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Protective Effects of Apple Peel Extract (*Malus sylvestris* Mill.) on the Sperm Quality and Testicular Histology in Mice Borax-Induced

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ABSTRACT

Borax, a toxic material, can disrupt the testes, hypothalamus, and spermatogenesis in the male reproductive system, leading to infertility. Apple peel contains quercetin, a substance crucial for boosting antioxidant levels and preventing free radical damage. The study aimed to assess the impact of apple peel extract on spermatozoa quality and testicular histology in borax-induced mice. The study utilized a Complete Randomized Design (CRD) with five treatment groups and five replications of each group: C- (without treatment), C+ (Borax), T1, T2, and T3, where borax was administered alongside various doses of apple peel extract: 0,2 mg, 0,4 mg, and 0,8 mg respectively. Testicular sections were collected for histopathological analysis and caudal epididymal sections for sperm quality assessment. Data were analyzed using ANOVA and DMRT tests using SPSS 23. The results showed that apple peel extract significantly increased the quality of mice spermatozoa in borax-induced mice ($p<0.05$). Apple peel extract to borax-induced mice also positively affected testicular diameter and weight, as well as the diameter of the seminiferous tubules and the number of Leydig cells. The optimal dose of apple peel extract in improving the quality of spermatozoa and testicular histology of mice was found to be0.4 mg / 20g BW.

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INTRODUCTION

Foods that are often added with borax include meatballs, rice cakes, noodles, crackers, and various other traditional foods (Yuliarti, 2007). Borax is added to food primarily for preservation purposes. Additionally, it is used to to enhance the texture of food, making it chewier, and improving its appearance, durability, and mouth feel (Alsuhendra & Ridawati, 2013). Borax is a soft crystalline substance with the chemical name of sodium tetrabonate ($Na_2B_4O_710H_2O$). It is also known by other names, such as sodium biborate, sodium pyrorate, sodium tetraborate, which are intended for use in non-food industries (Nurkhamidah et al., 2017). Borax should be used for purposes such as wood antifungal treatment, antiseptic applications, and as a cockroach repellent (Mayasari & Nanang, 2012). In the male reproductive system, the boric acid content in borax can inhibit spermatogenesis, leading to infertility (Mayasari & Nanang, 2012).

Borax is believed to induce oxidative stress through the production of free radicals, such as superoxide and hydrogen peroxide, that damage DNA (Utami, 2015). Boric acid can penetrate the blood-testis barrier and blood-brain barrier, disrupting the function of the testes and hypothalamus. The correlation shows that damage to the hypothalamus can create hormonal imbalances that can ead to testicular damage. Testicular interstitial tissue, where Leydig cells are found, produce testosterone, which is essential for spermatozoa differentiation. Research conducted by Rosa et al. (2016) showed that borax affects the quality of mouse spermatozoa at the dose of 2 mg / 10 g BW to adult mice for 35 days resulted in a decrease in the percentage of motility and an increase in the percentage of sperm membrane integrity. Research by Pramesti et al. (2016) also

showed that administering borax at the dose of 260 mg/Kg BW/day to the mice for 14 days caused a decrease in the number of Leydig cells.

To overcome this, it is necessary to have free radical scavengers. There are various ways to reduce exposure to free radicals that enter the body, one of which is to consume fruits and vegetables rich in antioxidants. Antioxidants are compounds that can inhibit oxidation reactions or a substance that can neutralize or capture free radicals (Murray et al., 2003). One of the antioxidant-rich fruits is apples, both the flesh and peel. Apples are often consumed with only the flesh, and some people have the habit of removing the peel, even though the highest antioxidant content in apples is found in the peel (Simamora, 2009).

Borax is a chemical compound that can generate free radicals. Borax that enters the body generates free radicals in the form of reactive oxygen species (ROS) (Verma & Nair, 2001)). Ascorbic acid serves as an antioxidant or reducing agent and has an important role in the male reproductive system. Low levels of ascorbic acid in the testes can lead to poor spermatogenesis, Leydig cell degeneration, seminiferous tubule degeneration, and decreased testosterone production. Apple peels contain catechins, procyanidins, floridzin, floretin glycosides, caffeic acid, chlorogenic acid, and quercetin glycosides (Mullen et al., 2007). The primary antioxidant in apple peel is quercetin glycoside. Apple peel contains quercetin, a substance essential for boosting antioxidant levels to prevent various diseases. The results of the study stated that only apple peel that contains quercetin, which means that apples can provide antioxidants equivalent to 1,500 mg of vitamin C from fresh apple extract of medium size apples (Pertiwi et al., 2016). The quercetin content in 100 grams of apples consists of approximately 4.42 mg of quercetin aglycone and 13.2 mg of glycosides (Coskun et al., 2004). Chinici et al. (2004) reinforced that in apple peel, the primary contributors to antioxidant activity are flavonoids and procyanidin, which account for 90% of the total phytochemical content. The high content of flavonoid compounds in apple peel can reduce free radicals in the body via the blood vessels and restore the control system of reproductive hormone (Wang et al., 2023).

