

Teratogenic Test of Jamblang Fruit Extract (Syzygium cumini) on the Fetus Mice (Mus musculus)

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Abstract

Jamblang fruit (Syzygium cumini) is used to treat diabetes mellitus, pain, inflammation, ulcers, and diarrhea, and in recent research, it has been proven to be used as a chemopreventive, radioprotective, and antineoplastic. Jamblang fruit contains several ingredients, namely: alkaloids, saponins, tannins, triterpenoids, flavonoids, polyphenols, and buchard. However, it is necessary to ensure whether jamun is safe for consumption by pregnant women, and a teratogenic test is needed. This research aims to determine the effect of jamblang fruit extract on maternal weight, number of fetuses, fetal length, fetal weight, hemorrhage, and morphological abnormalities in the fetus. This research is included in quantitative research using experimental methods with a Completely Randomized Design (CRD), which consists of 4 treatments and 5 replications. The experimental animals used were 20 female mice (Mus musculus) of the Balb/C strain, healthy mice aged 2 months, weighing 27-30 grams, and the mice were mature and ready to mate. Mice were divided into 4 treatment groups, namely the control group, the 100 mg/kgBB dose group, the 200 mg/kgBB dose group, and the 300 mg/kgBB dose group. Treatment doses of jamun fruit extract 70% ethanol are given on days 6 to 17 of pregnancy. Data analysis using SPSS showed that ethanol 70 % extract of jamblang fruit had no effect on maternal weight and fetal weight in mice (>0.05), but did affect fetal length, number of fetuses, hemorrhage, and morphological abnormalities in the form of open eyelids (p<0.05). Based on the research results, it can be concluded that administration of jamblang fruit ethanol extract can reduce fetal length and the number of fetuses. The 200 mg/kgBB and 300 mg/kgBB dose groups are doses that can cause teratogenic effects such as hemorrhage and open eyelids.

Keywords: Jamblang Fruit, Syzygium cumini, Fetus, Teratogenic, Teratogen, Hemorrhages

INTRODUCTION

Jamblang plant (*Syzygium cumini*) is classified as a species of the Myrtaceae family. People usually plant this plant around their homes so that jamblang plants are nicknamed plants that play a dual role, such as being a place of shelter and producing a lot of fruit. In addition to being a source of fruit, some studies have shown that jamblang is often used as a traditional medicine, namely a medicine for diabetes mellitus, a medicine for inflammation, a medicine for stomach diseases, and diarrhea. The use of jamblang as a medicine by some local people is only based on empirical evidence, even though there have been many studies that show the use of jamblang fruit as a medicine because of its bioactive content (Silalahi, 2018).

Based on the results of the questionnaire distributed to the entire community, especially Aceh Province in March 2023, it was found that from teenagers to pregnant women admitted to having consumed jamblang fruit. As many as 32.9% of respondents admitted to consuming jamblang because it tastes good, as many as 22.9% of respondents stated the reason for consuming jamblang fruit was because they thought it was beneficial for health, as many as 17.1% of respondents said the reason for consuming jamblang fruit was because the price was



affordable (cheap) and as many as 11.4% of respondents said that the reason for consuming jamblang fruit was because the fruit was easy to get. From the results of the questionnaire, it was also found that the public did not yet know whether the jamblang fruit was safe for pregnant women to consume.

Jamblang (Syzygium cumini) is a herbal plant or medicinal plant that has medicinal properties reported in various traditional and modern medicines. The bark, leaves, flowers, fruits, and seeds of jamblang (Syzygium cumini) are also used to treat many diseases in modern medicine. So far, Syzygium cumini is widely used to treat diabetes mellitus, pain, inflammation, ulcers, and diarrhea, and in recent studies, it has been proven that Syzygium cumini can be used as a chemopreventive, radioprotective, and antineoplastic (Vardhan et al., 2020).

