

## Histopathology Overview Uterus of the Mice (*Mus musculus*) ICR Female after Giving Extract of Papaya Seed (*Carica papaya*)

Nur Maghfirah Qadriati Putri M<sup>1</sup>, Zulkarnain<sup>2\*</sup>, St Aisyah Sijid<sup>3</sup>

<sup>1,2,3</sup> Jurusan Biologi Fakultas Sains dan Teknologi, UIN Alauddin Makassar.

### Abstract

The high maternal mortality rate (MMR) and infant mortality rate (IMR) are the main cases in the health sector. Efforts to prevent this are encouraged to utilize plants that contain antifertility compounds, for example with papaya seeds (*Carica papaya*) which are able to minimize the side effects of contraceptive use. This study aims to determine the description of uterine histopathology of female ICR mice (*Mus musculus*) after administration of papaya seed extract (*Carica papaya*). The stages are determining the dose of papaya seed extract (*Carica papaya*), making papaya seed extract (*Carica papaya*), preparation and treatment of experimental animals, as well as making and reading uterine histopathology preparations of mice. The results showed that the administration of papaya seed extract (*Carica papaya*) for 34 days orally at a dose of 0.5 ml/bb was able to disrupt reproductive cells in mice (*Mus musculus*) characterized by abnormal endometrial thickening along with the amount of dose given.

**Keywords:** Antifertility, Histology, Mice and Papaya Seeds

### Introduction

Indonesia is one of the developing countries that is experiencing increasing population growth every year. Indonesia is the fourth most populous country in the world after China, India and the United States. (Suartha, 2016). This is in line with the increase in maternal mortality rate (MMR) and infant mortality rate (IMR). Decentralization in the health sector has not had a good impact on efforts to reduce the maternal mortality rate (MMR) and infant mortality rate (IMR) so that it has become a major case in the health sector (Prihandini et al, 2016).

As for the government's efforts in handling these cases, the government

designed a family planning (KB) program to reduce maternal mortality rates (MMR), infant mortality rates (IMR) and regulate the rate of population growth. After the International Conference on Populations Development (ICPD) in Cairo in 1994 with the direct target of suppressing the natal rate. This program has been regulated in UU No 52 of 2009 which is under the auspices of the National Population and Family Planning Agency (BKKBN) aimed at improving the health and welfare of mothers and children and families as a whole, improving the quality of human resources (Zuhriyah et al, 2017).

The family planning program is often misinterpreted by most people as a

---

\*Corresponding Author: Zulkarnain, Email: [zulkarnainbio@uin-alauddin.ac.id](mailto:zulkarnainbio@uin-alauddin.ac.id), Biology Department, UIN Alauddin Makassar, Jl. H.M. Yasin Limpo No. 36. Romangpolong, Sombaopu, Kab. Gowa.

program that rejects the presence of children. However, this is not the case. While the goals and benefits of the family planning program are actually highly recommended in creating a healthy, prosperous and happy family (Zuhriyah et al, 2017).

One of the factors leading to the increasing maternal mortality rate is the lack of attention to birth spacing. The result of birth spacing that tends to be close will cause the risk of bleeding, miscarriage and even death. Therefore, steps that need to be taken to reduce MMR are by utilizing contraceptives.

Every couple needs to consider the contraceptives that will be used this is because each contraceptive has its own advantages and disadvantages. Side effects of contraceptives can include increased blood pressure, trigger bleeding and disruption of the menstrual cycle (Herlambang et al, 2018). The use of contraceptive methods can cause health losses. In an effort to minimize the occurrence of side effects from the use of contraceptives, people are encouraged to utilize natural ingredients. Natural ingredients derived from plants that contain antifertility compounds, for example papaya seeds (*Carica papaya*) (Adani et al, 2017).

Papaya seeds (*Carica papaya*) are one of the natural ingredients that are antifertile. They contain secondary metabolite compounds such as saponins, tannins, flavonoids, terpenoids, alkaloids, sterols and triterpenes that can suppress fertility by disrupting ovarian and uterine function. (Julaeha et al, 2016). Papaya seeds (*Carica papaya*) were used in this study because they are easily available economically priced and always considered worthless by most people.

