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Effect of White Ginger Rhizome Extract (*Zingiber officinale* var. amarum) on Pregnancy in Mice (*Mus musculus* L.) Early Post-implantation Stage

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Abstract: White ginger (Zingiber officinale var. Amarum) contains compounds in the antifertility group, namely flavonoids, terpenoids, and essential oils (gingerol and shogaol). Flavonoids can interfere with zygote division and implantation, while terpenoids and essential oils disrupt sperm transport and cause sperm to clot in male animals. This study aims to determine the effect of white ginger (Zingiber officinale var. Amarum) rhizome extract on the mouse (Mus musculus L.) early post-implantation stage. This research type is experimental and structured using a Completely Randomized Design (CRD) pattern. A total of 16 female mice weighing 20-30 g aged 2-3 months were divided into four treatments, namely K1 (control), K2 (0.7 mg/g BW), K3 (1.4 mg/g BW), and K4 (2.8 mg/g BW) white ginger rhizome extract. The extract is given orally at 0.5 mL/head/day on the 4th to 8th day of pregnancy. The mother's body weight is weighed every three days. On the 18th day of pregnancy, mice were sacrificed using chloroform and dissected to observe early post-implantation pregnancy parameters. Data were analyzed using Analysis of Variance (ANOVA) and Duncan's follow-up test with a confidence level of 95%. The research showed that white ginger rhizome extract significantly reduced the percentage of implantation (IM) and the percentage of live fetuses (FH), causing fetal growth to be hampered. The rate of implantation (IM) is K1 (1.8%), K2 (0.52%), K3 (0.49%). The percentage of live fetuses (FH) was K1 (1.06%), K2 (0.48%), and K3 (0.30%), while no fetus was found in K4. K3 treatment (1.4mg/g) causes fetal growth to be hampered. White ginger rhizome extract, on average, increases post-implantation mortality (KPI) and percentage of embryo resorption (ER) and reduces fetal weight. Based on the results of this study, it is concluded that white ginger extract has antifertility properties in female animals because it causes pregnancy disorders, so it is hoped that the use of white ginger in early pregnancy will be reduced.

Keywords: Early Post-Implantation; *Mus musculus*; Pregnancy; *Zingiber officinale*.

Introduction

Research to reduce fertility can be conducted on female rats because their reproductive tracts have many areas that can be disturbed, and their reproductive cycles are more easily disturbed or manipulated.[1]. The development of medicinal plants and spices in Indonesia is receiving more and more attention from the government and agricultural practitioners. This is mainly driven by increasing public awareness of using herbal resources, so herbal products are increasingly growing [2]. The government, in this case, the Department of Health, also supports the development of traditional medicine in Indonesia, especially in anticipation of high drug prices [4]. Efforts to treat conventional medicines are a form of community participation and are, at the same time, appropriate technology that has the potential to support health development. This is due, among other things, to the fact that the community has used traditional medicine for a long time, and its ingredients are widely available in all corners of the country [2]. Conventional medicine has relatively few side effects if used correctly. This accuracy includes the correctness of ingredients, the accuracy of dosage, the accuracy of time of use, the accuracy of method of use, and the accuracy in translating information about the use of traditional medicines [3].

Traditional medicine has relatively few side effects if used correctly. This accuracy includes the correctness of the ingredients, the correct dosage, the correct time of use, the correct method of use, and the accuracy in translating information about the use of traditional medicines [4]. White ginger is a widely used conventional medicinal ingredient (Zingiber officinale var. Amarum). The content of secondary metabolite compounds found in ginger plants mainly consists of flavonoids, phenolics, terpenoids, and essential oils. The essential oil in ginger combines terpenoid compounds consisting of zingiberene, bisabolene, cineole, citral, zingiber, and geranial [5]. Ginger's ability as a natural antioxidant cannot be separated from the level of total phenolic components

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contained therein. Gingerol and school have been identified as phenolic antioxidant components of ginger. Ginger's essential oils and phenolic compounds have been identified as compounds capable of preventing cell damage [6].

