



## Effect Of Passion Fruit (*Passiflora edulis*) Extract Administration on The Sex Ratio Of Mice (*Mus musculus*)

Naila Cahyani Kusuma Astuti Samodr<sup>a</sup>, Eka Aprilia<sup>a</sup>, Annisa Iriani<sup>a</sup>, Elphan Augusta<sup>b</sup>, Nur Alif Bahmid<sup>b\*</sup>

<sup>a</sup>Undergraduate Program in Veterinary Medicine, Faculty of Medicine, Hasanuddin University, Makassar 90245

<sup>b</sup>Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, Makassar 90245

\*corresponding author: [aliefbahmid02@gmail.com](mailto:aliefbahmid02@gmail.com)

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### Abstract

Passion fruit is known for its rich nutritional content. It contains several antioxidants that may influence the reproductive system of animals. Among these antioxidants is chrysin, a flavonoid compound abundantly present in passion fruit. This study aimed to determine the effect of passion fruit (*Passiflora edulis*) extract on the sex ratio of mice (*Mus musculus*) and to identify the optimal dose that may influence the proportion of male offspring. The research was conducted at the Animal Laboratory and Reproduction Laboratory of the Veterinary Teaching Hospital, Hasanuddin University, from May to July 2025. A total of 72 mice were divided into four groups: a control group (K0) and three treatment groups (P1, P2, P3) that received *Passiflora edulis* extract at doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW, respectively, administered orally for 28 days. Data on the offspring sex ratio were analyzed using ANOVA with a 5% significance level. The results showed that groups P1 and P2 tended to have a higher proportion of male offspring compared to the control, with the highest ratio observed in P2 ( $1.56 \pm 0.97$ ). However, the difference among treatments was not statistically significant ( $P > 0.05$ ). This trend may be associated with the presence of chrysin, a flavonoid compound in passion fruit that functions as an antioxidant and aromatase inhibitor, potentially enhancing Y-sperm viability and testosterone levels. In conclusion, the administration of *Passiflora edulis* extract tended to increase the proportion of male offspring, although the results were not statistically significant. Further research with a larger sample size, longer treatment duration, and tighter control of female reproductive variables is needed to confirm these findings.

*Keywords: Chrysin, mice, passion fruit, sex ratio*

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### Introduction

The sex ratio is the comparison between the number of male and female offspring born within a population or group. At birth, the probability of producing XY (male) and XX

(female) chromosome combinations is equal, or 50:50 (Nopianti et al., 2020). One method to modulate the sex ratio in mice is through the administration of natural herbal substances. These natural components include sodium, potassium, and the antioxidant chrysin (Dosom et al., 2023). Potassium and sodium can be used to increase the proportion of male offspring due to their alkaline properties, which enhance the ability of androsperm (Y-bearing sperm) to fertilize the ovum more rapidly than gynospem (X-bearing sperm), thus producing more male progeny (Riyanto, 2001). One fruit that contains all three of these components is passion fruit. Passion fruit possesses high nutritional value. It contains antioxidants such as beta-carotene, polyphenols, and flavonoids (Ahmad et al., 2024). Chrysin, a flavonoid widely found in passion fruit, acts as an aromatase inhibitor, preventing the conversion of androgens into estrogens and thereby promoting masculine characteristics (Hapsari & Saputra, 2024; Rosalina & Amelisa, 2020).

Passion fruit (*Passiflora edulis*) also has potential effects on the reproductive system of animals. A study by Silva et al. (2020) demonstrated that administration of passion fruit extract improves sperm quality and enhances reproductive hormone balance in male rats. Furthermore, its antioxidant content can protect germ cells from oxidative stress, a key factor influencing the sex ratio of offspring. Increased sperm quality may contribute to achieving the desired sex ratio in animal reproductive programs. Thus, passion fruit extract holds promise as a natural agent for modulating reproductive function and sex ratio in animals.

Research on sex ratio modulation has previously been conducted by Dosom et al. (2023) in a study titled “Sex Ratio in Rat Offspring Resulting from Bee Pollen Administration.” This research investigated the effect of bee pollen on the sex ratio of mice and determined effective dosages that produced significant results. The study reported that bee pollen administration increased the proportion of male offspring at a dose of 22 mg/day. Based on this background, the authors intend to conduct a study on “The Effect of Passion Fruit Extract Administration on the Sex Ratio of Mice.” Passion fruit contains sodium, potassium, and the antioxidant chrysin—components also found in bee pollen—which have been proven to significantly influence the sex ratio in mice.

