PROTECTIVE EFFECT OF CURCUMIN RIMPANG CURCUMA LONGA LINN. EXTRACT ON CHLORPYRIFOS POISONING IN WISTAR RATS

Azhari Muslim1*, Dadang2, Nastiti Siswi Indrasti3 and Yusman Syaukat4

1Natural Resources and Environmental Management Sciences, Bogor Agricultural University. Graduated School Building 2nd Floor, Baranangsiang Campus, Bogor 16144. West Java, Indonesia.
2Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, Dramaga Campus, Bogor 16002, West Java, Indonesia.
3Department of Agricultural Industrial Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Dramaga Campus, Bogor 16002, West Java, Indonesia.
4Department of Resource and Environmental Economics, Faculty of Economics and Management, Bogor Agricultural University, Dramaga Campus, Bogor 16002, West Java, Indonesia

*Corresponding Author : azharija6@gmail.com
(Date of Receiving : 05-02-2021; Date of Acceptance : 22-04-2021)

ABSTRACT

This study used an experimental method in the laboratory with a randomized design with five treatments and six repetitions. The parameters examined were cholinesterase (ChE), Aspartate Aminotransferase (AST), and serum Alanine Aminotransferase (ALT) of blood in the first and second treatment stages. Data were analyzed using factorial ANOVA test and LSD multiple comparison test with 95% confidence level with R software version 3.6.2. Wistar rats were given chlorpyrifos (6.75, 13.5, 27, 54) mg/kg BW/day orally once a day for 28 consecutive days, then further intervention with curcumin (27, 54, 108 and 216) mg/kg BW/day once a day for 14 consecutive days. A significant increase in ChE activity and a significant decrease in AST and ALT activity. This study shows that curcumin from Curcuma longa Linn rhizome extract provides a protective effect against chlorpyrifos poisoning in Wistar rats.

Keywords: Protective effect, curcumin, chlorpyrifos, Curcuma longa Linn.

INTRODUCTION

Organophosphate (OP) insecticide exposure is harmful to human health. Acute OP poisoning is a global threat to human health that causes more than 100,000 deaths per year (Mohajeri, 2017). Chlorpyrifos (CPF) is widely used in developing countries because it effectively controls plant pests (Rathod et al., 2017). Chlorpyrifos (O, O-diethyl-O-3, 5,6-trichloro-2-pyridylphosphorothioate) is an organophosphate pesticide that plays an essential role in pest control worldwide. CPF is often used to control plant pests in fruits and vegetables (Kopjar et al., 2018).

CPF exposure enters the human body through the skin, mouth, eyes, and respiration. Pesticides are distributed to the human body via the bloodstream and are excreted through urine, skin, and air (Naughton et al., 2018). The toxicity of CPF has received attention in various toxicological studies (Prakash et al., 2014). CPF causes liver dysfunction (Hasani et al., 2014). CPF causes sinusoid dilatation and decreases the function of the central vein and portal triad of the liver in test mice (Enzi et al., 2016). CPF causes damage to DNA (Sandhu et al., 2013), the reproductive system (Kim et al., 2016), hematotoxicity (Demir et al., 2011), genotoxicity (Eaton et al., 2008), and neurotoxicity (Khokhar et al., 2012). Cholinesterase inhibition (ChE) is the primary mechanism of CPF (Tripathi et al., 2010).