## Research Methods

This research is a quantitative study with an experimental approach to see the effect of papaya seed extract (*Carica papaya*) on uterine histopathology overview of mice (*Mus musculus*). This research was conducted on June 05-October 13, 2022 at the Green House, Zoology Laboratory, Department of Biology, Faculty of Science and Technology, State Islamic University of Alauddin Makassar and Anatomical Pathology Laboratory, RSUP Hasanuddin University. The tools used are microscope, oven, rotary evaporator, blender, scales, microtome, dropper pipette, petri dish, volume pipette, beaker, measuring tube, analytical balance, nipple bottle, rang, small shovel, cannula (sonde), spoit, surgical tools, refrigerator, funnel, slide warmer and glass object. While the materials used are mice (*Mus musculus*), old papaya seeds (*Carica papaya*), rice husks, tuloul solution, bouin solution, 30% alcohol, 40% alcohol, 50% alcohol, 60% alcohol, 70% alcohol, 80% alcohol, 90% alcohol, 96% alcohol, absolute alcohol, hematoxylin ehrlich, eosin, cotton bud, mayer's albumin, handscoon, NaCl 0.9%, 10% formalin, mask, aluminum foil and filter paper.

## Preparation of Test Animals

Mice (*Mus musculus*) were acclimatized for 4 weeks in cages and fed (AD1) and given water ad libitum. The cage was given saw dust as a bedding that is replaced every other day. Before treatment, the mice were fed for 1 day to obtain the same physiological conditions. Then the mice were weighed and labeled according to the treatment. The mice used were 2-3 months old with an average body weight of 20-30 gr.

## Preparation of Extract

Old papaya seeds (*Carica papaya*) were oven dried for 48 hours at 110oC. After drying, the papaya seeds were blended until they were powdered. The dried powder was

weighed as much as 250 grams and added with 96% ethanol as much as 1000 ml then the extraction process was carried out by maceration method for 24 hours. Then the extract was filtered and the filtrate was concentrated with a rotary evaporator. After that, the extract was diluted as needed.

### **The Treatment**

The extract was given orally every morning at 09.00 WITA at a dose of 0.5 ml/bb mice for 34 days. On the 35th day the mice were killed by dislocating the neck and the uterine organs were taken to make histopathology preparations.

### **Preparation of Histopathology Preparations**

First, the uterine organs were fixed in bouin's solution for 2 days. After 2 days the uterine organs were washed with 70% alcohol (1x60 min) then dehydrated with 70% alcohol (1x60 min), 80% (1x60 min) and 90% alcohol for 1 night. Then put in 96% alcohol and absolute alcohol (1x60 min) and then clarified in toluol solution for 1 night.

Second, the paraffin infiltration stage. The uterine organ was immersed in a mixture of toluol and paraffin solution in a ratio of 1:1 (30 min), followed by pure paraffin I, II and III for 45 min each.

Third, embedding step. Uterine organs are embedded in liquid paraffin arranged so that the direction of transverse cutting is then allowed to freeze in the refrigerator and form a block that is ready for incision with a microtome. Paraffin blocks containing uterine pieces are placed in the holder and then put into the refrigerator. Holder with paraffin blocks then mounted on a microtome then sliced 6 µm thick so that a row of slices forming a band. From the slices selected good then affixed to a glass object

that has been smeared with mayer's albumin and distilled water and then left for 24 hours in a slide warmer so that the attachment is strong enough.

Fourth, staining step. The first step is deparaffinization, glass objects containing uterine slices dipped in xylol solution until the paraffin dissolves all (15 minutes) then dried on filter paper. Next is the hydration process, the glass object that has been dried is put into alcohol with decreasing concentration starting from absolute alcohol, 96%, 90%, 80%, 70%, 60%, 50%, 40%, 30% and distilled water for 1 minute each. The third step is inserted into the dye jar containing hematoxylin ehrlich dye solution (7 seconds). and washed under running water (10 min). The fourth step, put in 30%, 50%, 60% and 70% alcohol for 1 minute each and then put in eosinY (2 minutes) and rinsed with 70%, 80%, 90%, 96% alcohol and absolute alcohol for 1 minute each. Fifth, Mounting. The preparation was put into xylol solution (15 minutes) and then dried on filter paper. Then mounted with Canada balsam and covered with cover glass. Furthermore, observations were made under a microscope to obtain a overview of uterine histopathology.

