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Detection of DNA Changes in the Parp-1 Gene in the Small Intestine Tissue of Male White Rats Exposed to Pesticides Using PCR

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A B S T R A C T

Excessive exposure to pesticides can damage DNA. This paper analyzes the effects of pesticide exposure on the small intestine tissue (ileum) of white rats (Rattus Norvegicus), focusing on DNA mutation, particularly in the Parp-1 gene. This paper was an experimental study with a posttest-only design. It involved 18 male white rats (Rattus Norvegicus), divided into 5 groups: dose 1 (1.1 ml) and dose 2 (2.2 ml) groups for 7 days of oral pesticide administration, dose 1 (1.1 ml) and dose 2 (2.2 ml) groups for 14 days of oral pesticide administration, and a control group. Blood samples were collected to measure acetylcholinesterase enzyme levels, and ileal tissue was collected for analysis of PARP-1 gene expression. DNA was isolated from the ileum of the small intestine, and amplification was performed using forward (5'-TGGCCAAAAAGAAATCTAAG-3') and reverse (5'-ATCCCCAGTACAGTAA TAAG-3') primers. Results found that the lowest average acetylcholinesterase levels were observed in the high-dose group (2.2 ml) with a treatment period of 14 days, namely 18%. In contrast, the highest average acetylcholinesterase levels were observed in the low-dose group (1.1 ml) with a 7-day treatment period, namely 49%. Furthermore, the PARP-1 gene was detected by band thickness (band) in samples ranging from 260 to 347, using the PCR method with PARP-1 gene primers. Thus, pesticide exposure changes PARP-1 gene expression in the small intestinal tissue of white rats (Rattus Norvegicus). It indicates that pesticides can cause DNA mutation, leading to damage in the rat's small intestinal tissue.

INTRODUCTION

Pesticides are highly harmful to health and the environment because they are pollutants that generate free radicals that damage organs, trigger cell damage, and cause premature aging and decreased fertility (Jayati et al., 2020). If pesticides enter the food chain, then toxic chemical residues from pesticides also enter the human body, if in the long term it can interfere with human health due to the large content of carcinogenic chemicals that can cause various diseases such as cancer, Parkinson's, mutations, congenital disabilities, CAIDS (chemically acquired deficiency syndrome), and several other disorders (Siswoyo et al., 2018).

The digestive tract consists of the mouth, pharynx, esophagus, stomach, small intestine, colon, and anus (Chairunnisa, 2021). The intestine is part of the digestive tract, a complex, multi-fold tube that extends from the pylorus to the ileocecal valve. The intestine fills the middle and lower parts of the abdominal cavity. Changes in the histological structure of the intestine can be affected by the entry of certain amounts and types of compounds, including food, into the intestinal organs, because these compounds undergo absorption, distribution, metabolism, and excretion (Ersawati et al., 2018).

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Excessive exposure to pesticides can disrupt the balance of enzymatic antioxidants. These disorders can increase free radical levels and trigger the production of reactive oxygen species (ROS) (Salampe et al., 2021). Increasing ROS levels can induce oxidative stress, leading to damage to various cellular molecules, including fats, carbohydrates, proteins, and DNA. If DNA damage is not repaired before replication, mutations will occur. DNA damage caused by carcinogenic materials can occur in the Parp-1 gene (ADP-ribose polymerase-1), which encodes a protein involved in DNA damage response, cell cycle arrest, and apoptosis. Under physiological conditions, PARP-1 is synthesized at low levels and increases in response to DNA damage (Karima & Widyarti, 2016). In the last decade, Parp-1 has been shown to play an important role in DNA damage repair in germ cells and in apoptosis in testicular germ cells. Several studies have shown the teratogenic and cytotoxic effects of peptides, including one that reports that intraperitoneal administration of high doses can cause changes in spermatogonial cells. This paper analyzes the effects of pesticide exposure on the small intestine tissue (ileum) of white rats (*Rattus Norvegicus*), focusing on DNA mutation, particularly in the Parp-1 gene.

METHOD

This paper was an experimental study with a posttest-only design. The sample in this study consisted of 20 rats weighing 200-250 grams, but 2 died during observation. Thus, this study involved 18 male white rats (*Rattus Norvegicus*), divided into 5 groups: 2 rats in the negative control group for comparison, and 16 rats divided into 4 groups, each consisting of 4 rats. These 4 groups were treated with the same dose, different for 7 days and 14 days. However, one rat in the 14-day group with high doses died during treatment. Blood samples were collected to measure acetylcholinesterase enzyme levels, and ileal tissue was collected for analysis of PARP-1 gene expression. Research procedures were:

Blood Preparation and Sampling

Blood was collected after the rats were orally administered pesticides. The authors prepared the equipment in advance, including blood collection tubes. Then, we anesthetized the rats by placing them in a container and adding cotton soaked in 10% ether solution. Blood was collected from the orbital sinuses of the eye using a capillary tube, then transferred to a Vacutainer tube.