Symptoms of chlorpyrifos poisoning are due to ChE inhibition, which is a mechanism of organophosphate pesticide toxicity. ChE hydrolyzes acetylcholine (ACh) in cholinergic and neuromuscular synapses (Banks et al., 2012). Acute and chronic CPF exposure has been shown to cause considerable liver damage. The accumulation of CPF causes endogenous acetylcholine stimulation of excess and interferes with nervous system activity (Verma et al., 2007). CPF causes apoptosis and DNA damage (Gupta et al., 2010). Research carried out in recent years has shown that CPF triggers this damage by forming free radicals (Eren et al., 2020). Turmeric rhizome (Curcuma longa Linn) is used as a food coloring and medicine. Curcumin is an active compound in turmeric rhizome (Venigalla et al., 2015). Curcumin is a natural polyphenol compound with anti-inflammatory, antioxidant, anti-tumor, immunomodulatory, and neuroprotective benefits (Daverey et al., 2016). Curcumin is an antioxidant that can clean free radicals...
(Eren et al., 2020). To date, although the toxic effects of chlorpyrifos have been well documented (Kopjar et al., 2018), very little information is available on the protective benefits of turmeric extract against chlorpyrifos poisoning. Therefore, this study was to investigate the benefits of turmeric rhizome extract against chlorpyrifos poisoning in Wistar rats.

MATERIALS AND METHODS

Ethical review statement

The trial protocol was approved by the Ethical Review Committee for Pre-clinical Research of the Integrated Research and Testing Laboratory of Gadjah Mada University Yogyakarta, Indonesia.

Experimental animal

This study used 30 healthy male Wistar rats weighing 180-220 grams. The rats were given a standard laboratory diet and drank ad libitum. During the acclimation and execution (Hassani et al., 2020), the rats were placed at constant temperature (23 ± 2 °C), relative humidity (60 ± 10%), and a cycle of light and dark for 12 hours and dark for 14 hours. Rats were handled following the Guidelines of the International Animal Ethics Committee (Thongsom et al., 2019).

Chemical material

Chlorpyrifos was obtained from Dow Agrosciences, Jakarta, Indonesia. Turmeric rhizome extract is obtained from Sidomuncul, Semarang, Indonesia. Each five mg of turmeric extract contains 1 mg of curcumin.

Curcumin efficacy test

Wistar rats were randomized before the research was carried out. The rats were randomly divided into five groups (6 in each group). The rats were estimated for seven days. Randomization uses a random number table. The study was divided into two stages, namely intervention with chlorpyrifos and curcumin.

In phase 1 of the study, Wistar rats were intervened with chlorpyrifos for 28 days (Kopjar N et al., 2018). The test group (P2, P3, P4, P5) is exposed to chlorpyrifos at a dose of 1/20 of 50% LD50. Chlorpyrifos was dissolved in corn oil (Tanvir et al., 2016). Group P1 is the control group.

Group I (P1): control (pellets + aquadest ad libitum)
Group II (P2): chlorpyrifos as much as 6.75 mg/kg BW/day.
Group III (P3): chlorpyrifos as much as 13.5 mg/kg BW/day.
Group IV (P4): chlorpyrifos as much as 27 mg/kg BW/day.
Group V (P5): chlorpyrifos as much as 54 mg/kg BW/day.

In Phase 2 of the study, Wistar rats intervened with curcumin for 14 days. Curcumin was dissolved in a carboxymethyl cellulose (Naik et al., 2010). The test group (P2, P3, P4, P5) was the group that was intervened with curcumin at a 27 mg/kg BW/day (Gupta et al., 2013). Meanwhile, the P1 group was the control group.

Group I (P1): control (pellets + aquadest ad libitum)
Group II (P2): 27 mg/kg BW/day
Group III (P3): 54 mg/kg BW/day
Group IV (P4): 108 mg/kg BW/day
Group V (P5): 216 mg/kg BW/day

Blood sampling of Wistar rats by heart was carried out at the end of the experiment. Drawing blood through the heart requires anesthesia. Combination of xylazine + ketamine, dose 16 mg + 60 mg, and injection application intraperitoneal (Parasuraman et al., 2010). The amount of blood drawn through the heart is 1 mL with a frequency of collection of one time (AVMA 2020).

The blood that was successfully obtained was left to stand for 30 minutes at room temperature and centrifuged at 3000 rpm for 10 minutes. The serum formed is separated from the sediment of blood cells using a pipette. I was checking the levels of cholinesterase (ChE), Aspartate Aminotransferase (AST), and serum Alanine Aminotransferase (ALT) by using the kinetic photometric test method according to the recommendation of the German Society of Clinical Chemistry (Thomas, 1998).

