ISSN 2515-8260 Volume 10, Issue 07, 2023

GUAVA LEAF (*Psidium guajava L.*) EXTRACTS AS AN ANTIFERTILITY MATERIALS FOR FEMALE MICE (*Mus musculus*)

Siti Nuryani, Sujono

Lecturer at Health Polytechnic, The Ministry of Health, Yogyakarta

Corresponding author :

Siti nuryani

Jurusan analis kesehatan

Poltekkes kemenkes Yogyakarta. Jl. Ngadinegaran

Email : suryaniajeng.2014@gmail.com

ABSTRACT

Background:

Guava leaves are used as medicine, among others, for blister burns and wounds, overcome ulcers and colds. Red guava leaf extracts 40 mg/ml/day and 80 mg/ml/day can reduce the number of spermatids in male white mouse models. The effect on spermatogenesis is caused by the content of guava leaf extract namely alkaloids, flavonoids, tannin, essential oil, avicularia, oleanolic acid, saponin and beta-sitosterol. Saponins have an effect especially on cells that develop like during spermatogenesis and oogenesis.

The study aimed to determine the effect of guava leaf extract on the process of oogenesis, especially on primary, secondary, de graf and atresia follicles.

Method : pre-experimental study used guava leaf extract 4mg / ml which was given orally every day to adult female mice for 4 weeks. A total of 10 mice were divided into 2 groups with 5 mice of each. The two groups were K for control and P for treatment. Each of the mice was sacrificed for their ovaries. The tissue processed by the Paraffin method was then prepared and stained with Hematoxylin Eosin in order to assess the number of primary, secondary , degraf and atresia follicles. The data were analyzed descriptively

The results of the study are as follows: the average number of follicles in normal animals without treatment is as follows primary 58, secondary 18, degraf 4 and atresia 48. The average number of follicles in the animals treated (P) is primary 38, secondary 24, de graf 2 and atresia 61

Conclusion: The administration of methanol extract of guava leaves with a dose of 4mg / ml daily for 4 weeks affects the number of primary, secondary, degraf, and atresia follicles.

Keywords : methanol extract, guava leaves (*Psidium guajava L.),* oogenesis

INTRODUCTION

Traditional medicine is an ingredient or material that has been used for treatment, which comes from plants, animals, minerals, sedan (galenic) or a mixture of this materials[2]. WHO recommends the use of traditional medicines, including herbs, because they have lower side effects than modern ones, making them safer for people to take. Herbal medicine is a natural medicinal ingredient whose preparation is still in the form of simple Simplicia, such as rhizome slices,

ISSN 2515-8260 Volume 10, Issue 07, 2023

dried leaves or roots. Meanwhile, to be called medicine, it must go through the test of preclinical, toxicity (safety), dose range, pharmacodynamics (usefulness) and teratogenic (safety of the fetus).

Guava leaves can be used empirically as medicine, among others, to cure diarrhea and wounds. Guava ethanol extracts of 10.5 mg and 21.0 mg doses did not show contraceptive antifertility effects in white rats, but at these doses guava leaf ethanol extracts had shown anti-transplantation effects in white rats[1]. Another study mentioned that red guava leaf extract with a dose of 40 mg/ml/day and 80 mg/ml/day did not reduce the number of spermatogonia cells in male white rats. The dose of red guava leaf extracts of 40 mg/ml/day and 80 mg/ml/day did not reduce the number of primary spermatocytes[3], but reduce the number of spermatids in male white mouse models.

The effect on the development of sperm or spermatogonia is caused by the content of guava leaf extract. The contents that are predicted as antifertility are alkaloids, flavonoids, tannins, essential oils, avicularia, oleanolic acid and beta-sitosterol. Alkaloids work to suppress the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) so that it will interfere with the process of spermatogenesis and consequently will also affect the quality and quantity of spermatozoa[6]. Flavonoid compounds inhibit the enzyme aromatase, an enzyme that catalyzes the conversion of androgens to estrogens which will increase testosterone levels[7]. High concentrations of the hormone testosterone will negatively give feed-back into the pituitary by not releasing FSH and LH. While the saponin compound is a bubbly solution as a steroid or glycosiditerpenoid. Saponins have an effect on the animal's reproductive system as an abortivum, inhibiting zygote formation and anti-implantation[8] and are cytotoxic, especially against developing cells such as during spermatogenesis and oogenesis[9].

