration stretching fields. The tape observed at 1658 cm^{-1} shows a stretched carbonyl. This is associated with stretching thymine (T) C2O. The ribbon 1613 cm^{-1} occurred due to scissorsing NH2. This is associated with adenine (A). Bands at 1492 cm^{-1} correspond to cytosine (C) base vibrations.[15] The bands at 1228 and 1087 cm^{-1} show asymmetric and symmetric vibrations of phosphate, respectively.[15] The absorption band in the spectral region of 1750–1450 cm^{-1} is assigned to stretch the vibrations of nucleotide base bonds in the field and is generally used for evaluation of pair-base interactions.[20]

DNA damage because of malathion has been studied through FTIR spectroscopy. In table 1, there is an absorbance decrease for DNA significantly between the Calf-Thymus DNA group and the Calf-Thymus DNA+Malathion group. This shows that malathion interacts with DNA through thymine, adenine, guanine, and cytosine. In addition, the absorbance value for phosphorus in Calf-Thymus DNA significantly increased. Phosphorus group in malathion is good substrate for nucleophilic attack, resulting in an increase in phosphorylation, which can cause DNA damage.[16]

This interaction of malathion and DNA can also be caused by nitrogen bases which have free electron pairs. This electron pair allows the rearrangement of nitrogen by the presence of malathion. This causes an increase in NH-deformation in nitrogen bases DNA base. This study is in accordance with the research of Yu Li *et al*.,[21] who explained that the chlorpropham herbicide can bind specifically to -NH in DNA so that the deformation –NH increases.

The research found that the absorbance of DNA + malathion oxidation increased by almost 250x. This oxidation begins with the reaction between the nitrogenous base of DNA and methyl parathion, which causes changes in hydrogen bonds in DNA bases. These changes result in deformation of the NH-groups and other groups, so that oxo compounds can be formed from DNA bases.

Kelakai in a wetlands endemic plant that can detain DNA damage. It contains neophytadiene which has the potential to prevent DNA damage due to malathion. Neophytadiene works by binding to DNA, between C-17 in hexadecanoic acid and dG-12A in DNA, so malathion cannot interact with DNA. In addition, the atomic contact energy of DNA-Malathion-hexadecanoic acid is greater than that of DNA-Malathion so that the DNA-Malathion bonds are more easily broken. The area of contact between DNA-Malathion- hexadecanoic acid atoms is larger than the others. The larger the contact area, the faster the reaction will be than the others.

Besides the physical aspects, there is also chemical aspect, which is the presence of hydrogen bonding. Hydrogen bonding occurs due to the intermolecular attractive forces that occur between hydrogen atoms with highly electronegative atoms (N, O, and F) and lone pairs of electrons from other highly electronegative atoms. This bonding can occur in DNA, through N-H and O-H bonds with a partial positive charge on the H and a partial negative charge on the N and O atoms.

CONCLUSION

FTIR spectroscopy can determine the interaction of DNA-Malathion and DNA-Malathion-Kelakai Extract. Kelakai

extract contains hexadecanoic acid and neophytadiene which can inhibit DNA damage due to malathion induced.

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