Consuming jamblang fruit is good for medicinal purposes, or consumed like regular fruit because of its slightly sour and unique taste. However, it is necessary to ensure whether jamblang is safe for consumption by pregnant women. During the pregnancy process, the fetus can experience serious problems due to teratogenic effects, including the use of drugs by pregnant women. Teratogenic effects are thought to be the effects of agents that are able to cross competition and can cause serious damage to the development of the fetus or embryo, and will cause abnormalities in the fetus or the newborn. Every pregnant woman may have a condition that can be transmitted during or during pregnancy. Some maternal diseases or medications used to treat these diseases may have teratogenic effects on the fetus and interfere with fetal development (Mohammadi, 2017). Although there has been a lot of scientific literature on the benefits of jamblang plants and scientific evidence on the teratogenic effects of various types of plants, there has been no scientific information regarding the teratogenic effects caused by jamblang fruit. Therefore, it is necessary to conduct a Teratogenic Test of Jamblang Fruit Extract (*Syzygium cumini*) on mouse fetuses (*Mus musculus*).

METHOD

This study uses an experimental method with a Completely Randomized Design (CRD) with a quantitative type study, consisting of 4 treatments and 5 replications (Fardhira *et al.*, 2022). The experimental animals used were female mice (*Mus musculus*) Balb/C strain, as many as 20 healthy female mice, 2 months old, weighing 27-30 grams, and mice Already Ready for mating (adult sex) (Duppa et al., 2022). Mice were divided into 4 treatment groups with a control group (P0), the first group (P1) ethanol extract of jamblang fruit at a dose of 200 mg/kgBW, and the third group (P3) ethanol extract of jamblang fruit at a dose of 300 mg/kgBW.



Administration treatment as much as 0.3 ml according to the given dose, administration is carried out on the 6th to 17th day of pregnancy (Sari, 2021).

Rare first conducted in research. This is taking Jamblang fruit samples obtained from the Aceh Besar area, namely from trees around Jalan Lamreh, District Grand Mosque, Aceh Besar District. Samples taken is old fruit are reddish black in color, and the fruit is washed clean with running water for 5 minutes. The fruit flesh is sorted from good and fresh fruit. The fruit flesh is softened by blending. A total of 1 kg of fruit is added with 1 liter of 70% ethanol into a maceration container, then covered and stored for 3x24 hours to protect it from light while stirring occasionally (Mapanao *et al.*, 2017). Stirring is done every 1x24 hours. The results of the maceration are filtered (filtered) using filter paper, and the filtrate is evaporated using a *rotary vacuum evaporator* using a temperature of 60°C °C until a thick solution is obtained (Dewi, 2021).

Next is the extract of Jamblang fruit was given to the mother mice using a tube (orally) using a gastric tube from the 6th to the 17th day of pregnancy (Suciyati *et al.*, 2020). Administration of extract jamblang fruit, namely orally, during the organogenesis period. The test animals were separated into 4 treatment groups with 5 replications. The control group that was not given treatment (P0) and 3 other groups were given a modified dose of jamblang fruit extract (*Syzygium cumini*) from Sari (2021), because guava belongs to the same family as jamblang fruit, namely the Myrtaceae family. The dose of jamblang fruit extract (*Syzygium cumini*) given was 100 mg/kgBW (P1), 200 mg/kgBW (P2), and 300 mg/kgBW (P3). Administration extract ethanol fruit jamblang given every day orally using a gastric tube as much as 0.3 ml for every mice in the morning, after weighing the parent body weight mice.

After pregnancy day 18 and done surgery so done measurements of parameters in the form of heavy parent mouse before and after surgery, including the number of living and dead fetuses, the length of the fetus, and the weight of the fetus. And also carried out observation in the form of hemorrhage and abnormalities The morphology of the fetus in the form of mouth, nose, eyes, tail, skin, earlobes, and number of front and back legs and it is seen whether the fetus is experiencing hemorrhage, the morphological parameters are then divided into two groups, namely normal and abnormal.