Organ DNA Isolation

The ileum tissue weighed up to 20 mg. The small intestine was homogenized in a mortar and pestle. Homogenate was mixed with 250 µl digestion buffer (100 mM NaCl; 10 mM Tris Cl, pH 8; 25 mM EDTA, pH 8; 0.5% (w/v) SDS; 0.1 mg/ml proteinase K), divortex, and incubated at 55°C. for 12-18 hours. Homogenate was mixed with 250 µl of CI (chloroform: isoamyl alcohol) solution and centrifuged

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at 4000 rpm, 4°C for 10 minutes. The formed supernatant layer was collected, along with a CI solution equal to the volume of supernatant taken (1:1), and centrifuged at 4000 rpm, 4°C for 10 minutes. For the DNA purification stage, the supernatant was transferred to a new endorf tube, then 1.5 volumes of 7.5 M ammonium acetate and 2 times the volume of absolute ethanol were added, and the mixture was centrifuged at 10000 rpm, 4°C for 10 minutes. The supernatant was discarded, the pellets were resuspended in 500 µl of 70% ethanol, and re-centrifuged at 10000 rpm, 4°C for 10 minutes. Next, the pellets were dried, aerated, and 50 µl of TE buffer was added until the DNA dissolved. The isolate was then stored at 4°C.

PCR (Polymerase Chain Reaction)

The ileum DNA sample was subjected to PCR to amplify the PARP-1 gene. The size of the PARP-1 gene amplification product was 254 bp (base pairs). The forward and reverse primers used were those reported by Widyarti & Wibi (2009), namely the forward (5'-GTTGTTTGTGTGTGTTTCCTA-3') and reverse (5'TTTTTAGCTCAAAAAATGCTTAA-3') primers. Table 1 describes the reaction composition for the PCR process.

Table 1. Reaction composition for PARP-1 gene amplification

Composition	Volume (µl)	Concentration
BSA	6	400 ng/µl
Primer forward	3	10 pmol/µl
Primer forward	15	10 pmol/µl
PCR Mix (KAPA 2G Fast Ready Mix)		
DNA template	2	400 ng/µl

Then, the PCR was performed using the TaKaRa PCR Thermal Cycler instrument with the following program (Table 2).

Table 2. PCR Program

Process		Temperature	Time
Initial Denaturation		95^0C	5 minute
Denaturation	1 Cycle	95^0C	
Annealing		55^0C	45 minute
Extension	33 Cycles	72^0C	
Extension	1 Cycle	72 ^{0}C	10 Minute

Elektroforesis Gel Agarosa 1.5%

Electrophoresis on a 1.5% agarose gel was performed to determine the success of the PCR. The tools used are plates, combs, and chambers. The plate installed with a comb was adjusted for flatness using a Waterpass. Next, agarose powder was weighed to 0.3 g in an Erlenmeyer flask and added to 1X TBE buffer to a final volume of 20 ml. The Erlenmeyer was covered with perforated plastic wrap (to allow evaporation) and heated in the microwave for 1-2 minutes at 50°C until it boiled and appeared clear. Then, we added 3 µl of EtBr and homogenized. The agarose solution was poured onto the plate and left to harden for 1 hour. After the agarose gel had hardened, the comb was removed, the plate was attached to the

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chamber, and 1X TBE buffer was poured until the gel was submerged. Amplified samples and DNA markers (O'GeneRulerTM 50 bp) were diluted to 3 µl each and added to each well. The electrode was connected to a power supply at 100 volts for 30-45 minutes. The electrophoresis gel was collected, visualized under a UV transilluminator, and documented with a camera.

RESULT

The results in this study were shown in Table 3, Figure 1, and Figure 2. It involved 15 blood and ileum tissue samples from pesticide-exposed rats and 2 from the control group. Table 3 presents the frequency distributions of ingested pesticides and AChE levels in rats.