The reading of the results was done by placing the organ slides on the microscope preparation table and the reading of the results was done using a computer that was connected to the microscope. Furthermore, the condition of the uterine organs of mice (*Mus musculus*) was observed during the treatment of papaya seed extract (*Carica papaya*). The indicators observed were the condition of the glands in the endometrium.

The data collected is qualitative data and presented in the form of figure

## Research Results and Discussion

The results of the study of the effect of papaya seed extract (*Carica papaya*) on uterine development of female mice are presented in Table 1 as follows

**Table 1**

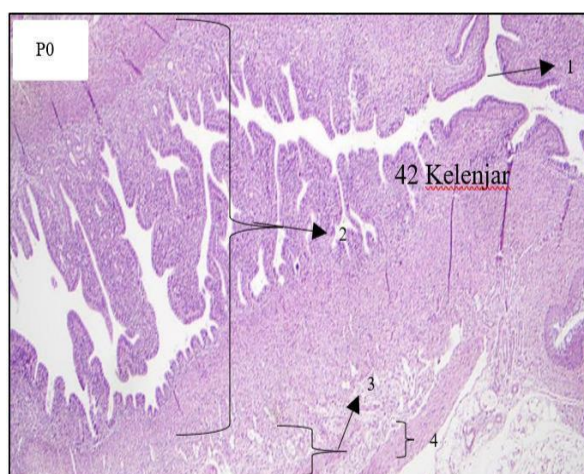
*Number of Endometrial Glands in Mice*

Treatment	Number of Endometrial Glands	
	Mice 1	Mice 2
P0 (Control)	42	42
P1 (Concentration of 10%)	62	57
P2 (Concentration of 20%)	110	137

Description: P0 group, untreated papaya seed extract; P1 group, was treated with papaya seed extract with a concentration of 10%; P2 group, was treated with papaya seed extract with a concentration of 20%

**Figure 1**

*Histology Structure of Mice Uterus in P0 Group*



Histology Structure of Mice Uterus in P0 Group (magnification of 400x) indicates normal cells (1) lumens (2) endometrium (3) myometrium (4) perimetrium

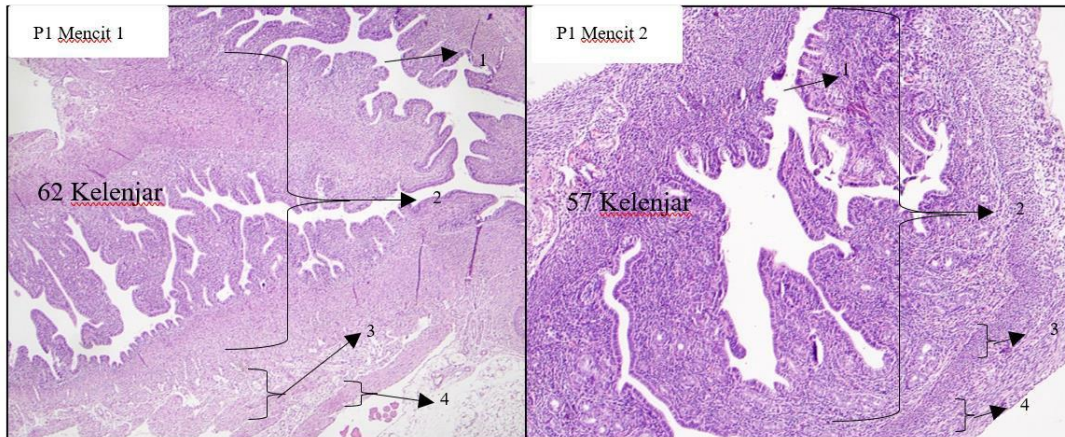
Uterine histology preparations of mice (*Mus musculus*) were observed using a microscope connected to a monitor to determine the effect of papaya seed extract on the number of endometrial glands per unit field of view in the uterine cross-sectional structure with 400x magnification and counted all the number of endometrial

glands in the endometrial layer using a counter as a counting aid.

