

## Effect of giving Extract Ethanol from Bitter Gourd Fruit (*Momordica Charantia* L) on the Quality and Quantity of Mouse Spermatozoa (*Mus Musculus*)

Zul Hendry \*

Yarsi Stickers Mataram , NTB Provincial Hospital

Email: [zulhendry.mtr@gmail.com](mailto:zulhendry.mtr@gmail.com)

Radliyatul Fahmi

Yarsi Stickers Mataram , NTB Provincial Hospital

Email: [radliyatulifahmi@gmail.com](mailto:radliyatulifahmi@gmail.com)

**Abstract.**Men's participation in the Family Planning (KB) program is still very minimal due to the limited choice of contraceptive methods. Efforts need to be made to develop contraceptive methods, one of which is using natural ingredients. Bitter melon (*Momordica charantia* L) is known to have potential benefits as a male antifertility agent. This research used an experimental design with a sample size of 20 mice divided into 4 groups randomly. Treatment groups 1-3 were given different doses, namely 100, 80, 60 mg of bitter melon ethanol extract/100g BW/day, and the control group was given distilled water for 35 days. The mice were sacrificed the day after the last dose, then spermatozoa were collected from the epididymis and their number, motility, viability and morphology were examined. The results of this study showed a significant decrease in the number and motility of spermatozoa and was directly proportional to the size of the dose given. In terms of viability parameters, there was a significant decrease at all doses. Meanwhile, for spermatozoa morphology, only a dose of 100 mg had a significant impact. The conclusion from the results of this research is that giving bitter melon fruit extract has an effect on the number, motility, viability and morphology of spermatozoa. The most effective dose is 100 mg/100 gBB/day.

**Keywords :** \_ Extract Ethanol , Bitter Gourd, *Momodica Charantia* L., Spermatozoa

### INTRODUCTION

One of the problems of developing countries is rapid population growth, which raises the risk of demographic instability and other problems. The population of Indonesia in 2017 was recorded at 261.8 million people. With a growth rate of 1.38% per year, it is estimated that Indonesia's population will reach 306 million people in 2035 (BPS, 2018).

The government continues to optimize the family planning (KB) program to control population growth, although participation is still dominated by women. Meanwhile, male participation is still very low, namely only 6.34% (Ministry of Health of the Republic of Indonesia, 2014). One of the reasons for the low participation of men in the family planning program is the limited choice of contraceptives that can be used, only condoms, vasectomies and hormonal ones.

On the other hand, Indonesia has abundant biodiversity and has potential as a medicinal ingredient, including birth control drugs for men. The advantages of using herbal ingredients include easy to obtain, cheap, and relatively low side effects and toxicity. One plant that has potential benefits as a male antifertility agent is bitter melon (*Momordica Charantia* Linn) (Agrahari, 2015; Kumar et al., 2010).

Bitter melon is traditionally used for family planning in several countries in Asia and Africa (Jerald et al., 2012). The results of phytochemical screening contain alkaloids, tannins, saponins, glycosides, steroids and polyphenolic compounds which are found in many fruits and seeds (Bakare et al., 2010; Behera et al., 2007; Villarreal-La Torre et al., 2020) .

The bitter taste of bitter melon is caused by the cucurbitacin content, namely momordicosides K and L. Cucurbitacin is a group of triterpene glycosides which has the basic structure of cyclopentane perhydrophenanthrene which is also a steroid and is thought to be a reversible inhibitor of spermatogenesis (Hernawati, 2015), and has anti-mitotic effects (Ilyas, 2014).

Bitter melon fruit contains saponin which is a surfactant and can disrupt cell membranes (Astuti et al., 2016). Saponin is a surface active compound characterized by having a bitter taste and producing foam when shaken in water. At low concentrations it often causes hemolysis (Sudarma, 2014). Disruption of the cell membrane can have an impact on spermatozoa motility, because motility itself is influenced by enzymes, membrane activity and surface activity.

Bitter melon extract has an impact on reducing the number of Leydig cells (Putri et al., 2019), affecting the structure of the testes and epididymis, including causing a decrease in testicular weight, diameter of the seminiferous tubules, thickness of the germinal epithelium of the seminiferous tubules, and thickness of the epididymis (Cholifah et al., 2014 ; Tumkiratiwong & Thong-asa, 2014).