## **Materials and Methods**

### **Ethical Clearance for Experimental Animals**

All animal procedures were approved by the Animal Ethics Committee of the Hasanuddin University Veterinary Teaching Hospital, Makassar, Indonesia, under Ethical Clearance Certificate No. 028/UN4.1.RSHUH/B/PP36/2025. All protocols complied with institutional and international standards for the humane treatment of laboratory animals.

### **Research Subjects**

The population in this study consisted of male and female mice maintained at the Animal Laboratory of the Hasanuddin University Veterinary Teaching Hospital. The test animals were obtained from a mouse breeding facility near Makassar, with male mice weighing 20–40 grams and female mice weighing 25–45 grams. Samples were drawn from the population of male mice using the Simple Random Sampling method. A total of 6 mice were used for each treatment group, resulting in 24 male mice. Additionally, female mice were placed with the males, with two females introduced into each male mouse cage to prevent infertility issues or the possibility that females were not in estrus (Dosom et al., 2023). Thus, the total number of animals used in this study was 72 mice, consisting of 48 females and 24 males.

## Study Design

This study was a quantitative experimental design aimed at determining the sex ratio of mice following the administration of passion fruit extract and to identify the dose that could influence the resulting sex ratio. The sampling technique used was purposive sampling, which selects subjects based on predetermined criteria.

## Research Variables

Independent variables: dosage of passion fruit extract and sex of offspring. Controlled variables: mouse strain, age, and body weight. Dependent variable: sex ratio of newborn mice.

## Data Collection Methods

**Preparation of Passion Fruit Extract** The extract was prepared from *Passiflora edulis L.* sourced from Malino, Gowa Regency, South Sulawesi. A total of 5 kg of passion fruit was sun-dried for 3 days, followed by oven drying for 2 additional days until completely dry. The dried material was ground using a chopper into fine granules. The resulting simplicia was placed into a glass container and macerated with 750 mL of 70% ethanol. Maceration was conducted for 3 days in a closed, light-protected environment, with stirring every 24 hours. The mixture was then filtered, and the residue was remacerated with another 750 mL of 70% ethanol. On the third day, a final filtration was conducted using 500 mL of 70% ethanol, resulting in a clearer extract solution. The combined filtrate was concentrated using a rotary evaporator for 2 days to remove the ethanol solvent, yielding a thick extract. The extract was then freeze-dried to obtain a more solid extract powder.

## Experimental Treatment

Before treatment, mice underwent a 7-day acclimatization period during which they received uniform feed and housing conditions. After acclimatization, mice were assigned to four groups:

Control group (K): received no passion fruit extract

Treatment 1 (P1): 100 mg/day passion fruit extract

Treatment 2 (P2): 200 mg/day passion fruit extract

Treatment 3 (P3): 400 mg/day passion fruit extract

The extract was administered to male mice for 28 days. After 14 days of extract administration, two female mice were placed into each male mouse cage for mating. Observation continued until day 28 to determine pregnancy status in females. Pregnancy was identified by changes in abdominal coloration and enlargement. Once pregnancy was confirmed, males and females were separated. Pregnant females were maintained throughout the gestation period (19–20 days) until parturition (Ihwan et al., 2020). Newborn fetuses were observed until their sex could be identified. The gestation length in mice varies slightly between strains, ranging from 451 to 493 hours (approximately 18.8–20.5 days), with minimal variation within a strain.

## Determination of Offspring Sex

Sex identification of mouse pups at gestational age 16.5–18.0 days was performed by examining external genital morphology, focusing on dimorphic characteristics. The most accurate feature is the presence of the urethral seam in males or the proximal urethral meatus in females at the base of the genital tubercle. Males exhibit a raised longitudinal urethral seam, whereas females possess a small opening without a raised structure. Male genitalia also show more prominent preputial swellings, giving a narrower base and a more elongated polygonal shape, while females display a wider, more symmetrical genital base. A dark spot associated with the urethral plate may also be visible in females

until approximately GD 17.5, although this feature alone is less reliable. By combining multiple morphological markers, sex identification accuracy approaches nearly 100% during this gestational window (Murdaugh et al., 2018).

### Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) with IBM SPSS Version 26 at a 95% confidence level ( $\alpha = 0.05$ ) to determine significant differences. SPSS was specifically used to assess whether there were statistically significant differences between male and female offspring ratios among treatment groups.