MATERIALS AND METHODS

Ethical approval

The study was approved by the Committee of the Research Ethics of Universitas Negeri Padang, Indonesia (approval No. 06.02/KEPKH-UNP/V/2024).

Extract Preparation

Apples were obtained from the market in Padang. To prepare the apple peel ethanol extract, the maceration method was used. The apple peels were washed thoroughly, then dried in the oven at 40-60°C until the apple peels dry. In the next stage of the extraction process, the dried apple peels were ground into a powder using a blender. The powdered apple peel (100 grams) was measured and placed into a 1-liter Erlenmeyer flask." The dried apple peels were added with 96% ethanol to make a total volume of 1 liter and the mixture was stirred until homogeneous. The mixture of apple peels and ethanol was allowed to stand for 24 hours, during which a precipitate formed. Then, the top layer, consisting of ethanol (solvent) and the active compounds, was then separated through filtration using filter paper (repeated three times). The mixture was put into a one-liter evaporation flask, which was then connected to the evaporator (Heidolph Rotary Evaporator Hei Vap Core HL/G3). Then the water bath was filled with water to the brim and the temperature was set according to the boiling point of the solvent. Evaporation was carried out until the extract thickened. Lastly, the weight of the extract was measured.

Animals

This experimental study used 25 male mice, aged 3-4 months and weighing 20-30 grams. Mice were acclimated in the Animal Division in Biology Laboratories, Faculty of Mathematics and Natural Science Universitas Negeri Padang, for 7 days to allow them to adapt to new environmental conditions. Animals were fed with standard pellet chow and water ad libitum. After 7 days, the mice were divided into 5 treatment groups.

Experimental Design

The study utilized Complete Randomized Design (CRD) with 5 treatment groups, each consisting of five replicates. The negative control treatment group (C-) was the mice group without treatment, the positive control (C+) was the mice group induced by borax at the dose of 28 mg/20gBW/day, The first treatment group (T1) was the mice group induced by borax at the dose 28mg/20gBW/day + apple peel extract at 0.2 mg/20 g BW/day. The second treatment group (T2) was the mice group induced by borax at dose

of 28mg/20gBW/day + apple peel extract at 0.4mg/20gBW/day. The third treatment group (T3) was the mice group induced by borax dose at 28mg/20gBW/day + apple peel ethanol extract at 0.8 mg/20g BW/day for 21 days. The dose of borax was given orally to mice in 0.1 ml of aquadest, while the apple peel extract was given orally to mice 1 hour after exposure to borax with a volume of 0.5 ml.

Quality of spermatozoa Assessment

Mice were euthanized by dislocation of the neck, then dissected to collect the parts of testicles and epididymis on day 22. The cauda epididymis was separated and diluted with 1 ml of 0.9% NaCl. The suspension was homogenized, and the quality of spermatozoa was observed. Furthermore, 10 µL of sperm samples was assessed for motility, viability, morphology, and sperm number. The number of spermatozoa cells was calculated by the following formula:

$$\text{Number of spermatozoa} = \frac{N}{2} \times 10^5 \text{ spermatozoa/ml suspension}$$

(Rahma & Ahda, 2021)

Motility of spermatozoa was observed according to WHO (1994), which categorizes motility as follows:(A) spermatozoa move forward in straight line and fast, (B) spermatozoa move slowly or have difficulty advancing in a straight line, (C) spermatozoa exhibit non-progressive movement, (D) spermatozoa are stationary or immobile. Sperm motility was examined in 4-6 fields of view, with 100 spermatozoa observed sequentially. The sperm were then classified into motility categories, and the percentage of motile spermatozoa was calculated.

$$\text{Motility} = \frac{A+B}{\text{Total of sperm}} \times 100\%$$

(Fatmawati et al., 2016)

Viability of spermatozoa was assessed by placing a drop of the sperm suspension on the glass of the object and adding eosin (Merck), and allowing it to dry. Then, it was covered with a glass cover and observed using a microscope with a magnification of 400 x. Live sperm were colorless, while dead sperm on the head were red (pink). Furthermore, the number of live (colorless) spermatozoa were counted in in a sample of 100 spermatozoa. The value was expressed as a percentage.