White ginger is a medicinal plant in the form of a pseudo-stemmed clump. White ginger is widely used as an antioxidant and a cooking spice to warm the body. Based on the rhizome's shape, color, and size, there are three known types of ginger: large white ginger, small white ginger, and red ginger. The essential oil content of red ginger ranges from 2.58-3.72%, while the oleoresin content can reach 3%. The main constituents of ginger oleoresin are flavonoid derivative compounds such as gingerol and school [4]. Red ginger (Zingiber officinale Var. rubrum) is an anti-vomiting agent in pregnancy. Even though red ginger has side effects, pregnant women are not recommended to consume high doses of red ginger because it can increase the risk of miscarriage [7].

The white ginger rhizome contains two main parts: essential oil, which carries the aroma, and oleoresin (gingerol and shogaol), which has been identified as ginger's flavonoid component, giving it a spicy taste [8]. Oleoresin provides an intense aroma ranging from 4-7% and has excellent potential as an antioxidant. Terpenoids and essential oils can act as antifertility in male animals; that is, they can prevent the sperm transportation process, while essential oils can coagulate sperm, thereby reducing sperm motility and viability, causing sperm cells not to reach the egg and fertilization can be prevented [9]. The initial post-implantation stage is the pregnancy stage, where organogenesis occurs, namely the stage of formation of body organs. This stage is susceptible to materials or substances that induce morphological abnormalities. The advanced post-implantation stage is where organogenesis is actively taking place so that if a toxic effect occurs at this stage, the embryo's growth can be disrupted. The organogenesis stage is a stage that is susceptible to toxic or teratogenic effects. Most mouse embryos become susceptible on day eight and end on day 12 of pregnancy.

White ginger rhizome has the potential to be used as an antifertility agent because it contains bioactive compounds in the form of alkaloids, tannins, flavonoids, terpenoids, phenols, saponins, and steroids. This research was carried out at the early post-implantation stage. The early post-implantation stage is when the embryo is implanted in the endometrial wall of the uterus; at this stage, the embryo undergoes differentiation, mobilization, and organogenesis. The post-implantation stage is susceptible to the effects of active substances, such as alkaloids, flavonoids, and saponins [10]. Giving red ginger extract to mice (Mus musculus L.) caused embryo resorption, which caused the embryos to fail to develop [11]. Red ginger causes abortion and fetal mutation and increases the risk of bleeding in pregnant rats (Rattus rattus) [11]. The results of this study are expected to provide information on the effects of white ginger as an antifertility ingredient with doses that can interfere with pregnancy and its impact on the fetus so that it can be considered in its use.

Research Methods

This research was carried out in March-April 2021 at the Biology Laboratory of the Zoology Unit and continued in the mouse cage house, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari.

Preparation of test animals. The mice's body weight was weighed and labeled according to treatment and acclimated for seven days. During acclimation and therapy, mice were given food and drink ad libitum and fasted for one day before treatment so that physiological conditions were the same. The mice used in this study were 2-3 month-old mice with an average body weight of 20-30 grams and had a regular estrus cycle ranging from 4 to 5 days. In the afternoon, at around 16.00-17.00 WITA, the mice were mated, then at 07.00-08.00 WITA in the morning, a vaginal examination was carried out. The presence of sperm in a vaginal smear indicates that copulation has occurred and is declared as day 0 of pregnancy [13].

Extraction. Making ginger rhizome extract is done by cleaning the ginger rhizome, slicing it thinly, and then drying it. After drying, the ginger was blended and weighed 100 g, soaked in 500 ml of 70% ethanol. The ginger rhizomes were left for 48 hours, then filtered, and then evaporated using a rotary evaporator until no more ethanol dripped and a concentrated extract was obtained in solid form [14].

Determination of dosage. A preparation or substance is said to be toxic if it causes death at a dose of 5000 mg/kg bw. This study used varying doses and did not exceed the lethal dose, namely 100 mg/g BW. The doses used in this study were 35 mg/kg BW, 70 mg/kg BW and 140 mg/kg BW. Determination of the dose in mice with a body weight of ± 20 grams given a suspension dose of 0.5 ml using the formula: mouse body weight x treatment dose. A dose of 35 mg/kg BW is equivalent to 0.7 mg/g BW, 70 mg/kg BW is equivalent to 1.4 mg/g BW, and 140 mg/kg BW is equivalent to 2.8 mg/g BW. White ginger extract was administered using a syringe orally with a volume dose of 0.5 ml per animal. Administration is carried out on the 6th to the 9th day of pregnancy. Every three days, the parent's body weight was weighed. At 18 days of gestation, the treated mice were anesthetized using chloroform and underwent surgery [10].