Reagean kit and autoanalyzer

The ChE, AST and ALT reagean kits used are DiaSys Diagnostic System products. Kenza 240TX is an autoanalyzer instrument for checking ChE, AST and ALT.

Concentrations of ChE, AST and ALT

The method used was a kinetic photometric test according to the German Society of Clinical Chemistry (DGKC). ChE, AST and ALT levels are expressed in U/L (Thomas, 1999).

Data analysis

Factorial analysis of variance (ANOVA) to see the effect of interaction interactions on Wistar rats. The next analysis is the least difference multiple comparison test (LSD) with software R version 3.6.2. The criteria for testing the factorial ANOVA hypothesis is if F-count > F-table or p-value <0.05, then H0 is rejected. The results of the value analysis were as mean values ± standard deviation (SD). The difference with p <0.05 was accepted as statistically significant. Alpha in this study was 5%. Different letters indicate a significant difference (Bate, 2014).

RESULTS AND DISCUSSION

ChE activity

ChE is a marker of CPF poisoning in rat blood. (Barski, 2018). ChE blockage resulted in decreased ChE activity, synaptic acetylcholine accumulation, excessive neuronal stimulation and cholinergic symptoms of poisoning (Roszczenko et al., 2013).

The results of the analysis of ChE activity in Table 1 show that the doses P1 with P2, P1 with P3, P1 with P4, P2 with P3, P2 with P4, P3 and P4 are significantly different at the 5% significance level. The doses of P1 and P3 were also not significantly different at the 5% level. The dose of P3 with a level of 1573.667 ± 33.869 U/L was the highest ChE activity. P2 dose with a level of 1374.583 ± 46.439 U/L was the lowest ChE activity.

Table 1 : Differences in ChE activity in Wistar rats

<table>
<thead>
<tr>
<th>Group</th>
<th>ChE Activity (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1577.417 ± 19.481</td>
</tr>
<tr>
<td>P2</td>
<td>1374.583 ± 46.439</td>
</tr>
<tr>
<td>P3</td>
<td>1412.333 ± 35.576</td>
</tr>
<tr>
<td>P4</td>
<td>1500 ± 26.215</td>
</tr>
<tr>
<td>P5</td>
<td>1573.667 ± 33.869</td>
</tr>
</tbody>
</table>

Note: It differences in ChE activity in Wistar rats. Each value was expressed as mean ± SD with p <0.05. Data were analyzed using the factorial ANOVA test and LSD multiple comparison test. Different letters indicate a significant difference (p <0.05).
The results of research by Yadav et al., 2012 concluded that there was a significant increase in ChE activity in Wistar rats after treatment with dichlorvos and curcumin.

The results of research by Hosseini et al., 2018 shows that CPF causes toxicity to the lung organs with the mechanism of lipid peroxidation, free radical production, lipid peroxidation and decreased antioxidant enzymes. Curcumin at a dose of 100 mg/kg and 300 mg/kg with vitamin E can increase ChE activity (significant difference) through the binding mechanism of reactive oxygen species (ROS) and increase in antioxidant enzymes.

AST activity

The liver is an activation organ for detoxifying chemicals, pesticides and drugs. Several research results prove that chlorpyrifos causes liver damage characterized by increased activity of the enzymes AST, ALT, ALP and LDH (Barski et al., 2015).

The results of the analysis of AST activity in Table 2 show that the doses P1 and P2 and the doses P3 and P4 are significantly different. The doses of P1 with P2, P1 with P3, P2 with P3, P1 with P4, P3 with P4 and P2 with P4 with P3 were not significantly different. The P3 dose shows the highest AST activity. Meanwhile, the P1 dose showed the lowest AST levels. The addition of curcumin levels tends to show a decrease in AST levels.