$$\text{Viability} = \frac{\text{Number of live sperm}}{\text{Total of sperm}} \times 100\%$$

(Maria et al., 2020)

Sperm morphologists were observed by preparing a smear slide, followed by drying and fixing the sample with 96% methanol for 5 minutes. is the slide was stained using Giemsa solution and allowed to stand for 30 minutes. It was washed in running water and observed using a microscope with a magnification of 400x. To determine the percentage of spermatozoa morphology, the following formula can be used:

$$\text{Morphologist} = \frac{\text{Number of normal sperm}}{\text{Total of sperm}} \times 100\%$$

(Maria et al., 2020)

Histological Assessment

Mice were euthanized by dislocation of the neck, then dissected to collect parts of the testicles and epididymis (day 22). Testicular organ harvesting was done by making an incision in the abdominal area over the testes while the mouse was in a supine position. Then the testicular organs were taken by cutting the epididymis and removing the connective tissue and fat. The weight of the testicle was measured using an electronic scale, while the diameter of the testicle was measured using a caliper.

The testis was washed with phosphate-buffered saline and then fixed in Bouin's solution for 24 hours. The tissue samples were dehydrated a routine alcohol-based method and embedded in paraffin. A five-micrometer-thick section was stained with hematoxylin-eosin and histologically analyzed using light microscopes (Carl Zeiss, Inc., Germany) The diameter of the seminiferous tubules and the number of Leydig cells were investigated at 400x magnification.

The assessment of Leydig cells was performed by counting them in ten random microscopic fields in the interstitial compartment located between seminiferous tubules. The number of Leydig cells was calculated in 5 fields of view, and the average total number of Leydig cells was calculated for each treatment group (Januarti et al, 2021).

Measurement of the diameter of the seminiferous tubule, as described by (Sripratiwi, 2019), was carried out using a micrometer device on

the ocular lens. The diameter was measured between two opposite points on the middle line, specifically at the basal membrane of the seminiferous tubule. The selected tubules were those with a round cross-section and approximately the equal size. The measurement of seminiferous tubule diameter was conducted using a microscope at 400x magnification. The diameter of both the longest and shortest seminiferous tubules was measured in micrometers, and the average of these measurements was calculated (Larasati et al., 2015).

Statistical analysis

Observational data on spermatozoa quality and testicular histology were statistically analyzed through the ANOVA *(Analysis of variance)* test using the SPSS 23 ((SPSS Inc., Armonk, NY, USA). If the ANOVA test results indicated a significant difference at 5% level, Duncan's Multiple Range Test (DMRT) was carried out.

RESULTS AND DISSCUSION

Quality of Spermatozoa

The results showed that treatment group 2, which received apple peel extract at a dose of 0.4 mg/20grBW/day, exhibited an improvement in the quality of borax-induced mouse spermatozoa compared to treatment groups 1 and 3. In the positive control group, a decrease in the quality of spermatozoa of male mice was observed when borax was given, compared to the negative control group. The results of the study on the effect of apple peel extract on the quality of spermatozoa in borax-induced male mice are shown in Table 1.

The average number of spermatozoa in male mice after borax induction (C+) was lower compared to the negative control group (C-) without borax treatment, with values of 47.5 x 10^5/mL and 86.5 x10^5/mL, respectively (Table 1). An increase in the number of borax-induced spermatozoa in mice was observed after administration of apple peel extract. T2 showed the highest average number of spermatozoa at 86.5 x10^5/mL. The results showed that the administration of apple peel extract was able to increase the number of spermatozoa in borax-induced mice. The results of statistical analysis showed that the administration of apple peel extract had a significant effect on the number of spermatozoa ($P<0.05$). This suggests that the antioxidant content in apple peel extract can reduce free radicals caused by borax. In a study conducted by Octaviany et al, (2015), the administration of apple peel extract demonstrated that its antioxidant content in apple peel extract can reduce SGPT levels in rat induced with CCl4. The antioxidant activity contained in apple peel extract functions by stimulating the antioxidant defense system, such as superoxide dismutase, catalase, glutathione, glutathione reductase, stabilizing bio membranes by reducing fat peroxidation and capturing free radicals.

According to Utami (2015), borax can cause a decrease in testosterone levels in the blood circulation, inhibit spermiation and decrease sperm count. Free radicals generated by borax can damage DNA through the peroxidation of PUFA found in the membrane of spermatid cells. Spermatid cells are formed from the meiotic division stage of spermatogenesis. Then, the spermatic cells no longer undergo further division, but instead differentiate into spermatozoa cells, which are subsequently released during ejaculation. If the number of spermatic cells decreases, no cells will differentiate into sperm cells, leading to a reduction in sperm count (Sabilla et al., 2020).