Statistical data analysis using ANOVA (Analysis of variance) test using statistical software social science package (IBM SPSS) for Windows, Version 22 (IBM CorpArmonk, NY, USA). Then the results are considered insignificant if P > 0.05. The significance of the data obtained is classified into two categories, namely p < 0.05 according to the p value obtained (Atallah *et al.*, 2021). Then, if the results have a significant effect, a further statistical test is



carried out, namely the Duncan Test, to see which groups are different from each other (Sianturi et al., 2020).

RESULTS AND DISCUSSION

Extraction results in fruit jamblang as much as 1000 g of fruit fresh jamblang is obtained, heavy extract thick as much as 200 g, which can be seen on Table 1.

Table 1. Yield Results Extract Fruit Jamblang

Sample	Results		
Sample	Fruit weight	Extract weight	Yield
Extract fruit jamblang	1000 g	200 g	20%

Based on Table 1, it is known that the yield produced is 20% and the resulting jamblang fruit extract has a dark purple or black color and has a distinctive odor. Yield results a sample need to be counted because for know the amount of extract obtained during the extraction process. Result data yield is also available the relation to the compound active from A sample, if amount the resulting yield, the more Lots so compound active that are contained in an extract, also increasingly many (Hasnaeni et al., 2019). Based on the results data yield, a large 20% extract yield of fruit jamblang already fulfills conditions, where the condition yields an extract thick according to Indonesian Pharmacopoeia (2017), namely mark the yield is not marked as not enough from 10%.

Influence extract fruit jamblang (*Syzygium cumini*) against mice, after performing surgery on day 18 of pregnancy, obtained data results in Table 2

Table 2. Average Body Weight of Parent Mouse

Treatment Group	Average Weight Gain Parent ± SD	P Value
Control group (P0)	36.68 ± 5.18 a	
Dose 100 mg/kgBW (P1)	34.90 ± 4.04 a	0.21
Dose 200 mg/kgBW (P2)	34.80 ± 3.54 a	
Dose 300 mg/kgBW (P 3)	33.86 ± 3.49 a	

Description: N data value followed by the same letter notation indicates not significantly different in Duncan's test, p > 0.05

Based on Table 2, the results of statistical tests from *the Analysis of Variance* (ANOVA) on the body weight of the mother mice during treatment from the 6th to the 17th day of pregnancy after being given jamblang fruit extract treatment, there was no real difference. Between group control (P0), group dose 100 mg/kgBW (P1), group dose 200 mg/kgBW (P2), and group dose of 300 mg/kgBW (P3), where the p value (p>0.05, p = 0.21).



Table 3. Average 1	Number of Fetuses

Test Group	Number of Parents	Number of Fetuses (Percentage)		Mean Number of Fetuses ± SD	P- Value	
	(Tail)	Life	Dead	Resorption	of retuses ± SD	vaiue
Control (P0)	5	51 (100%)	0	0	10.2 ± 2.28 a	
100 mg/kgBW (P1)	5	39 (99 %)	1	1	7.80 ± 0.83 b	0.02
200 mg/kgBW (P2)	5	48 (100%	0	0	$9.40\pm0.54~^{ab}$	0.02
300 mg/kgBW (P3)	5	39 (100%)	0	3	$7.80\pm0.83^{\ b}$	

Description: N data value followed by the same letter notation indicates not significantly different in Duncan's test p<0.05

Based on Table 3, ANOVA (*Analysis of variance*) results on the number of mouse fetuses in each treatment, both the control group (P0), dose 100 mg/gBW (P1), dose 200 mg/kgBW), and dose 300 mg/kgBW (P3), there was a significant difference where p<0.05 (p = 0.02). Furthermore, Duncan's follow-up test was carried out to determine the most influential group, then the results were obtained in the control group (P0) and the group that given a dose of treatment 200 mg/kgBW (P2) differs across dose groups 100 mg/kgBW (P1) and a dose of 300 mg/kgBW (P3).