Based on the description above, the process of spermatogenesis is affected by the hormones of FSH and LH, and the excretion of these hormones is affected by the content of guava leaf extract. The hormone also affects the process of oogenesis in female mice[9]. Therefore, it is necessary to study the effect of guava leaf extract on the development of eggs in their ovaries.

This research is aimed at supporting the Healthy Living Community Movement (GERMAS) as a systematic and planned action carried out jointly by all components of the nation with awareness, willingness and ability to behave in a healthy manner to improve the quality of life. The implementation of the GERMAS must start with the family because the family is the smallest part of the community that forms the personality. The first indicator of a healthy family is family planning. Family planning by preventing pregnancy can be done by using contraceptives such as birth control pills, injectable birth control, and spiral birth control. Most people do not want their bodies entered by foreign objects, are not suitable to use contraception or are afraid of the side effects. Preventing pregnancy is naturally possible to be an alternative choice, for that it needs to be examined the effect of guava leaf extract on the development of ovarian follicles in female mice.

ISSN 2515-8260 Volume 10, Issue 07, 2023

RESEARCH METHODS

The study was included in the type of pre-experimental research[16] because researchers applied guava leaf extract which was given orally to mice to assess the number of primary, secondary and de graf follicles. A total of 10 mice were used, and divided into 2 groups with 5 mice of each.

The design of the research is *Static Group Comparison*

	Treatment	Postest
Experimental Group	X	O2
Control Group		O2

In Which:

X = treatment of extracts

O2 = the number of primary, secondary and de graf follicles.

The independent variable is the dose of guava leaf extract of 4 mg/ml as the administration of 1 ml solution containing 4 mg with a duration of 4 weeks (28 days). Dependent variables are the number of primary, secondary and de graf follicles. Primary follicles are young follicles with small size characteristics and still surrounded by layers of follicular layers. Secondary follicles are the development of primary follicles marked by the emergence of small fluid-filled spaces. De graft follicles are a development from secondary to mature characterized by an increasingly large antrum space with a thinner layer of granulomas. Atresia follicle is a degenerative state so that the follicle perishes and is not visible.

To look for the Ethical Feasibility of Research, it was done by submitting a Decent Ethics statement obtained after a request to the Ethics Committee of Poltekkes Yogyakarta, Ministry of Health.

Extracts were made at UGM LPPT with procedures in accordance with local SOPs. The making of guava leaf extract with 70% methanol was carried out at LPPT UGM Yogyakarta, with procedures in accordance with SOP.

10 mice were used as an experiment with criteria: Adult female mice weighing 25-30 grams adapted for 1 month in the laboratory. Before being administered an extract, the female mice were first exposed to male mice for 1 hour (male mice only looked at each other without contact so that the same fertile period occurred). During adaptation and treatment all mice were given standard feed and drink adlibitum. Mice were divided into 5 groups, N for normal and P for treatment. Group N was only given 0.5% Na-CMC and group P with guava leaf extract of 4mg/ ml/day in the morning. After 28 days, decapitation and ovarian organs were taken, then put in 10% formalin as a fixative.

The Making of histological preparations is with the Fixation, Dehydration, Embeding, Sectioning, and Staining stage. This stage is carried out at the Anatomy Pathology Laboratory of UGM. Then, the observations were made. Observation of each preparation was carried out by 2 students who were blinded. Students have previously been given an explanation and given training to identify

ISSN 2515-8260 Volume 10, Issue 07, 2023

primary, secondary and de graf follicles. Each preparation was counted to find out the number of primary, secondary and de graf follicles in both ovaries.

FINDINGS

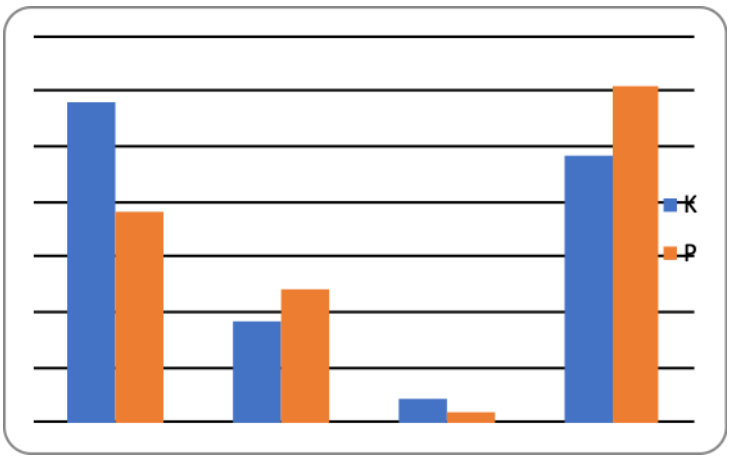
The administration of guava leaf extract for 4 weeks at a dose of 4 mg/ml for 28 days to female mice gives the results as in the table:

Table: Number of mice follicles by giving guava leaf extract of 4mg/ml for 28 days

	G. K					Average	G.P					Average
Mice	1	2	3	4	5		1	2	3	4	5	
Primary	10	18	8	16	6	58	16	2	0	8	12	38
Secondary	2	2	0	4	10	18	8	8	0	6	2	24
de graf	1	1	1	0	1	4	1	0	0	0	1	2
atresia	11	6	9	9	12	48	15	16	1	17	16	61

Based on the table, the average number of follicles in normal animals (N) without treatment are as follows: primary 58, secondary 18, de graf 4 and atresia 48. Then, the average number of follicles in animals with treatment (P) is primary 38, secondary 24, de graf 2 and atresia 61.

A description of the differences in each type of follicle in the Control and Treatment group as in the graph below:



The follicles in the normal group decreased consecutively from the primary, secondary and degraf follicles, while the atresia follicles were slightly higher than the primary ones.

Primary follicles in the treatment are less than the control, so does the de graf follicle. Atresia follicles show a large number in the treatment group.

Discussion

ISSN 2515-8260 Volume 10, Issue 07, 2023

Guava leaf was chosen as research material because the extract of guava leaf has known its effect on spermatozoa in Wistar rats. The selection of mice (Mus musculus) for experimental animals is the same as other experiments such as the antifertility effect of black tea extract, although there are studies using rats.

Female mice were used as research to evaluate the process of oogenesis, in particular, count the types of eggs or follicles. Normally, the primary follicles will be more numerous than the other follicles. But in the course of time, these primary follicles develop and some are damaged. The smallest amount is mature follicles (de graf) where these mature follicles will be ready to come out of the ovary to meet the spermatozoa that fertilize it. Cells that experience atresia in the treatment are more in numbers. Atresia follicles can be experienced by eggs in each stage. Cell damaging material in guava leaf extract will increase the number of atresia cells.

Quercetin in flavonoid inhibits the cytochrome P-450 III A enzyme in the process of hydroxylation of estradiol 17β to estrone and subsequently to estriol. The cytochrome P-450 III A enzyme is a terminal oxidase component enzyme that is responsible for the oxidation reaction of the drug. Estradiol 17β, estrone and estriol are hormones that work in female reproduction. Where estradiol 17β has the function of maintaining the female genital tract system and stimulation of the mammary glands, while estrone and estriol are the conversions of estradiol which includes natural estrogenic steroids (1). Estradiol 17β, estrone and estriol are hormones found in women for female reproductive development. These three hormones are the estrogen family. Flavonoids can inhibit many oxidation reactions, both enzymes and non-enzymes (Robinson, 1991). By inhibiting a number of enzymatic reactions in the body, this will inhibit the number of processes of cell development in the body, including sex cells during oogenesis[19].

Saponins have cytotoxic properties, especially against cells that have developed as during spermatogenesis and oogenesis[9]. Oogenesis in the treatment appeared to experience developmental obstacles due to the saponin content in guava leaf extract in the study. Saponins affect the release of the Luteinizing hormone and directly inhibit the work of genes responsible for the process of steroidogenesis. Saponins inhibit the development of granular cells that are regulated by the hormone FSH (follicle-stimulating hormone), so that in this study secondary follicles in each group decreased in number. As a result, the atresia follicles increase.

Guava leaf extract dose of 4mg/ml/day affects the development of follicles in the ovary in primary, de graf and atresia. The effect of stunted development can be seen from the increasing number of atresia follicles compared to normal conditions. The cause of atresia is caused by compounds that are toxic to follicle cells so that atresia cells increase in number.

Different results on secondary follicles that are larger than the control can occur, possibly because each individual mouse has a different physiological state, for example, differences in the amount and content of hormones that will cause different fertility.

ISSN 2515-8260 Volume 10, Issue 07, 2023

Hormone and fertility levels are research constraints or limitations. In addition, it is necessary to review using the appropriate number of mice for statistical calculations. Hormone tests also need to be examined to provide a clear picture of the involvement of hormones in the treatment with guava extract on the oogenesis process.

Antifertility compounds in principle work in 2 ways, namely through cytotoxic or cytostatic effects and through hormonal effects that inhibit the rate of sex cell metabolism by disrupting the balance of the hormonal system[18].