The results of the observation of the potential of apple peel extract on spermatozoa motility showed that the average motility of spermatozoa mice after borax induction (C+) was 30%, which was lower compared to the percentage of spermatozoa motility in negative control group (C-), which was 47.6%. The motility of borax-induced spermatozoa in mice increased after administration of apple peel extract. The highest average was found in the T2 treatment, which was 47.6%. The results of observation of the potential of apple peel extract on spermatozoa motility showed that the administration of apple peel extract was able to increase the motility of spermatozoa in mice. The results of statistical analysis showed that the administration of apple peel extract affected spermatozoa motility ($P<0.05$). The motility of spermatozoa is considered normal if the forward motility exceeds 40% (Dcunha et al., 2020). Based on the results of this study, normal motility was observed in T2, T1, and T3 treatment groups, as their motility percentages were above 40%. These results confirm that the antioxidant content in apple peel extract can reduce free radicals caused by borax. This statement is consistent with the research by Wolfe et al. (2003) which stated that the high content of phenolic compounds, antioxidant activity, and antiproliferative activity of apple peels suggest potential health benefits when

consumed and establish apple peels as a valuable source of antioxidants.

The decrease in spermatozoa motility can be attributed to the toxic properties of borax, which inhibit the activity of ATP-ase enzymes in the sperm cell membrane (Rosa et al., 2016). This ATP-ase enzyme, located in the middle piece of spermatozoa, plays a crucial role in maintaining internal homeostasis of sodium and potassium ions. Since sperm motility depends on the composition of sodium and potassium ions, any disruption in their composition results in a decline in motility (Purwaningsih, 2000).

The viability of spermatozoa in mice after borax induction (C+) had a value of 24.2%, showing a decrease in viability in the positive control group (C+) compared to the negative control group (C-), which had a value of 60.6%. The viability of borax-induced spermatozoa in mice increased after administration of apple peel extract. The highest average was found in the T2 treatment, which was 60%. Observation of the potential of apple peel extract showed that the administration of apple peel extract was able to increase the viability of mouse spermatozoa. Living spermatozoa are marked by a bright color on the head and dead spermatozoa are reddish-purple when stained with eosin staining (Figure 1). The results of statistical analysis showed that the administration of apple peel extract had a significant effect on the viability of spermatozoa ($P<0.05$). According to (Hafez, 2000), the percentage of viability of spermatozoa is considered normal when the percentage exceeds 50%. In this study, normal viability was observed in the T2, T1, and T3 treatment groups, as their viability percentages were all greater than 50%. This shows that the antioxidant content in apple peel extract is able to reduce free radicals caused by borax. The analysis of the phenolic composition of the extract from Jonagold apple peels indicated that it is mainly composed of quercetin, catechin, epicatechin, and anthocyanins, such as cyanidin-3-O-galactoside, phloridzin, phloretin, and chlorogenic acid. Quercetin, the main flavanol, is characterized by its pentahydroxyflavone structure, with five hydroxy groups positioned at the 3-, 3′-, 4′-, 5-, and 7-positions. Its antioxidant activity is mainly dependent on its effect on glutathione level, enzymatic activity (mainly acetylcholinesterase and butyrylcholinesterase), signal transduction pathways, and reactive oxygen species scavenging (Thilakarathna et al., 2013; Balasuriya et al., 2012; Xu et al., 2019).

The results of observation of spermatozoa morphology showed that the average percentage of normal spermatozoa morphology in mice after exposure of borax (C+) was 24%, which was smaller compared to the percentage of normal spermatozoa morphology in mice. Observations of abnormalities are seen from spermatozoa that have abnormal shapes such as no spermatozoa head, large head shape, broken tail and coiled tail (Figure 2). The morphology of normal spermatozoa in borax-induced mice improved after administration of apple peel extract. The highest average was found in T2, which was 47.6%. The morphology of spermatozoa is one of the important factors supporting the ability of spermatozoa. Observations from the treatment groups showed that borax induction caused abnormalities in spermatozoa, although these were fewer in number following apple peel extract administration.