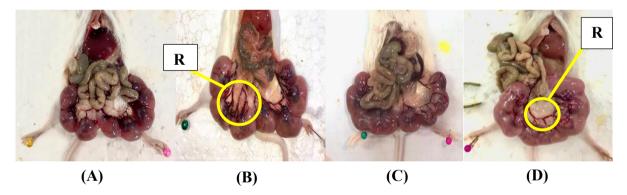


Figure 1. Fetus Image Control Group (P0); Dose 100 mg/kgBW (P1); Dose 200 mg/kgBW (P2); Dosage 200 mg/kgBW (P3)

Based on Figure 1, it is known that in the 100 mg/kgBW dose group (P1), one fetus died, and resorption was found in the uterus, namely in the group dose 100 mg/kgBW (P1), 1 fetus, and group dose 300 mg/kgBW (P3), 3 fetuses experienced resorption.



Table 4. Statistical Test Table Weight and Length of Mice Fetus

Treatment Group	Average Weight fetus	P Value	Average Length Fetal Body ± SD	P Value
Control (P0)	$1.15\pm0.36~^{\mathrm{a}}$		$2.13 \pm 0.12a$	
Dose 100 mg/kgBW (P1)	$1.00\pm0.02~^{\rm a}$	0.42	$2.10 \pm 0.10 a$	0,000
Dose 200 mg/kgBW (P2)	0.85 ± 0.14 a		$1.90 \pm 0.04b$	
Dose 300 mg/kgBW (P3)	0.82 ± 0.05 a		$1.70\pm0.08c$	

Description: N data value followed by the same letter notation indicates not significantly different in Duncan's test p<0.05.

Table 4 shows the statistical test *of Analysis of Variance* (ANOVA) on fetal weight, it can be seen that there is no difference in each group, both the control group (P0), the 100 mg/kgBB dose group (P1), the 200 mg/kgBW dose group (P2) and the 300 mg/kgBW dose group (P3). Meanwhile, after conducting the ANOVA test on fetal length, there was a significant difference between the control group (P0) and the 100 mg/kgBW dose group (P1), against the 200 mg/kgBW dose group (P2) and the 300 mg/kgBW dose group (P3) with a p <0.05 (p = 0.000). To find out which group is the most different, a Duncan test was conducted, where the results of the Duncan test showed that the length of the fetus in the control group (P0) and the 100 mg/kgBB dose group (P1) was control (P0) and group dose of 100 mg/kgBW (P1) was significantly different on the length of the fetus in the group dose 200 mg/kgBW (P 2) and group dose of 300 mg/kgBW(P3). Where the average body length of the fetus in group P 2 is 1.90 cm and P3 is 1.70 cm, where groups P 1 and P 3 have the smallest number of fetuses, namely 39 fetuses.

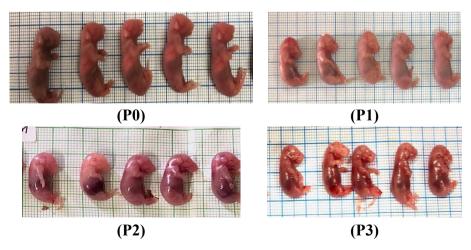


Figure 2. Fetus Image Control Group (P0); Dose 100 mg/kgBW (P1); Dose 200 mg/kgBW (P2); Dosage 200 mg/kgBW (P3)



Table 5. Results of Statistical Test of Mouse Fetal Hemorrage

Treatment	Number of parents	Average Number of Fetus Hemorrhage (tail)	P Value
Control (P0)	5	0.00 ± 0.00 a	
100mg/kgBW (P1)	5	$60~\pm1.86~^{\rm a}$	0.00
200 mg/kgBW (P2)	5	$7.20 \pm 3.11^{\ b}$	0.00
300 mg/kgBW (P3)	5	10.20 ± 1.48 b	

Description: N data value followed by the same letter notation indicates not significantly different in Duncan's test p<0.05

Table 5 shows the results of the analysis using *Analysis of variance* (ANOVA), with different results in each dose group compared to the control group (p < 0.05, p = 0.00). The results of the Duncan test statistically show that the number of fetuses with hemorrhage in the 100 mg/kgBW dose group and the control group was different from the number of fetuses with hemorrhage in the dose groups given jamblang fruit extract of 200 mg/kgBW and a dose of 300 mg/kgBW. This can be seen from the increasing number of fetuses experiencing hemorrhage.