Treatment (Mean±SD)	Male (Mean±SD)	Female (Mean±SD)	Sex Ratio Male/Female
K	8,25 ± 3,86 <sup>a</sup>	8 ± 1,4 <sup>a</sup>	1,03 ± 0,43 <sup>a</sup>
P1	6,25 ± 3,94 <sup>a</sup>	4,75 ± 2,5 <sup>a</sup>	1,44 ± 0,73 <sup>a</sup>
P2	7,75 ± 3,3 <sup>a</sup>	6 ± 3,16 <sup>a</sup>	1,56 ± 0,97 <sup>a</sup>
P3	8 ± 1,82 <sup>a</sup>	7,5 ± 2,6 <sup>a</sup>	1,10 ± 0,19 <sup>a</sup>

## Results and Discussion

### Result

This study was conducted to evaluate the effect of passion fruit extract on the sex ratio of mice. The data obtained were based on observations of the number of male and female offspring born to the mother mice after treatment. In addition, an analysis was performed to assess the tendency of sex ratio shifts across the different treatment doses administered. Throughout the study period, all test animals remained in good health, with no signs of stress or mortality that could influence the results. Copulation and pregnancy progressed normally in all treatment groups. After the gestation period was completed, the number of male and female pups was recorded to determine the sex ratio within each treatment group.

The observations are presented in tabular form and described to illustrate the tendency of passion fruit extract dosage to influence the increase in the proportion of male offspring compared with female offspring.

**Table 1.** Sex Ratio of Mice.

The table shows that the male-to-female ratio was calculated by comparing the number of male and female offspring in each treatment group. A ratio value of 1.0 was used as a reference point, indicating a balanced condition in which the numbers of male and female pups are relatively equal. The results demonstrated that the positive control group (K+) had a ratio of  $1.03 \pm 0.43$ , indicating that the numbers of males and females were nearly balanced. Groups P1 ( $1.44 \pm 0.73$ ) and P2 ( $1.56 \pm 0.97$ ) exhibited a tendency toward producing more males than females, with P2 showing the highest ratio. Meanwhile, group P3 ( $1.10 \pm 0.19$ ) returned to a condition close to balance. Although variation in ratio values was observed among the treatment groups, the statistical analysis indicated that all groups shared the same superscript, meaning that differences in the male-to-female ratios among treatments were not significant ( $P > 0.05$ ). Therefore, the administration of passion fruit extract in this study did not have a significant effect on the offspring sex ratio, and the observed fluctuations likely reflect natural biological variation rather than treatment influence).

## Discussion

### Effect of the Antioxidant Chrysin on Sex Ratio

Passion fruit extract contains chrysin, a flavonoid with strong antioxidant activity. Chrysin is known to protect sperm cells from free radical-induced damage and improve testicular function by enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Ahmad et al., 2021). The results of this study showed an increase in the number of male offspring at doses P1 (100 mg/kg) and P2 (200 mg/kg), which is presumed to be related to the role of chrysin in improving the viability of Y-bearing sperm, which are more susceptible to oxidative damage. Enhanced survival of Y-sperm increases the likelihood of successful fertilization by these sperm. These findings are consistent with the study by Dosom et al. (2023), which used bee pollen as a source of natural antioxidants and reported a significant increase in the proportion of male offspring. The mechanism reported also involved improved motility and viability of Y-bearing sperm due to protection from oxidative stress. The present findings support the theory that oxidative balance in the male reproductive system plays an essential role in determining the fertilization efficiency of Y-sperm.

A study by Dwiastuti et al. (2021) further explained that flavonoid supplementation can increase testosterone levels and enhance spermatogenic enzyme activity in male mice. Optimal testosterone levels are crucial for the production of high-quality sperm, thereby increasing the probability of fertilization by Y-sperm. However, at excessively high doses, such as in the P3 group, antioxidant effects may shift toward pro-oxidant activity, disrupting hormonal balance and reducing sperm quality. Kokosar et al. (2015) reported that at higher doses (400 mg/kg BW), the sex ratio tends to return to equilibrium. This phenomenon may be attributed to the physiological effects of high-dose flavonoid intake, which can suppress luteinizing hormone (LH) and testosterone secretion through negative feedback mechanisms. Reduced hormone levels may interfere with spermatogenesis and decrease sperm quality, including that of Y-sperm. Thus, although chrysin functions as an antioxidant, high doses can exert the opposite effect on reproductive performance.

### Effect of Testosterone on Sex Determination in Male Mice

In male mice, testosterone acts as a mediator of masculinization, not as a determinant of primary sex. Genetic sex determination begins with the expression of the SRY (Sex-determining Region Y) gene on the Y chromosome, which induces testis formation. The expression of the SRY gene begins at 10.5 days post-conception (dpc), reaches its peak at 11.5 dpc, then declines sharply at 12.5 dpc (Wilhelm & Koopman, 2012). After the SRY gene is expressed, a molecular cascade occurs that leads to Sertoli cell differentiation and testis formation. Differentiated Sertoli cells secrete the morphogen DHH (Desert Hedgehog), which induces Leydig cell specification. These Leydig cells then synthesize testosterone using four steroidogenic enzymes, namely P450 side chain cleavage (SCC), 3- $\beta$ -hydroxysteroid dehydrogenase type 2 (HSD3B2), cytochrome P450 17-hydroxylase (CYP17), and 17 $\beta$ -hydroxysteroid dehydrogenase 3 (17 $\beta$ HSD3) (Wilhelm & Koopman, 2012).