Table 1. Quality of mice spermatozoa with borax treatment and apple peel extract

Group Treatment	Quality of spermatozoa			
	Number of Spermatozoa Mean ± SD	Motility (%) ± SD	Viability (%) ± SD	Morphology (%) ± SD
C-	$86.5 \times 10^{5a} \pm 2.3 \times 10^{5}$	$47.6^{a} \pm 1.14$	$60.6^{a} \pm 2.70$	$54.2^{a} \pm 2.38$
C+	$47.5 \times 10^{5c} \pm 1.3 \times 10^{5}$	$30.0^{c} \pm 1.58$	$24.2^{b} \pm 8.87$	$24.0^{d} \pm 1.58$
T1	$59.25 \times 10^{5b} \pm 6.6 \times 10^{5}$	$45.0^{b} \pm 1.58$	$59.4^{a} \pm 2.07$	$38.8^{c} \pm 2.58$
T2	$86.25 \times 10^{5a} \pm 3.1 \times 10^{5}$	$47.6^{a} \pm 1.14$	$60.0^{a} \pm 2.54$	$47.6^{b} \pm 0.70$
T3	$62.5 \times 10^{5a} \pm 9.3 \times 10^{5}$	$45.0^{b} \pm 1.00$	$55.0^{b} \pm 3.80$	$43.0^{c} \pm 3.84$

Note: The difference *between superscripts* in the same column shows a real difference ($p<0.05$) in the DMRT test, while the data without *superscript* in the same column shows no real difference in results ($p>0.05$).

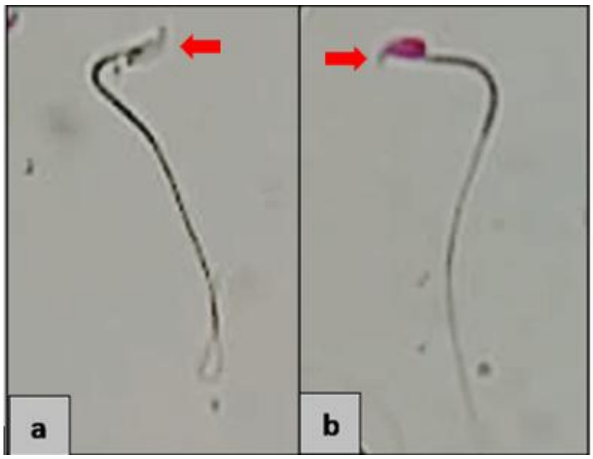

Figure 1. Viability of borax-induced male mouse spermatozoa and apple peel extract (a) live spermatozoa, (b) dead spermatozoa. (➡) shows spermatozoa viability (400x magnification)

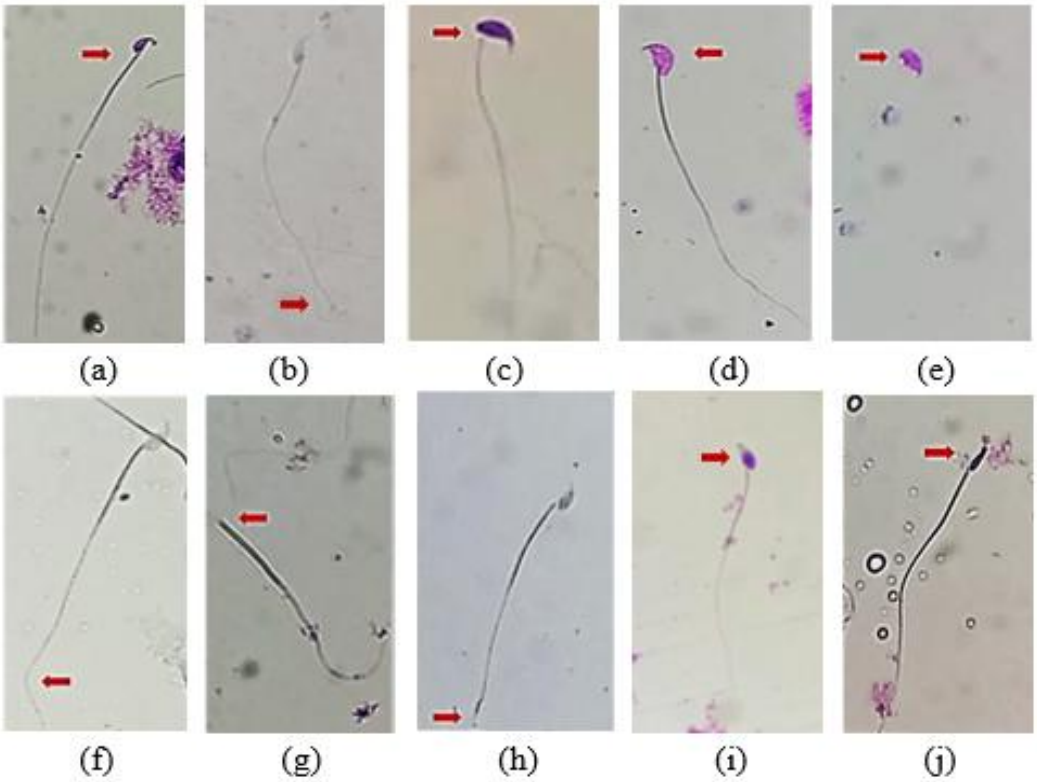

Figure 2. Morphology of male mouse spermatozoa exposed to borax and apple peel extract (a) normal sperm, (b) circular tail sperm, (c) twisted neck sperm, (d) large head sperm, (e) tailless sperm, (f) twisted middle sperm, (g) headless sperm, (h) folded tail sperm, (i) hookless sperm, (j) tapered head sperm.
Note:(➡) shows the condition of spermatozoa in male mice (400x magnification)

