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PROTECTIVE EFFECTS OF GUAVA FRUIT ON THE HISTOPATHOLOGY REPRODUCTIVE ORGANS OF RATS EXPOSED TO 2,3,7,8 TETRACHLORODIBENZO-P-DIOXIN

Efek Proteksi Buah Jambu Biji Pada Histopatologi Organ Reproduksi Tikus Yang Dipapar 2,3,7,8 Tetraklorodibenzo-P-Dioksin

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Abstract

Dioxin Toxicity has been studied to affect reproductive organ damage, so it can increase oxidative stress in the body. Guava fruit has several antioxidant compounds that can help break the chain of oxidative stress. The aim of this study was to determine the potential of guava fruit (*Psidium guava L.*) on Spermatogenik cell, Sertoli cell, and Leydig cell white rats exposed by TCDD. A total of twenty five white rats randomly divided to 5 groups, the Control group (K) which was given aquadest a dose 0.5 ml, Treatment group 0 (P0) which was given TCDD 100µg/KgBW solution a dose 0.5 ml, Treatment group 1 (P1) which was given guava fruit juice with a concentration of 25% a dose 0, 5 ml, treatment group 2 (P2) which was given guava fruit juice with a concentration of 50% a dose 0.5 ml, treatment group 3 (P3) which was given guava fruit juice with a concentration of 100% a dose 0.5 ml and the three groups were given a TCDD solution of 100µg/KgBW a dose 0.5 ml four hours before the treatment dose was given with 5 replicates in each group. Based on the results showed from this study that the administration of guava fruit juice at concentration 100% can efficiently affect the number of

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Spermatogenik cells consisting of Spermatogonium cells, Spematocyte cells, Spermatid cells in male white rats (Rattus norvegicus) exposed to 2,3,7,8, Tetrachlorodibenzo-P-dioxin.

Keywords: Antioxidant, guava fruit, spermatogenic cell

Abstrak

Toksisitas dioksin telah diteliti dapat mempengaruhi kerusakan organ reproduksi, sehingga dapat meningkatkan stres oksidatif dalam tubuh. Buah jambu biji memiliki beberapa senyawa antioksidan yang dapat membantu memutus rantai stres oksidatif. Tujuan dari penelitian ini adalah untuk mengetahui efek proteksi buah jambu biji (*Psidium guava L.*) pada gambaran histopatologi sel Spermatogenik, sel Sertoli, dan sel Leydig pada tikus putih yang dipapar TCDD. Sebanyak dua puluh lima ekor tikus putih dibagi secara acak ke dalam 5 kelompok, yaitu kelompok Kontrol (K) yang diberi aquadest dosis 0,5 ml, kelompok Perlakuan 0 (P0) yang diberi larutan TCDD 100µg/KgBB dosis 0,5 ml, kelompok Perlakuan 1 (P1) yang diberi sari buah jambu biji konsentrasi 25% dosis 0,5 ml, kelompok Perlakuan 2 (P2) yang diberi sari buah jambu biji konsentrasi 50% dosis 0,5 ml, kelompok Perlakuan 3 (P3) yang diberi sari buah jambu biji konsentrasi 50% dosis 0,5 ml. 5 ml, kelompok perlakuan 3 (P3) yang diberi jus buah jambu biji dengan konsentrasi 100% dosis 0,5 ml dan ketiga kelompok tersebut diberi larutan TCDD 100µg/KgBB dosis 0,5 ml empat jam sebelum dosis perlakuan diberikan dengan 5 kali ulangan pada masing-masing kelompok. Berdasarkan hasil penelitian ini menunjukkan bahwa pemberian jus buah jambu biji pada konsentrasi 100% secara efisien dapat mempengaruhi jumlah sel Spermatogenik yang terdiri dari sel Spermatogonium, sel Spematosit, sel Spermatid pada tikus putih jantan (Rattus norvegicus) yang terpapar 2,3,7,8, Tetraklorodibenzo-P-dioksin.

Kata kunci: Antioksidan, buah jambu biji, sel spermatogenik

INTRODUCTION

Environmental contamination is mostly generated through by-products of human activities, specifically, from industrial processes and household industries. The household industry contributes to pollution through home heating systems and the use of detergents, exhaust from cars and cigarette smoke containing harmful chemicals such as dioxins.