CONCLUSION

The Administration of guava leaf methanol extract at a dose of 4 mg/ml daily for 4 weeks affects the number of primary, secondary, de graf and atresia follicles.

REFERENCES

1. Ariani, SRD. Endang Susilowati, Elfi Susanti VH , Setiyani , Uji Aktivitas Ekstrak Metanol Daun Jambu Biji (Psidium guajava L.) sebagai Antifertilitas Kontrasepsi pada Tikus Putih (Rattus norvegicus*) Indo. J. Chem*., 2008, 8 (2), 264 – 270
2. Kementerian Kesehatan RI. , http://www.kemkes.go.id/index.php?pg=brokenlink. Diunduh 6 Desember 2017
3. Ervi Husni DAN Sukesi. Efek Zat aktifb ekstrak daun jambu biji merah (Psidium guajava L.) terhadap spermatogenesis pada tikus putih jantan Ratus Norvegicus*). Jurnal Ners* Vol. 11 No. 2 Oktober 2016: 269- 276
4. Tarigan RV., Pandapotan N., Wijaya SS., Aktivitas Antifertilitas Ekstrak Etanol Daun Jambu Biji (*Psidium Guajava L.*) berdasarkan Analisis Semen dan Tampilan Imunohistokimia Cyclooxygenase-2 pada Testis Mencit (*Mus Musculus L.*) , *Jurnal Ilmu Kefarmasian Indonesia*, September 2016, hlm. 219-225
5. Wien W, Dian S. 2007. Informasi tanaman obat untuk kontrasepsi tradisional diambil dari: URL:http: //www kalbefarmafile/10/InformasiTanamanObatUntukKontrasepsi120pdf. diakses 18 April. 2014
6. Hartini. Pengaruh dekok daun jambu biji merah terhadap jumlah kecepatan dan morfologi spermatozoatikus putih jantan, *tesis* Medan: Program Studi Biomedik Universitas Sumatera Utara; 2011
7. Kapsul, JW. 2007 *Biologi, Erlangga*, Jakarta, hlm 14 – 17.
8. Rusmiati dan Susetyarini, E. Efek Senyawa Aktif Daun Beluntas Terhadap Kadar Testosteron Tikus Putih (Rattus norvegikus) Jantan. *Jurnal GAMMA*.. September 2009: Vol.V No 1 . 21-27.
9. Nurliani A. dan Rusmiati, S.H.., Perkembangan sel spermatogenik mencit (Mus musculus L) setelah pemberian ekstrak kulit kayu durian

ISSN 2515-8260 Volume 10, Issue 07, 2023

(Durio ziberthinus murr.*). Jurnal Berk. Penel. Hayati*, 2005. 11, pp.77–79

10. Dalimartha , Setiawan . *Atlas Tumbuhan Obat Indonesia* Jilid III. *2003* .Jakarta:Puspa Swara
11. Erfan Yudapraja, 2010, Pengaruh Konsentrasi ethanol dalam penyarian maserasi dau jambu biji terhadap kadar Tanin secara spektrofotometri, *KTI*, Akademi Farmasi Mitra Sehat Mandiri
12. Tamzil Azis, Sendry Febrizky, Aris D. Mario. 2014. Pengaruh jenis pelarut terhadap persen Yiel alkaloid dari daun salam India (Murraya koenigii) Teknik Kimia , No. 2, Vol. 20, hal. 5
13. Indriani Susi.2006. Aktifitas ekstrak daun jambu biji *(Psidiun guajava L.). J.II.Pert.Indon.* Vol. 11 (1). Hal 13-17
14. Rahim Andri. 2018 . *Histologi Ovarium*. http://andryrahim.blogspot.com diunduh pada 20 Februari 2018
15. Notoatmodjo, S. 2010. *Metodologi Penelitian Kesehatan.* Jakarta: Rineka Cipta.
16. Priyandari Y, Ekstrak Daun Jambu Biji Merah terhadap Hormon Reproduksi dan sel leidigtikus jantan , *Journals of Ners Community*. 2015 vol 6 no.1 hal 14-27
17. Yuliani, S., L. Udarno & E. Hayani.. Kadar Tanin Dan Quersetin Tiga Tipe Daun Jambu Biji *(Psidium guajava*). *Buletin Tanaman Rempah dan Obat.* 2003. 14(1):17-24
18. Herdiningrat, S. 2002. Efek Pemberian Infus Buah Manggis Muda (GarciniaMangostana Linn) Terhadap Spermatozoa Mencit (Mus musculus). Majalah Andrologi Indonesia. 10 (4): 130.