Observation. After dissecting the mice, both ovaries were removed and placed in a 0.9% NaCl solution. The uterus is removed from the mother's body and then cut on the side opposite the implantation site. The amniotic sacs were opened individually, and observations were made on both uterine horns regarding live and dead fetuses [15]. The number of fetal implantations can be calculated by looking at the fetuses that have successfully implanted in the uterine endometrium, both live and dead fetuses. A fetus that is fully developed and responds to touch is

categorized as a living fetus, while a fetus that is fully developed but does not respond to touch is categorized as a dead fetus. Then, malformations were observed in live fetuses and dead fetuses. Malformations characterize physical defects in the fetus.

The corpus luteum is observed separately by removing the bursa covering the ovary using tweezers and scissors. Then, the corpus luteum was counted, and the protrusion of the corpus luteum in the ovaries was observed using a stereo microscope [15]. The data obtained were then calculated for the parameters per mouse parent using the formula [16] as follows:

1. Percentage of Post-Implantation Deaths
$$\%KPI = \frac{\text{Number of implantations} - \text{number of live fetuses}}{\text{Number of implantations}} \times 100\ \%$$
2. Implantation Percentage
$$\%IM = \frac{\text{Number of implantations}}{\text{Number of Corpus luteum}} \times 100\ \%$$
3. Percentage of Live Fetus
$$\%FH = \frac{\text{Number of live fetuses}}{\text{Number of implantations}} \times 100\ \%$$
4. Percentage of Dead Fetuses
$$\%FM = \frac{\text{Number of dead fetuses}}{\text{Number of implantations}} \times 100\ \%$$
5. Percentage of Embryo Resorption
$$\%ER = \frac{\text{Number of resorption embryos}}{\text{Number of implantations}} \times 100\ \%$$

Post-Implantation Mortality Percentage (PMR)

Data analysis

Data were analyzed using Analysis of Variance (ANOVA) and Duncan's advanced test with a confidence level of 95% [17].

Results and Discussion

Early post-implantation pregnancy is the pregnancy of mice after the embryo attaches to the uterine endometrium wall, which occurs on the 6th-9th day of pregnancy. The parameters observed were the percentage of post-implantation mortality (KPI), the percentage of embryo resorption, the percentage of dead fetuses, the percentage of live fetuses, and fetal malformations. The mother's body and fetal weight were used as additional materials to strengthen the data obtained.

Table 1. Percentage of Post-Implantation Mortality (PMR), Embryo Resorption (ER), and Fetus After Administration of White Ginger Rhizome Extract in the Early Post-Implantation Period

Treatment	Average Early Postimplantation Period				
	IM (%)	KPI (%)	ER (%)	FM (%)	FH (%)
K	100 ± 0	0 ± 0[a]	0 ± 0	0 ± 0[a]	100 ± 0[d]
P1	97.20 ± 4.84	26.10 ± 6.71[b]	0 ± 0	26.10 ± 6.71[b]	71.06 ± 7.73[c]
P2	92.30 ± 7.70	37.76 ± 4.18[b]	2.76 ± 4.79	35 ± 2.94[c]	56.80 ± 2.59[b]
P3	90.90 ±1.85	57.13± 10.33[c]	10.13 ± 11.16	46.83 ± 2.87[d]	33.86 ± 11.96[a]

Note: Different letters indicate significant differences in the BNT test at a 95% confidence level

Table 1 shows that the average percentage of implantation in mice treated with P3 was lower, namely 90.90%, compared to those treated with K, P1, and P2. Giving white ginger rhizome extract affects early post-implantation pregnancy. The decrease in implantation percentage is caused by the content of active compounds such as flavonoids, terpenoids, and essential oils contained in white ginger rhizome extract, which interferes with the proliferation of embryonal cells, which occurs at the cleavage stage of embryogenesis. This means that at this stage, the new blastomeres will be implanted in the endometrial wall of the uterus, thereby disrupting the implantation process. This is to research [18] that the cause of the decrease in the implantation percentage value in mice is due to disruption of the growth of the inner cells in the blastocyst wall, which form embryo cells, and the outer cells, which are disturbed in the uterine wall which form the placenta. Similar research was also mentioned by [10] that the triterpenoid and flavonoid compounds in the leaf extract of L. camara interfered with the proliferative process of embryonic cells at the cleavage stage of embryogenesis, so they did not reach the blastocyst stage entirely, and as a result, the embryo could not be implanted.