The results of the observation showed that the apple peel extract with the dose of 0,4 mg was able to improve the morphology of mouse spermatozoa. The results of statistical analysis showed that the administration of apple peel extract had a significant effect on the normal morphology of spermatozoa ($P<0.05$).

According to Dillasamola (2021), spermatozoa are considered fertile if the percentage of abnormal spermatozoa is below 40%. In this study, normal results were observed in T2 and T3, because the morphological percentage was below 40%. This shows that the antioxidant content in apple peel extract can reduce free radicals caused by borax. Disruptions during spermatogenesis due to borax induction cause a decrease in the normal morphology of spermatozoa, and this reduction becomes even

more pronounced during maintenance in the epididymis, due to a lack of energy (Kaspul, 2004).

The results of ANOVA statistical analysis showed that the treatment of apple peel extract resulted in a significant difference of P<0.05 in the observation parameters of spermatozoa quality in borax-induced mice. Therefore, further tests were carried out by DNMRT, which showed that the T2 treatment showed an improvement in the quality of borax-induced spermatozoa in mice. Apple peel extract can improve the quality of borax-induced spermatozoa because apple peel is rich in antioxidant content. Previous studies have also investigated apple peel extracts, although with different exposures. Administration of apple peel extract var. Rome beauty dose of 0.12 mg/kg BW can reduce SGPT enzyme in rats (Rattus norvegicus) induced by CCl4 to normal value (Octaviany et al., 2017)

Testicular Histology

The results of the study on the effect of apple peel extract on the testicular histology of borax-induced male mice are shown in Table 2.

The highest average testicular weight was found in the C- group, at 131.32 mg, while the lowest average testicular weight was found in the C+ group, at 94.81 mg (Table 2). The weight of the testicles in T1 was relatively similar to that of T3, but lower than that of T2. The highest testicular diameter was found in the C- group which was 6.49 mm and the lowest average testicular diameter was found in the C+ group, at 5.65 mm. The testicular diameters in T1 and T3 were similar to each other, but both were lower than that of T2.

The weight of the testicles in this study was not much different from the typical weight of normal testicles in mice. The administration of certain drugs or substances that can affect spermatogenesis lead to changes during the division or development of germ epithelial cells until they become spermatozoa (Samplaski and Nangia, 2015). Microscopic alterations in the spermatogenesis process can be seen through the size and number of cells within seminiferous tubules. These changes will affect the thickness of the epithelium and the diameter of the seminiferous tubules. On a macroscopic level, testicular weight can serve as an indicator of these alterations. A disorder in testicular function, cell damage or testicular atrophy will obviously result in a decrease in testicular weight. If the function of other organs is not impaired, the decrease in testicular weight would be the primary change observed. Therefore, it would be more appropriate to assess the deterioration of testicular function based on the ratio of testicular weight to body weight. The administration of apple peel extract was able to increase the weight of borax-induced testicles in mice, although it could not fully restore it to normal levels. This shows that the antioxidant content in apple peel extract can increase borax-induced changes in testicular weight.

The highest diameter of the seminiferous tubule was observed in the C- group, which was 16.67 mm and the lowest was found in C+ group, which was 10.18 mm. the diameters in T1 and T3 were relatively similar to each other but lower than that of T2. This shows that the highest seminiferous tubule diameter was found at T2. The administration of apple peel extract was able to increase the diameter of the testicles of mice. The results of statistical analysis showed that the administration of apple peel extract had a significant effect on testicular histology (P<0.05).