Fetal Leydig cells in mice do not require LH (luteinizing hormone) to stimulate androgen production and are capable of producing sufficient androgen levels without LH stimulation to induce masculinization of the male fetus (Wilhelm & Koopman, 2012). The testosterone produced then mediates the process of masculinization of the developing embryo,

including the differentiation of the Wolffian duct into the epididymis, vas deferens, and seminal vesicles (Hatkevich & Wilhelm, 2024). A recent study by Hurtado et al. (2024) showed that the timing of Sry expression is very crucial in determining gonadal fate. Deletion of the microRNA cluster miR-17~92 induces complete male-to-female sex reversal in XY mice due to delayed SRY expression. This confirms that although testosterone is important for masculinization, testosterone production depends on the genetic cascade initiated by SRY, and disturbances in SRY expression timing can prevent testis differentiation and adequate testosterone production.

### **Comparison with Previous Studies**

The results of this study share similarities with the study by Dosom et al. (2023), which examined the effect of bee pollen administration on the sex ratio of rat offspring. The study showed an increase in the male ratio of 1.47 in the treatment group compared to the control, although the result was not statistically significant. Bee pollen contains flavonoids such as quercetin and kaempferol, which have antioxidant mechanisms similar to chrysin, namely protecting sperm from oxidative stress. Thus, in both bee pollen and passion fruit extract, antioxidants play an important role in increasing the survival of Y-sperm. A study by Rahman et al. (2022) also mentioned that administration of flavonoids from citrus peel extract can increase sperm motility and improve the male birth ratio in mice. These results strengthen the hypothesis that flavonoids have an important role in maintaining redox balance in sperm, which can ultimately influence the sex of offspring.

The study by Ahmad et al. (2021) showed that chrysin at moderate doses (50–100 mg/kg BW) is able to improve sperm quality and testosterone levels in male rats. This effect is related to increased activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which fight free radicals in testicular tissue. However, administration of high doses of chrysin causes the opposite effect, namely a decrease in the number of normal sperm due to disturbances in hormonal regulation. These results are in line with this study, in which moderate doses showed a tendency to increase the male ratio, whereas high doses did not produce positive effects. A study by Musa et al. (2020) also reported that supplementation with antioxidant vitamins E and C can improve sperm quality and increase Y-sperm viability in male mice. However, increased Y-sperm viability does not always correspond directly to an increase in the male offspring ratio produced. This shows that the sex of offspring is influenced not only by sperm quality, but also by female internal factors such as reproductive tract pH, ovulation timing, and uterine physiological conditions at fertilization. Thus, although the results of this study did not show statistically significant differences, the directional tendency of the results supports the theory that chrysin, as an antioxidant flavonoid, may contribute to increasing the likelihood of male offspring.

Overall, administration of natural antioxidants such as chrysin can improve the oxidative condition of the male reproductive system and increase the chances of successful fertilization by Y-sperm. However, these effects greatly depend on dose, timing of administration, and interactions with hormonal factors and the internal environment of the female. Therefore, further research with larger sample sizes, longer treatment durations, and more controlled dose arrangements is needed to strengthen and clarify the influence of chrysin on sex ratio.

## Conclusion

The administration of passion fruit (*Passiflora edulis*) extract in mice showed a tendency to increase the ratio of male offspring, especially at the dose of 200 mg/kg BW; however, the results were not statistically significant ( $P>0.05$ ). The antioxidant chrysin contained in passion fruit plays a role in protecting sperm from oxidative stress and may increase the chances of fertilization by Y-bearing sperm. The dose that produced the most optimal response in this study was 200 mg/kg BW, whereas the high dose (400 mg/kg BW) did not provide a positive effect on the sex ratio.

## Suggestions

Further research should be conducted with a larger sample size and a longer treatment period to obtain statistically significant results. Analysis of testosterone hormone levels and antioxidant enzyme activity such as SOD and GPx needs to be carried out to clarify the mechanism of action of chrysin in influencing sex ratio. Female physiological factors such as reproductive tract pH and ovulation timing should be controlled so that the results of the study are more representative. The dose of 200 mg/kg BW can be used as an initial reference in future studies investigating the effect of passion fruit extract on the reproductive system.

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