The average percentage of post-implantation deaths from mice and those treated with white ginger rhizome extract at gestation age 6 to 9 days can be seen in Table 1, showing that in mice in the P3 group, it was higher, namely 57.13% compared to treatments K, P1 and P2. Disruption of the growth of each embryonic cell will weaken the ability of the embryonic and placental cells, making it easier for teratogenic substances to enter the mouse's mother's body. This is stated by the statement [10], which states that in mice, blastomeres will be implanted on

day 4 of pregnancy and end on day 6 of pregnancy. The table above shows that the percentage of post-implantation deaths given white ginger rhizome extract at various doses increased the rate of post-implantation deaths. It can be seen that the higher the dose, the higher the percentage of post-implantation deaths. This is by the statement [19] that foreign substances enter mammalian embryos through the placenta. Chemical and physical agents with small molecular weight can enter the embryo quickly across the placenta. These foreign substances can affect the fetus so that a fetus that is not yet fully developed cannot metabolize the foreign substance properly, resulting in pregnancy problems[10]. In general, the higher the concentration of a formulation, the higher the active ingredients it contains[20].

The average percentage of embryo resorption, as shown in Table 1, shows that white ginger rhizome extract influences the average percentage of embryo resorption. This can be seen in the P2 and P3 treatments, where the average rate of resorbed embryos was 2.70% and 10.13%, respectively, whereas in the K and P1 treatments, no resorbed embryos were found. The resorption embryos found in this study were red lumps embedded in the uterus during organogenesis. This is the most sensitive period for foreign compounds entering the mouse mother. In the organogenesis period, differentiation occurs, growth and formation of organs. This is stated by the statement [21] stating that there is no longer totipotency during this organogenesis period, so tissue damage is not repaired, and further development does not occur. As a result of this, the fetus dies, and red lumps form. The discovery of fetuses experiencing late growth, death, and resorption due to cell disorders. Another statement concluded by [10] states that other factors that cause the fetus to survive in the womb until delivery are hormonal balance, availability of nutrients, temperature, and the mother's body metabolism.

Table 1 shows that the average percentage of dead fetuses in the P3 group was higher, namely 46.83%, when compared with treatments K, P1, and P2. The average rate of fetuses killed increased along with increasing doses of the extract used. The increase in dead fetuses is caused by secondary metabolite compounds contained in white ginger rhizome extract, thereby disrupting fetal development. Based on Table 1, the average percentage of live fetuses for mice in treatment P3 was lower, namely 33.86%, when compared with treatments K, P1, and P2. The percentage of live fetuses is due to increased dead fetuses and resorbed embryos.

The decrease in the percentage of live fetuses is caused by white ginger rhizome extract given during the organogenesis period. At this stage, the embryo undergoes growth differentiation to form organs so that the embryo is more susceptible to teratogenic compounds such as alkaloid compounds. The alkaloid compounds in ginger rhizome extract can affect fetal development, causing the fetus to experience a decline in life. This is based on the statement [22] that alkaloids can cause a significant increase in preimplantation losses, the number of implantations, and the number of live fetuses, which decrease significantly. Another study conducted [19] stated that the cause of the low life expectancy of live fetuses was that mice who were given teratogenic substances experienced impaired cell growth in the mother's body. This is to what was stated by [10] that alkaloid compounds can interfere with cell replication and inhibit mitosis at the metaphase stage by inhibiting the formation of mitotic spindle threads, resulting in chromosomes breaking, spreading, or clumping and causing cell death, as a result of which fetal development is inhibited and eventually becomes stunted.

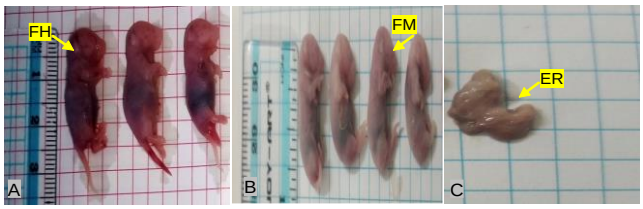

Figure 1. Appearance of a mouse fetus after administration of white ginger rhizome extract. (A) A live fetus (FH) that is bright red and has a beating heart and blood vessels after being removed from the amniotic sac. (B) A dead fetus (FM) that looks pale and does not react to touch. (C) A resorption embryo (ER) is the remaining embryonic tissue undergoing maceration.