Table 3. Frequency distribution based on ingested pesticides and AChE levels in rats

Combination of Interventions	Acetylcholinesterase Levels (%)					
	Dose	1	2	3	4	Average
7 Days	Dose 1 (1.1 ml)	44%	34%	69%	48%	49%
	Dose 2 (2.2 ml)	31%	24%	39%	43%	34%
14 Days	Dose 1 (1.1 ml)	32%	23%	23%	32%	27%
	Dose 2 (2.2 ml)	21 %	18%	16%	-	18%
	Control A		59%			
	Control B		43%			

In addition, Figure 1 indicates the amplification of the Parp-1 Gene in the mouse ileum (*Rattus Norvegicus*) after 7 days of intervention.

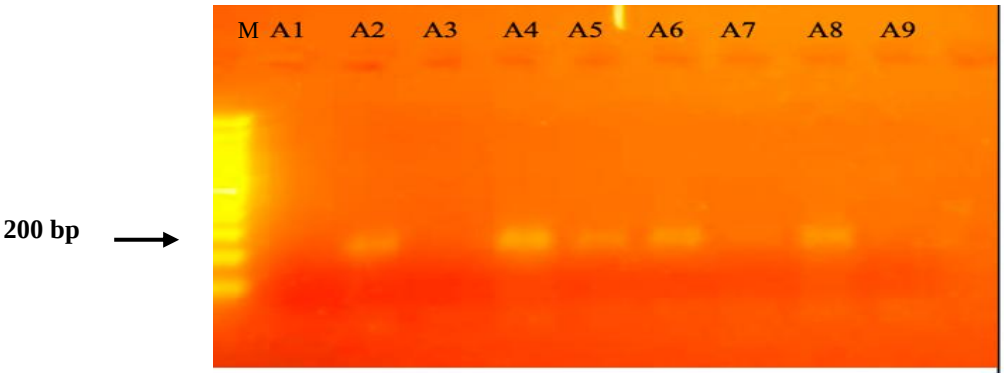

Figure 1. Results of amplification of the parp-1 gene in mouse ileum (*Rattus norvegicus*)

Furthermore, Figure 2 exhibits the amplification of the Parp-1 Gene in the mouse ileum (*Rattus Norvegicus*) after 14 days of intervention.

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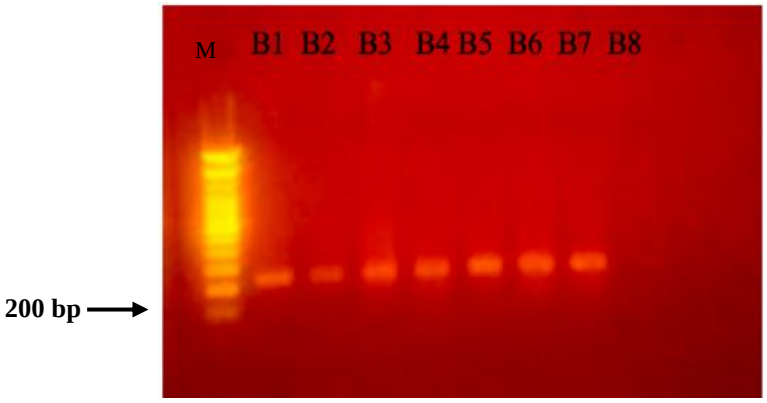

Figure 2. Results of amplification of the parp-1 gene in mouse ileum (*Rattus norvegicus*)

DISCUSSION

Results found that the lowest average acetylcholinesterase levels were observed in the high-dose group (2.2 ml) with a treatment period of 14 days, namely 18%, which fell within the 0-25% range and was categorized as severe poisoning. In contrast, the highest average acetylcholinesterase levels were observed in the low-dose group (1.1 ml) with a treatment period of 7 days, namely 49%, which fell within the 25-50% range and was categorized as moderate poisoning. The standard for acetylcholinesterase enzyme levels in blood is: normal levels: 85-100%; mild poisoning: 50-85%; moderate poisoning: 25-50%; and severe poisoning: 0-25% (Muawanah et al., 2025).

In addition, based on the visual analysis, the PARP-1 gene was detected by band thickness (band) in samples ranging from 260 to 347, using the PCR method with PARP-1 gene primers. The presence of bands indicates PARP-1 expression, which is induced by pesticides containing carcinogenic substances. According to research (Karima & Widyarti, 2016), the parp-1 gene was amplified by carcinogenic chemical exposure in the rat testicular organ, leading to histological structural changes.

CONCLUSION

Thus, pesticide exposure changes PARP-1 gene expression in the small intestinal tissue of white rats (*Rattus Norvegicus*). It indicates that pesticides can cause DNA mutation, leading to damage in the rat's small intestinal tissue.

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