Observation abnormality Morphology is done starting from the size of the head, as well as 2 eye protrusions that are still closed, 2 nostrils, 2 ears, toes, mouth, and nose. The results of the study showed that jamblang fruit extract caused morphological abnormalities in the form of open eyelids at a dose of 200 mg/kgBW and a dose of 300 mg/kgBW. However, no morphological abnormalities were found in all jamblang fruit extract treatment groups, either at a dose of 100 mg/kgBW, a dose of 200 mg/kgBW, and a dose of 300 mg/kgBW, such as head size, 2 nostrils, mouth, 2 ears, and toes.

Table 6. Statistical Test Results: Open Eyelid Disorder

Treatment	Number of Fetuses	Eyelid Open	P Value
Control (P0)	51	$0.00\pm0.00~^{a}$	_
Dose 100 mg/ kgBW (P1)	39	$0.00\pm0.00~^a$	0.03
Dosage 200 mg/ kgBW (P2)	48	$1.00\pm1.00~^{b}$	
Dosage 300 mg/ kgBW (P3)	39	$1.60 \pm 1.60^{\ b}$	

Description: The data value followed by the same letter notation indicates not significantly different in Duncan's test p<0.05

Table 6 shows the results of the ANOVA test on abnormalities in the form of open eyelids; there was a significant difference in the jamblang fruit extract treatment group compared to the control group (p < 0.05 p = 0.03). The results of the Duncan statistical test showed that the abnormalities in the form of open eyelids from the control group and the 100 mg/kgBW



jamblang fruit extract dose group were significantly different from the 200 mg/kgBW and 300 mg/kgBW jamblang fruit extract treatment groups. It can be seen in Appendix 17 groups that experienced the most abnormalities in the form of open eyelids, namely, a dose of 300 mg/kgBW.

The content of jamblang fruit extract that is suspected of affecting the reduction in the number of fetuses compared to the control group is the alkaloid compound. After testing several different solvents, it was proven that jamblang fruit extract contains secondary metabolite compounds of alkaloids (Widyastuti *et al.*, 2021).

The average body weight and length of the fetus in the treatment group were still within the normal range. Based on the literature, the body length (head-tail) of normal mice is around 19-23 mm, and the average weight of normal mouse fetuses ranges from 0.5-1.5 g. However, although the weight and body length of the fetus normal range, declines weight and body length of the fetus if compared to with group control. A decrease in estimated fetal weight and length is caused by several factors. First, content alkaloid and flavonoid compounds in fruit jamblang It is thought to increase uterine muscle activity, thereby disrupting the process of distributing important nutrients to the fetus through the placenta, which results in low fetal weight and length (Elwuar et al., 2020).

Second, the content of the teratogenic jamblang fruit extract, namely flavonoids, has the ability to act as an iron metal chelator, which causes iron deficiency. Flavonoids are included in the phytoestrogen group, namely sources of estrogen derived from plants, which are non-steroidal compounds and have estrogenic activity. Flavonoids can inhibit FAS (*Fatty Acid Synthase*) by blocking acetyl-CoA and malonyl-CoA, which are substrates of acyl-transferase, which have the potential to inhibit genes that play a role in adipogenesis, thereby reducing the number of adipocytes. Iron deficiency during pregnancy can cause fetal weight loss. However, the results of the study showed that fetuses exposed to flavonoids (*quercetin*) had an average weight that was not significantly different from the control group. Third, other ingredients influence it heavy fetal body during organogenesis, namely tannin. Tannin is thought to bind to protein, so it can interfere with protein absorption. This is thought to be able to reduce fetal weight.