There were 210 dioxin compounds found, of which only 17 were toxic. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been studied and is known as the most toxic compound among dioxin compounds. TCDD has the highest toxicity among other dioxin compounds. The toxicity effect of TCDD has been known to disrupt endocrine activity in producing hormones, including reproductive hormones. According to (El-Gerbed et al., 2015), many toxins in the environment that accumulate in the body have been shown to affect spermatogenics in rats which can cause low spermatozoa count, abnormal spermatozoa morphology and poor semen quality.

TCDD toxicity in the body can cause oxidative stress, therefore ingredients are needed that function to break the oxidative chain caused by TCDD, such as antioxidant compounds in fruits such as guava, tomatoes, etc. These fruits have high antioxidant content that can protect organ damage from toxic substances (Wahyuningtyas et al., 2023). (Bouchoukh et al., 2019) stated that guava fruit has good antioxidant abilities in the Free Radical Scavenging (FRSA) and Ferric Reducing Ability (FRAP) tests.

Based on research conducted by (Meles et al., 2021), the results show that giving red guava fruit extract to mice can help lung alveolar recovery due to exposure to cigarette smoke. This is because the quercetin content in red guava fruit can help the alveolar recovery process in the

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lungs of male rats due to exposure to cigarette smoke and the high vitamin C content in guava fruit can break the ROS chain by blocking ROS production through caspase 3.

The antioxidant content of guava fruit as an antioxidant has been widely studied. However, there has not been much research on the antioxidant potential of guava fruit extract in preventing damage to the male reproductive system due to exposure to certain compounds.

RESEARCH METHODS

Research Ethics Statement and Research Design

This study is an experimental laboratory study using a completely randomised design (CRD) on 25 male white rats divided into five treatments and each treatment has four replicates with an ethical number No: 101/EA/KEPK/2023. This study used guava fruit juice treatment. According to the results of research by (Alifia et al., 2023) the concentration of white guava fruit juice (*Psidium guajava L.*) which is categorised into three concentration levels, 25%, 50%, 100% of these concentrations are sequentially divided into three groups P1, P 2, P3. The juice was administered orally as much as 0.5 ml/head to male rats. TCCD preparation in 1 ml ampoules was taken as 100µg/KgBB/day dissolved in corn oil in accordance with the dose calculation. Corn oil (Mazol Codaa Switzerland AG) was used as the solvent for TCDD.

Data collection

The Control group (K) which was given aquadest a dose 0.5 ml, Treatment group (P0) which was given TCDD 100µg/KgBW solution a dose 0.5 ml, Treatment group (P1) which was given guava fruit juice with a concentration of 25% a dose 0,5 ml, treatment group (P2) which was given guava fruit juice with a concentration of 50% a dose 0.5 ml, treatment group (P3) which was given guava fruit juice with a concentration of 100% a dose 0.5 ml and the three groups were given a TCDD solution of 100µg/KgBW a dose 0.5 ml four hours before the treatment dose was given with 5 replicates in each group.

Data Analysis

The data obtained in this study were analysed using non parametric analysis using Anova. Normal data distribution and homogeneous variance if the p value > 0.05. If the data distribution is normal, it is continued by using the One-Way Anova Test if there is a real difference Post-Hoc Duncan.

RESULTS AND DISCUSSION

The ANOVA test results on the histopathological picture of spermatogenic cells showed that there were significant differences in each group ($p<0.05$). Statistical data tests were carried out using anova test and Duncan's multiple range test for the observation of the count of Spermatogenic cells in table 1.

Based on the analysis results in Table 1. The table is the result of spermatogonia cells in each treatment, it is found that the count of spermatogonia cells in group P3 has a mean number of spermatogonia cells which is 59.120a ± 3.71376 this result is greater than the control group which is 46.840b ± 8.97931 and group P3 has a mean number of spermatogonia cells which is 59.120a ± 3.71376 which is not significantly different from the count of spermatogonia cells in group P2 57.920a ± 2.60615. Meanwhile, between the treatment group P1 the count of spermatogonia cells is 47,200b ± 2,05913 and the control group has an mean number of Spermatogenic cells 46,840b ± 8,97931 not significantly different from each other but very significantly different from the mean number of Spermatogenic cells more than P0. The lowest mean number of spermatogenic cells was the P0 group 34.240c ± 3.13177.

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Based on the analysis results in Table 1 The table is the result of spermatocyte cells in each treatment, it is found that the count of spermatocyte cells in group P3 has the highest mean number of spermatocyte cells 76,320a ± 3,15785 among the Control group with the mean number of spermatocyte cells 75,280a ± 7,71051, and group P2 with the mean number of spermatocyte cells 71,400a ± 8,99111 and P1 with the mean number of spermatocyte cells 69,080a ± 3,27292. However, the mean number of spermatocyte cells in the treatment of the Control group, group P1 and group P2 with group P3 is not significantly different. group P3 has a mean number of spermatocyte cells which is 76,320a ± 3,15785 more than group P0 which has a mean number of spermatocyte cells 48,600b ± 4,04475. The lowest mean number of spermatocyte cells is the P0 group.