**Table 2: Differences in AST activity in Wistar rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>AST activity (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>76.33 ± 2.53c</td>
</tr>
<tr>
<td>P2</td>
<td>83.42 ± 3.87a</td>
</tr>
<tr>
<td>P3</td>
<td>81.33 ± 6.51ab</td>
</tr>
<tr>
<td>P4</td>
<td>79.00 ± 6.73bc</td>
</tr>
<tr>
<td>P5</td>
<td>79.83 ± 5.27ac</td>
</tr>
</tbody>
</table>

Note: It differences in AST activity in Wistar rats. Each value was expressed as mean ± SD with p <0.05. Data were analyzed using the factorial ANOVA test and LSD multiple comparison test. Different letters indicate a significant difference (p <0.05).

ALT activity

The results of the analysis in table 3 below show that the doses P1 with P2, P1 with P3, P2 with P4, P2 with P3, P1 with P3, P1 with P4, P2 with P3, P1 with P4 and P1 with P3 differ significantly at the 5% significance level. The doses of P1 with P4, P1 with P3, P2 with P3, P1 and P2 were not significantly different at the 5% significant level. The P2 dose with the highest ALT activity. The dose of P1 with the lowest ALT activity.

**Table 3: Differences in ALT activity in Wistar rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT activity (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>35.33 ± 2.87a</td>
</tr>
<tr>
<td>P2</td>
<td>43.00 ± 1.81b</td>
</tr>
<tr>
<td>P3</td>
<td>41.25 ± 3.16bc</td>
</tr>
<tr>
<td>P4</td>
<td>38.67 ± 5.19ac</td>
</tr>
<tr>
<td>P5</td>
<td>37.00 ± 5.86a</td>
</tr>
</tbody>
</table>

Note: It differences in ALT activity in Wistar rats. Each value was expressed as mean ± SD with p <0.05. Data were analyzed using the factorial ANOVA test and LSD multiple comparison test. Different letters indicate a significant difference (p <0.05).

CPF causes changes in liver damage biomarkers in rat serum (Mohajeri et al., 2017). The liver is a key organ of metabolism, excretion, detoxification of therapeutic agents and environmental pollutants. The activities of AST and ALT are liver enzymes. AST and ALT enzyme activities are biomarkers for determining hepatotoxicity (Hussain, 2013). The results of this study indicate a beneficial protective role of curcumin in mice exposed to chlorpyrifos. These results were evidenced by a decrease in AST and ALT activity from the highest curcumin dose to the lowest curcumin dose (P5 to P1). Our results showed that administration of curcumin to chlorpyrifos-induced rats resulted in a decrease in AST and ALT. This is in accordance with the results of research by Yadav et al., 2011 showed that curcumin administration resulted in decreased AST activity in rats after being induced by dichlorvos. Decreased AST and ALT activity indicates hepatoprotection of curcumin against dichlorvos toxicity. ALT is an enzyme cytosol that is more specific for the liver. AST is a mitochondrial enzyme found in the liver, skeletal muscle and kidneys. The increase in AST and ALT activity is due to leakage of cytosolic liver cytosolic transaminases into the bloodstream. The increase in AST and ALT may be due to the formation of reactive oxygen species and biomarkers of liver damage and liver dysfunction. The results of a study by Hassani et al., 2014 showed that the protective effect of curcumin led to a decrease in AST and ALT activity. The protective function of curcumin is by neutralizing reactive oxygen species, inhibiting superoxide anions and forming hydroxyl radicals and binding free radicals.

**CONCLUSION**

It can be concluded that the curcumin from the rhizome extract of *Curcuma longa* Linn shows a protective effect against chlorpyrifos poisoning in Wistar rats.

**Conflict of interest statement**

The author states that there is no conflict of interest

**Acknowledgements**

The author would like to thank the Head of the Health Development and Empowerment Agency of the Ministry of Health of the Republic of Indonesia for the research funding.

**REFERENCES**


Protective effect of curcumin rimpang Curcuma longa linn. extract on chlorpyrifos poisoning in wistar rats