Parent Body Weight

Supporting data in this study measured the mother's body weight, which experienced changes during white ginger rhizome extract administration. The average body weight of the parents between treatments is presented in Table 2 below.

Table 2. Changes in Body Weight of Mice (Mus musculus L.) Between Treatments of White Ginger Rhizome Extract

Treatment	Average ± SD
K	18 ± 3^{a}
P1	17.66 ± 1.52^{b}
P2	15.66 ± 0.57^{b}
P3	11 ± 2^{b}

Note: Different letters indicate significant differences in the BNT test at a 95% confidence level

Based on the ANOVA test and Duncan's advanced test (α= 0.05), it showed a significant value for the parent's body weight. Table 2 shows that the average change in maternal weight from the beginning to the end of pregnancy in treatment P3 decreased to 11 g compared to treatments K, PI, and P2. The average weight loss of the parents was caused by the active tag compounds contained in the white ginger rhizome extract in the form of terpenoid, tannin, and flavonoid compounds. This is to the statement [23] that terpenoid compounds can cause a bitter taste, thereby reducing the appetite of pregnant mice. In contrast, tannin compounds can precipitate protein on the surface of the small intestine because they easily bind to

protein, thereby reducing food absorption so that the obesity process can be inhibited.

Fetal Weight

Table 3. Changes in Fetal Body Weight between White Ginger Rhizome Extract treatments

Treatment	Average ± SD
K	1.43 ± 0.02^{d}
P1	1.18 ± 0.04^{c}
P2	0.93 ± 0.05^{b}
P3	0.75 ± 0.01^{a}

Note: Different letters indicate significant differences in the BNT test at a 95% confidence level

Based on the ANOVA test and Duncan's test (α= 0.05) show that there is a significant difference between the control and white ginger rhizome extract treatments. Table 3 shows that the higher the extract dose, the higher the average reduction in fetal weight. The average fetal body weight in treatment P3 was 0.75 lower than in treatments K, P1, and P2. This is caused by the high levels of compounds from white ginger rhizome extract, which results in the fetus experiencing developmental delays. The white ginger extract also causes fetal malformations in mice. The following are the forms of mouse fetal malformations found when treated with white ginger rhizome extract.

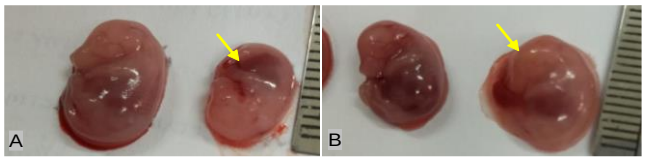

Figure 2. Fetal malformations after administration of white ginger rhizome extract. A stunted fetus; B. fetal haemorrhage

The results of the observations showed that malformations were indicated by the body size of the fetus being smaller compared to controls, and hemorrhagic fetuses were found in the head in the P3 dose group (2.8 mg/g BW); this was caused by the influence of white ginger rhizome extract given during the organogenesis period. Thereby causing delays in fetal development. This organogenesis phase is the most sensitive phase for the occurrence of specific physical defects, so this phase is also called the teratogenic period [10]. Inhibition of fetal growth and development, which is indicated by a decrease in the weight and length of the fetus, can occur if a chemical agent affects cell proliferation and interactions or reduces the synthesis rate of nucleic acids and proteins during the embryogenesis period. Secondary metabolite compounds contained in white ginger rhizome extract can inhibit cell division. If cell division is inhibited, fetal development will be disrupted. Alkaloids are thought to inhibit cell division in osteoprogenitor cells so that osteoblast formation is disrupted, and as a result, calcium absorption by these cells is hampered [22]. Alkaloids can interfere with cell replication and inhibit mitosis (cell division) at the metaphase stage by inhibiting the formation of the mitotic spindle, causing chromosomes to break, spread, or cluster and causing cells to die.

Conclusion

This study concludes that white ginger rhizome extract (Zingiber officinale var. Amarum) has an antifertility effect on pregnancy in mice (Mus musculus L.) at the early post-implantation stage.

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