Papaya seed extract (*Carica papaya*) was used to look at the condition of endometrial hyperplasia. Papaya seeds (*Carica papaya*) contain a number of compounds toxic to the reproductive system. These compounds are used as a reference to measure the potential or

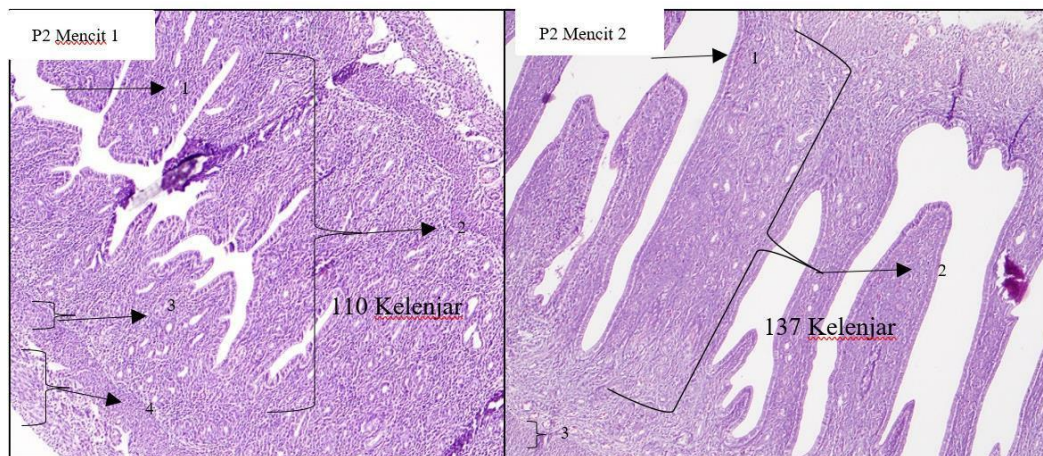
determine the possibility of antifertility derived from plants.

**Figure 2**  
*Histopathology Structure of Mice Uterus in P1 Group*



Histopathology Structure of Mice Uterus in P1 Group (magnification of 400x) shows the cell (1) lumens (2) endometrium (3) myometrium (4) perimetrium

**Figure 3**  
*Histopathology Structure of Mice Uterus in P2 Group*



Histopathology Structure of Mice Uterus in P2 Group (magnification of 400x) shows the cell (1) lumens (2) endometrium (3) myometrium (4) perimetrium

The results showed that the P0 group (Figure 1) had the best histopathology picture compared to other treatments. Histology of the uterus of the P0 group shows the condition of endometrium is normal, fertilization can occur because the hormone estrogen stimulates the growth of glands and tissues in the uterine muscle so

that it is filled with blood vessels and becomes an ideal place for the embryo or fetus to grow under normal conditions. According to [Udayani et al, 2021] Normal uterine histology conditions, the three layers of the endometrium are spread evenly, these conditions occur because the hormone estrogen increases endometrial growth

under normal conditions and stimulates the growth of uterine muscle tissue, especially the cause of muscle cell hypertrophy that occurs in the fertile period.

During pregnancy, glands and blood vessels in the endometrium play a role in providing oxygen, nutrients and other substances for the fetus to continue developing. The endometrium joins and forms the placenta with the outer layer of the embryo.

The uterus consists of three layers based on histology. These layers are the perimetrium, myometrium and endometrium. Perimetrium is outermost part of the uterine lining, consisting of Tunica Adventitia and Tunica Serosa and lined by mesothelium and composed of simple squamous epithelium. The myometrium consists of bundles of smooth muscle fibers separated by elastic fibers and collagen and contains many blood vessels. This layer is the thickest layer of the uterus. The endometrium consists of 2 layers, namely epithelium (ciliated columnar epithelium) and lamina propria or stroma containing tubular glands simplex and tissue containing many blood vessels (Udayani et al, 2021).