Flavonoid content, such as in fruit jamblang, can change the track axis of the hypothalamus-pituitary and significantly lower the level of estrogen and progesterone can cause abortion. These hormonal changes can cause an imbalance in embryo implantation and can result in a decline in fetal weight and mortality after implantation (Mehrabani *et al.*, 2019). Other causes suspected to decrease the length of the fetus are because inhibition of the



ossification process because calcium absorption by osteoblasts being disturbed, so that decrease in fetal length. Alkaloid content found in fruit crown god (*Phaleria marcocarpa*) is also found in fruit jamblang (*Syzygium cumini*) is suspected can hinder division cells in osteoprogenitor cells so that formation of osteoblasts is disturbed and calcium absorption by the nucleus cells is inhibited (Widyastuti *et al.*, 2006).

Causes of hemorrhage in group dose 200 mg/kgBW (P2) and group dose 300 mg/kgBW (P3). It is suspected that this is due to repeated administration of high doses of jamblang fruit extract, which causes an imbalance in the blood's osmotic pressure. In normal conditions, the embryo can develop in an isotonic fluid amniotic fluid or isotonic blood. Substance content foreign like compounds in fruit jamblang can cause vessels blood broken imbalance osmotic. This is can cause disturbance pressure on the fetus and viscosity fluid in the embryo, so that result in the rupture of blood vessels and causing bleeding in the fetus (Rusdi et al., 2021).

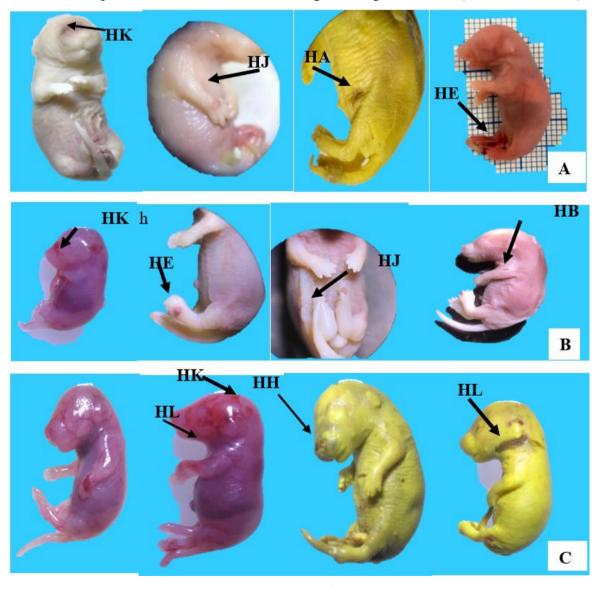


Figure 3. (A) Hemorrhage Group Dose 100 mg/kgBW; (B) Hemorrhage Group Dose 200 mg/kgBW; (C) Hemorrhage Groub Dose 300 mg/kgBB;



Informatioan: (HK) Head Hemorrhage; (HH) Nasal Hemorrhage; (HL) Hemorrhage of the Neck; (HB) Hemorrhage of the Body; (HE) Hemorrhage of the Tail; (HJ) Hemorrhage of the Finger.

Figure 3 shows that the fetus with petals eye open was found at a dose of 200 mg/ kgBW and a dose of 300 mg/ kgBW. It is suspected reason for the opening of petals opens the content in fruit jamblang or happens in a way spontaneously. In addition, other literature states that disabled petals eye open happens consequence of mutation spontaneous autosomal recessive mutation, so that it happens in a random and not yet known manner (Lea *et al.*, 1998). Research by Angelina et al. (2022) on the influence of ethanol extract leaf regards *Syzygium polyanthus*) on mouse fetus morphology shows that two external malformations are hemorrhage and eyelids eye open. Opening the petals of eye allegedly happens spontaneously. This is because of only 1 fetus (1.63%) and only in the low group, namely, a dose of 5 mg/kgBW. While the fetus with petals eye open was not found in the group dose was tall.

CONCLUSION

Ethanol extract of jamblang fruit (*Syzygium cumini*) affects the number of fetuses and fetal length p<0.05, but does not significantly affect the weight of the mother mouse and fetal weight P>0.05. Ethanol extract of jamblang fruit (*Syzygium cumini*) has a teratogenic effect on fetal morphological abnormalities in the form of hemorrhage and open eyelids. The most fetal morphological abnormalities appeared at doses of 200 mg/kgBW and 300 mg/kgBW.

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