Table 2. The effect of apple peel extract on testicular weight, testicular diameter, seminiferous tubule diameter and number of leydig cells on mice induced borax

Group Treatment	Testicular Histology			
	Testicular Weight (mg) ± SD	Testicular Diameter (mm) SD ±	Diameter of Seminiferous Tubules (mm) ± SD	Number of Leydig Cells mean ± SD
C-	$131.32^{d} \pm 4.94$	$6.49^{d} \pm 1.63$	$16.66^{c} \pm 1.64$	$253.40^{d} \pm 1.14$
C+	$94.81^{a} \pm 3.65$	$5.58^{a} \pm 1.34$	$10.18^{a} \pm 5.10$	$228.80^{a} \pm 3.27$
T1	$104.15^{c} \pm 1.93$	$5.89^{c} \pm 1.59$	$11.57^{a} \pm 1.66$	$241.80^{b} \pm 2.58$
T2	$101.60^{c} \pm 4.28$	$6.24^{c} \pm 2.40$	$15.03^{b} \pm 1.16$	$251.40^{d} \pm 1.14$
T3	$103.92^{d} \pm 2.32$	$6.24^{d} \pm 3.63$	$14.07^{b} \pm 2.57$	$247.80^{c} \pm 1.30$

Note: The difference *between superscripts* in the same column shows a real difference (p<0.05) in the DMRT test, while the data without *superscript* in the same column shows no real difference in results (p>0.05)

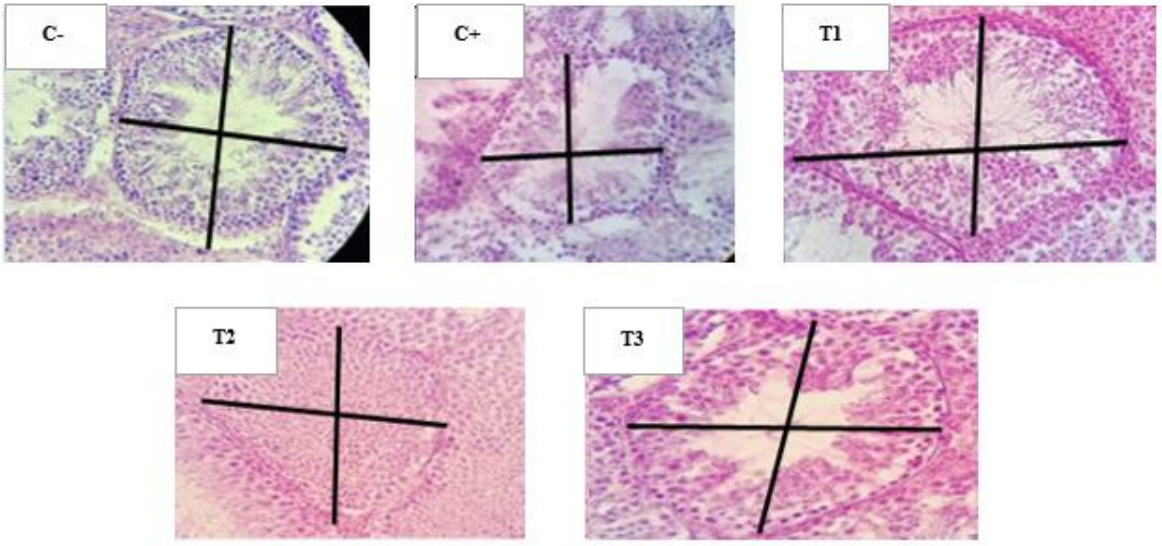

Figure 3. Histological overview of the seminiferous tubule (400x magnification) C-: Negative control (No treatment) C+: Positive control (Borax 28 mg/kg BW/day) T1: Treatment 1 (Borax 28 mg/kg BW/day + 0.2 mg/20 gr BW/day) T2: Treatment 2 (Borax 28 mg/kg BW/day + 0.4 mg/20 gr BW/day) T3: Treatment 3 (Borax 28 mg/kg BW/day + 0.8 mg/20 gr BW/day)