This can be seen in the P1 group in figure 2. an increase in the number of endometrial glands in mice there are 62 glands while in mice 2 there are 57 glands. This treatment was given papaya seed extract (*Carica papaya*) for 34 days at a concentration of 10% at a dose of 0.5 ml/BB mice. This is caused by an increase in estrogen levels in the blood so that it disrupts the secretion process of GnRH.

According to Alfian et al (2018) which states that flavonoids are compounds that have similar structures to the hormone estrogen but do not stimulate these

receptors. This causes the amount of free estrogen to increase in the blood so that it will cause negative feedback on the secretion of GnRH (Gonadotropin releasing hormone). Disruption of GnRH secretion will suppress the hormone FSH which stimulates the growth of granulosa cells in de graaf follicles

Saponins have antiestrogenic effects that interfere with the binding of natural estrogen to its receptor. Saponin has mechanism of damaging cell membranes by increasing permeability resulting in cell leakage followed by the release of intracellular material. Saponins form complex bonds with membrane sterols. The bound membrane sterols will be released from the cell membrane, causing disruption of ion transport and cell membrane permeability (Mwangengwa et al, 2021).

Alkaloids has antiproliferative properties against reproductive cells so that they can interfere with the performance of the ovaries. Alkaloids can also suppress hormones in the reproductive system that are needed in the process of spermatogenesis and oogenesis (Harlis et al, 2017). Tannin has cytotoxic effects on developing cells such as ovum cells (Harlis et al, 2017). The two principles of action of antifertility agents, the first is cytotoxic, which is by damaging cells and the second is hormonal, by disrupting the function of hormones.

P2 treatment in figure 3 occurs an increase in the number of endometrial glands in mice 1 there are 110 glands while in mice 2 there are 137 glands. This treatment was given papaya seed extract (*Carica papaya*) for 34 days at a concentration of 20% at a dose of 0.5 ml/BB mice. This is due to the antiestrogenic effect contained in papaya seeds (*Carica papaya*).

According to Adani et al (2017) which states that the mechanism of entry of antifertility compounds from papaya seed extract starts from the absorption of compounds by the intestine wall which contains capillary vessels. The capillaries then deliver blood to in the portal vein. The compounds in the test material are absorbed into the intestinal wall where there are capillaries. Capillaries drain blood into veins that join larger veins and eventually enter the liver as a portal vein. Antifertility compounds from the portal vein are flowed throughout the body, including into the endometrium of mice (*Mus musculus*).

The inhibition of fertilization process is caused by abnormal uterine endometrial morphology which results in failure of embryo implantation in mice (*Mus musculus*). Inhibition of proliferation of cells that make up the uterine wall, as well as disruption of the function of the hormone progesterone in the thickness of the endometrium and the development of blood vessels in the endometrium, will arise due to disruption of estrogen synthesis. Ethanol extract of papaya calina leaves shortens the estrus phase and prolongs the proestrus, metestrus, and diestrus phases during the 30-day treatment. Ethanol extract of papaya leaves contains compounds from the alkaloid, flavonoid, glycoside, tannin, triterpenoid, steroid and saponin groups (Setiawan et al, 2021).

According to Nita (2019) which states that papaya seeds (*Carica papaya*) contain chemical compounds that are phenols, triterpenoids, alkaloids, flavonoids and saponins. Therefore, papaya seeds have antifertility properties and can be used as a safe contraceptive.

## Conclusion

Based on the results of the research obtained, papaya seed extract (*Carica papaya*) is able to disrupt the cells reproduction in mice (*Mus musculus*) which can be seen in the increase in the number of endometrial glands along with the addition of the extract concentration. P0 has 42 endometrial glands, P1 mice 1 has 62 endometrial glands, mice 2 has 57 endometrial glands, P2 mice 1 has 110 endometrial glands and P2 mice 2 has 137 endometrial glands. Based on this, papaya seed extract (*Carica papaya*) can be an alternative in preventing conception with minimal side effects due to abnormal cell growth in the endometrium.