Based on the results of the analysis in Table 1 the results of spermatid cells in each treatment, it was found that the mean number of spermatid cells in the control group had the highest mean number of spermatid cells 110.760a ± 16. 46232 but not significantly different from group P3 which has an mean number of spermatid cells of 104.080ab ± 4.22280. group P3 has an mean number of spermatid cells of 104.080ab ± 4.22280 the mean number of spermatid cells of group P3 is more than group P2 which has an mean number of spermatid cells of 96.760bc ± 4.75689. Meanwhile, the P1 group which has an mean number of spermatid cells is 92.160c ± 5.36917 with the mean number of P2 groups which are not significantly different from each other but are significantly different from the mean number of spermatid cells more than P0. the lowest mean number of spermatid cells is P0 62.480d ± 4.07087.

The histopathological examination results used Olympus CX-22 microscope with 400x magnification and supported by optilab application for documentation. The count of Spermatogenic cells consisting of the count of spermatogonium cells, spermatid cells and spermatocyte cells were counted based on the mean of five field of view on the microscope of each replicate treatment group. The histopathological picture of Spermatogenic cells can be seen in Figure 1.

The results of the study showed that when observing the number of spermatogenic cells consisting of spermatogonia cells and spermatocyte cells in the treatment groups given guava fruit juice with concentrations of 25% (P1), 50% (P2) and 100% (P3), the results were no different. significant compared to the control, however, the results of research on the number of spermatid cells in the treatment group given Guava Fruit Juice with concentrations of 25% (P1), 50% (P2) and 100% (P3) had slightly different results from the control with the highest value, namely number of spermatid cells in the control treatment. This is because, this research was only carried out for 14 days or one phase in the spermatogenesis cycle.

Damage to spermatogenic cells due to exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can be minimized by administering antioxidants. This is in line with the research results that the number of spermatogenic cells consisting of spermatogonium cells, spermatocyte cells and spermatid cells saw an increase in the number of cells due to the administration of guava fruit. In this research, guava fruit with a concentration of 100% was able to improve the structure of spermatogenesis cells and inhibit the process of spermatogenic cell necrosis in the seminiferous tubules. One of the antioxidant compounds contained in guava fruit is the phenolic group, such as Proteuchautic acid, Ferrulic acid, Absorbic acid (vitamin C) and α tocopherol (vitamin E), which are compounds that are able to break the chain of oxidative stress so that cell damage can be minimized. This is in accordance with the statement of (Wahyuningtyas et al., 2023) that TCDD toxicity in the body can cause oxidative stress, therefore materials are needed that function to break the oxidative chain caused by TCDD such as antioxidant compounds.

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One of the compounds, namely Protocatechuic acid, is an antioxidant compound that comes from the phenolic group. This compound is able to inhibit the process of cell damage by inhibiting the expression of the P53 protein. P53 protein expression is related to the process of cell apoptosis. This is in line with research conducted by (Varì et al., 2015) Protocatechuic acid prevents apoptosis induced by Low Density Lipoprotein Oxidation (oxLDL) by activating c-Jun NH2-terminal kinase (JNK) and nuclear factor erythroid 2-related factor signals. 2 (Nrf2)in Macrophages. This is because Protocatechuic acid reduces the excess production of ROS induced by oxLDL and especially the initial increase in ROS by inhibiting the expression of the P53 gene which is induced by ROS.

CONCLUSION AND SUGGESTION

Conclusion

The administration of guava fruit juice with a concentration of 100% orally effectively maintains the number of spermatogenic cells and reduces the risk of exposure to TCDD compounds.

Suggestion

Based on the research that has been done, the suggestions are, Guava fruit can be used as an alternative source of herbal ingredients that contain antioxidants to reduce the effects of free radicals and increase fertility. Further studies need to be done with guava fruit at higher doses and length of time to give guava fruit to determine whether there are side effects.