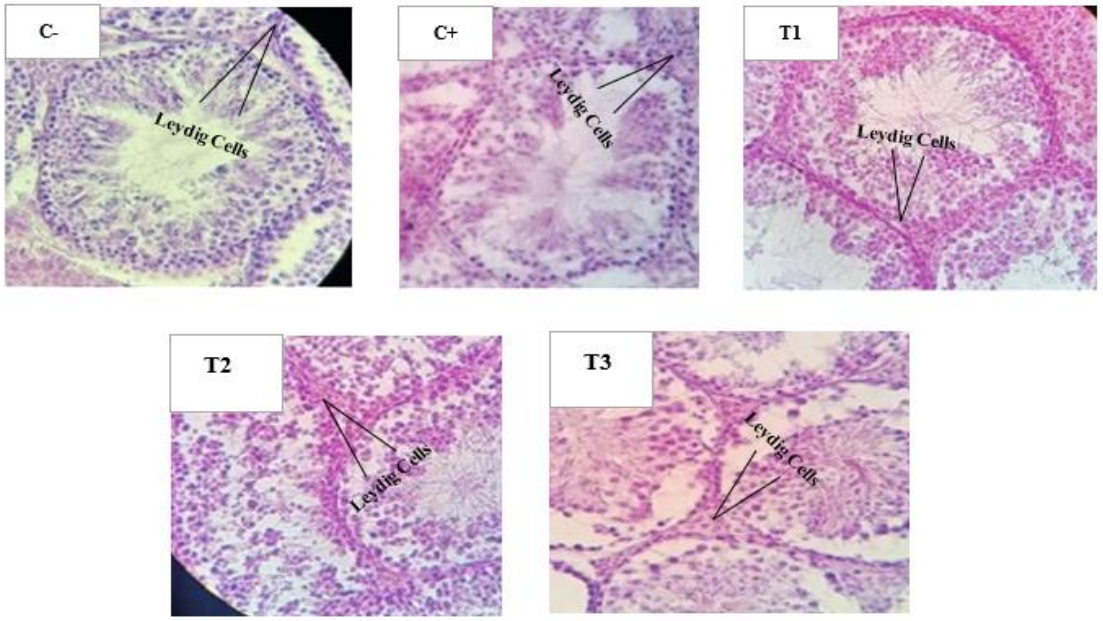

Figure 4. Histological description of the number of leydig cells (400x magnification)

This shows that the antioxidant content in apple peel extract can increase the diameter of the testicles. An overview of the testicular histology of borax-induced mice, with the administration of apple peel extract, is shown in Figure 3.

A decrease in FSH will affect the growth of seminiferous tubules, which serve as a place to start spermatogenesis (Toelihere, 1981). The decrease in FSH will also affect the activity of the Sertoli cells, resulting in a decrease in the production and secretion of ABP into the lumen of the seminiferous tubule. The ROS content in borax was successfully neutralized by the antioxidant content in apple peel extract which was shown by an increase in average diameter and significant yield statistically. Simultaneous administration of apple peel extract with borax can compensate for the effect of borax-induced radicals on the testicles, specifically the reduction in the diameter of seminiferous tubules. Apple peel extract can significantly increase endogenous antioxidant enzymes in the body. Hsu

et al. (2021) reported that quercetin treatment significantly reversed the reduced levels of SOD and the increased levels of CAT and GSH, indicating that quercetin could modulate the activity of antioxidants in the NaIO3-treated mice. This suggests that borax can reduce the diameter of the seminiferous tubules, which is consistent with previous research showing that the administration of borax (10mg/head) in *Mus musculus* mice can reduce sperm motility and sperm cell membrane integrity (Rosa et al., 2016).

The effect of administration of apple peel extract containing dominant antioxidant compounds on the diameter of the seminiferous tubules, the thickness of the seminiferous tubule epithelial layer, and the weight of the testicles in male mice showed results that were not significantly different. The number of Leydig cells in the testicles decreased in the C+ treatment group (228.8 cells), compared to the negative control group (C-), which was 253.4 cells. The number of Leydig cells in T1 was relatively similar to that of T3 but lower than that of T2. These shows the highest number of Leydig cells was observed in T2.

The decrease in Interstitial cell-stimulating hormone (ICSH) disrupts the activity of Leydig cells to produce testosterone. Testosterone is the main testicular hormone. ICSH and testosterone together promote further growth of sex cells (Hardjopranjoto, 1995). To overcome the decline in Leydig cells, apple peel extract which contains natural antioxidants from the flavonoid group, was utilized due to its beneficial effects on cellular health. Antioxidants are compounds that can protect other molecules from oxidation by free radicals. Scientifically, the human body produces antioxidants. However, these compounds are often not enough to protect the body, making the supplementation of external antioxidants necessary necessary (Moniharapon et al., 2016).

Based on the results of the study, the administration of apple peel extract was able to improve testicular weight, testicular diameter, the diameter of seminiferous tubules, the number of Leydig cells in borax-induced mice. However, these improvements did not differ significantly from the negative control group, which consisted of mice without any treatment. Wang et al., (2023) reported apple peel extract enhanced the expression of spermatogenesis-related genes. There was increasing the expression of oxidative stress- and apoptosis-related genes, thereby effectively reducing oxidative damage in the testes.

CONCLUSION

Based on the results of the study, it can be concluded that apple peel extract can improve the quality of spermatozoa and testicular histology in borax-induced mice. The optimal dose of apple peel extract in improving the quality of spermatozoa and testicular histology n borax-induced mice was 0.4mg/20gr BW/day. Future research should determine whether specific components in apple peel extract, such as quercetin and chlorogenic acid, are responsible for the beneficial effects.

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