## Suggestion

It is necessary to conduct further research with the addition of independent variables similar to papaya seed extract which can be used as a comparison in determining which effect is better for preventing conception and determining the tolerance limit of mice at what concentration.

## References

- Adani, M. F., Sitaswi, A. J., & Isdadiyanto, S. (2017) Efek Antifertilitas Ekstrak Biji papaya (*Carica papaya*) dengan Pelarut Air terhadap Bobot Anak Mencit (*Mus musculus*). Buletin Anatomi dan Fisiologi. 2 (1)
- Alfian, M. A., Sitaswi, A. J., Djaelani, M. A (2018) Efek Antifertilitas Ekstrak Air Biji Pepaya (*Carica papaya*) terhadap Jumlah dan Diameter Folikel de graaf
- Mencit (*Mus musculus*) Betina. Jurnal Prolife: Jurnal Pendidikan Biologi, Biologi dan Ilmu Serumpun 5 (1)
- Harlis, W. O., & Septiana, A. (2017)

- Gambaran Histologi Testis Mencit (*Mus musculus*) setelah Pemberian Ekstrak
- Tumbuhan Brotowali (*Thospora crispa*). *Biowallaceae* 4 (1)
- Herlambang, H., Fitri, A.D & Kusdiyah, E. (2019) Peningkatan Pengetahuan Masyarakat dan Pemasangan Alat Kontrasepsi dalam Rahim (AKDR). *Jurnal MEDIC (Medical Dedication)* 2 (1) <https://doi.org/10.22437/medicaldedication.v2i1.5896>
- Julaeha, E., Permatasari, Y., Mayanti T., Dianti, A. (2015) Antifertility Compound From the Seeds of *Carica papaya*. *Procedia Chemistry* 17 (1)
- Mwangengwa, M. L., Bakari, G. G., Kanuta, N. L., & Max, R. A (2021) Antifertility Effects of Crude Extract from *Acasia nilotica* Pods and *Albizia lebbeck* Stem Bark in Female Multimammate Rats, *Mastomys natalensis*. *Journal of Physiology and Pathophysiology* 12 (1)
- Nita, S. H., & Hayati, L. (2019) Mekanisme Antifertilitas Fraksi Biji Pepaya pada Tikus Jantan. *Sriwijaya Journal of Medicine* 2 (1)
- Prihandini, S. R., Pujiastuti, W., & Hastuti, T. P (2016) Usia Reproduksi Tidak Sehat dan Jarak Kehamilan yang Terlalu Dekat Meningkatkan Kejadian Abortus di Rumah Sakit Tentara Dokter Soedjono Magelang. *Jurnal Kebidanan* 5 (10)<https://doi.org/10.31983/jkb.v5i10.1147>
- Setiawan, H., Wulandari, S. W., Nurwidyantary, F.E., & Dewantari, I. (2021) The Effect of Calina Papaya Leaf Ethanol Extract on Estrus Cycle and Uterus Morphology of Wistar Rats. *Biosaintifika: Journal of Biology & Biologu Education* 13 (3) <https://doi.org/10.15294/biosaintifika.v13i3.31343>
- Suartha, N. (2016) Faktor-faktor yang Mempengaruhi Tingginya Laju Pertumbuhan dan Implementasi Kebijakan Penduduk di Provinsi Bali. *Jurnal Kependudukan dan Pengembangan Sumber Daya Manusia* 12 (1)
- Udayani, D. P. C., Jawi, I. M., Sugiritama, W., & Arijana, I. G. K. N. (2021) Ekstrak Etanol Ubi Ubi Jalar Ungu (*Ipomea batatas* L) Meningkatkan Ketebalan Miometrium dan Jumlah Kelenjar Endometrium. *Jurnal Medika Udayana* 10 (10)
- Zuhriyah, A., Sofyan, I., & Bambang, B. R., (2017) Kampung Keluarga Berencana dalam Peningkatan Efektivitas Program Keluarga Berencana. *HIGEIA (Journal of Public Health Research and Development)* 1 (4)