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Figure

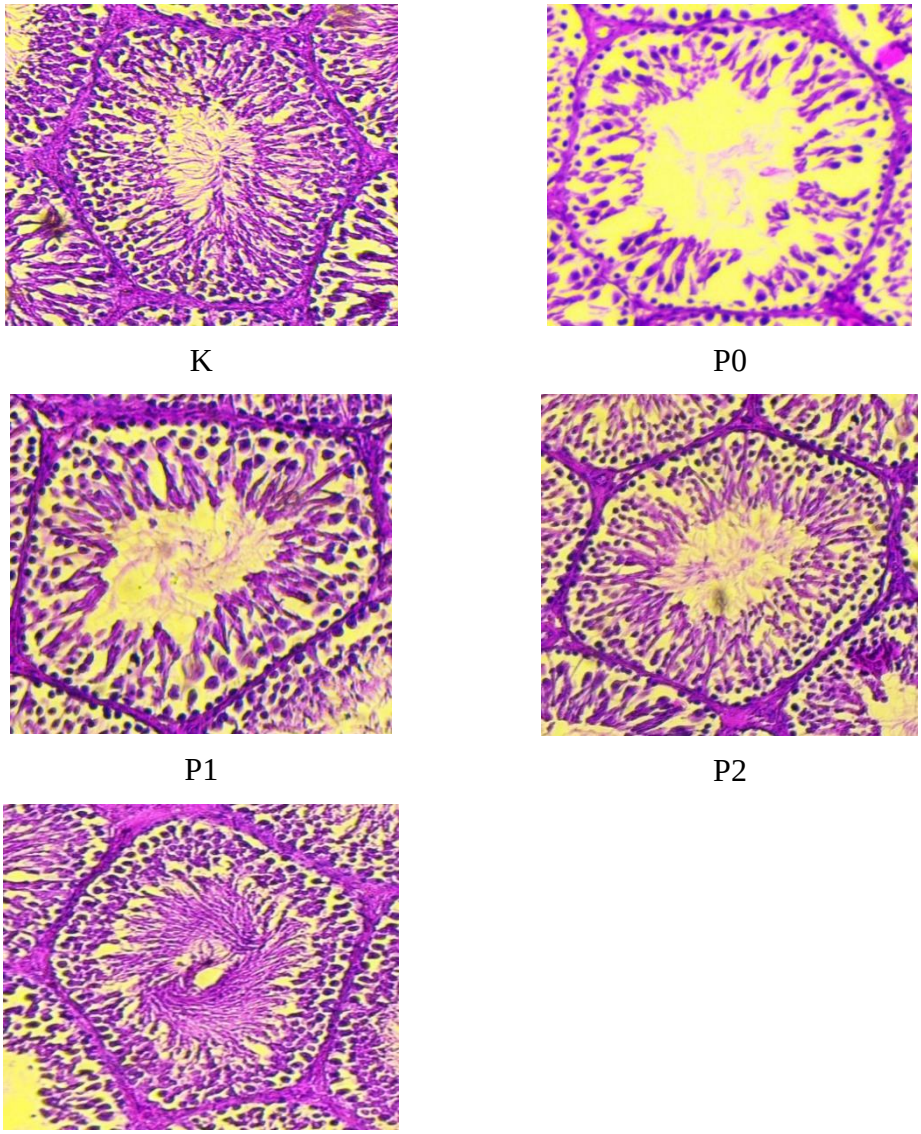

Figure 1. Histopathological picture of Spermatogenic cells in each treatment. The picture describes the stages of spermatogenic cells: In the basal part of the seminal basal are a spermatogonia cell, in the middle part, the cell shape is larger and the nucleus is clearly visible are a spermatocyte cell, in the area near the lumen of the seminiferous tubules are a spermatid cell.

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Table

Table 1. Mean and deviation of the count of spermatogonia, spermatocyte and spermatid cells in each treatment group

Treatment	Spermatogonia Cells Mean±SD	Spermatosit Cells Mean±SD	Spermatid Cells Mean±SD
K	$46.840^{b} \pm 8.97931$	$75.280^{a} \pm 7.71051$	$110.760^{a} \pm 16.46232$
P0	$34.240^{c} \pm 3.13177$	$48.600^{b} \pm 4.04475$	$62.480^{d} \pm 4.07087$
P1	$47.200^{b} \pm 2.05913$	$69.080^{a} \pm 3.27292$	$92.160^{c} \pm 5.36917$
P2	$57.920^{a} \pm 2.60615$	$71.400^{a} \pm 8.99111$	$96.760^{bc} \pm 4.75689$
P3	$59.120^{a} \pm 3.71376$	$76.320^{a} \pm 3.15785$	$104.080^{ab} \pm 4.22280$

^{abc}superscript different in the same column indicates significant difference ($p<0.05$)

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