Bitter melon extract also affects the accessory glands, namely the seminal vesicles and prostate (Ilyas, 2018). There is an increase in cholesterol levels and accumulation of sudanophilic lipids which indicates an obstacle in steroidogenesis (Joshi et al., 2011). It is suspected that this effect is related to the precursor substances contained in bitter melon fruit extract, namely cucurbitacin, triterpenoids and steroids.

Spermatozoa are haploid cells that originate from the development and differentiation of germ stem cells in the testes which are easily affected by various factors such as the presence of foreign substances including components in *Momordica Charantia* extract (Harlis et al., 2015). This influence can include the quantity and quality of spermatozoa, including number, motility, morphology and viability.

This research was carried out with the aim of identifying the effect of administering bitter melon fruit extract at doses of 600, 800, and 1000 mg/kg BW/day on the number, motility, morphology and viability of spermatozoa.

## **METHOD**

### *1. Research design*

This research uses a true experimental design with The Randomized Posttest Only control Group Design, where data is collected after treatment or intervention (Ariel et al., 2022).

The research was carried out by dividing the samples randomly into four groups where 3 groups were given ethanol extract of bitter melon fruit in graded doses, and 1 group served as a control.

### *2. Ethanol extract of bitter melon fruit*

Fresh fruit was purchased from local farmers in Gerung District, West Lombok, who were identified and validated at the Advanced Biology Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University with number: 2/UN18.7/LBL/2022.

After wet sorting is carried out to separate the fruit and seeds, chopping and drying are carried out using an oven at a temperature of 30-40 degrees Celsius. Ethanol extract was made using the maceration method at the Pharmacy Laboratory, Faculty of Medicine, Mataram University.

### *3. Treatment Animals*

The animals used in the research were 20 male mice (*mus musculus*) aged 10-12 weeks with a body weight of 30-40 grams, which were obtained from the Pharmacy Laboratory, Faculty of Medicine, Mataram University. Mice were placed in closed plastic cages

measuring 40x30x15 cm with a base covered with husk 1-2 cm thick which was replaced every 3 days. Food and drink were provided ad libitum using commercially available rat food.

Before being given treatment, acclimatization was carried out for two weeks under standard laboratory conditions. Lighting uses room lights with a duration of 12 hours of light and 12 hours of darkness. Meanwhile, the room temperature and humidity are left within the natural range.

#### 4. *Experimental Protocol*

Mice were divided randomly into four groups (I to IV), where each group consisted of 5 mice. Bitter melon fruit extract is given via the oral route with a duration of 1 mouse spermatogenesis cycle, namely 35 days (Costa et al., 2018). The doses were given in stages, namely 100 mg/100gbb (K1), 80 mg/100gbb (K2), and 60 mg/100gbb (K3) while the controls were given Aquades (K4).

bitter melon fruit extract was carried out for 35 days once a day between 10.00 – 12.00 WITA every day.

#### 5. *Measurement of the number, motility, morphology and viability of spermatozoa*

Mice were sacrificed one day after the last dose of bitter melon fruit extract. The euthanasia process was carried out using the cervical dislocation method after previously carrying out light anesthesia using intra-peritoneal ketamine hydrochloride.

After surgery, the cauda epididymis of the testis is removed and placed in 1 ml of 0.98% NaCL physiological fluid to allow the spermatozoa to swim so that their motility can be observed. To count the spermatozoa, they are placed in a Neubauer using a pipette.

Motility is determined by counting the number of spermatozoa in the visual field minus the number of immotile spermatozoa and then multiplying by 100%. The morphology of spermatozoa was determined by Giemsa staining with normal morphology and multiplied by 100%. The viability of spermatozoa is determined using the eosin Y staining method and counting viable spermatozoa, namely colorless or not absorbing color, then multiplied by 100% (WHO, 2010).

#### 6. *Statistic analysis*

To analyze differences in average number, motility, morphology and viability of tozoan sperm, One Way ANOVA multiple comparison was used. If an average difference is found between groups, then proceed with the LSD (*Least Significant Difference*) test.

## RESULTS AND DISCUSSION

### RESULTS

Table 1 . The effect of administering bitter melon fruit extract on the number, motility, viability and morphology of spermatozoa

Parameter	Dosage Group			
	I (100 mg)	II (80 mg)	III (60 mg)	IV (0 mg)
Tozoan Sperm Count (x10 <sup>6</sup> )	28.66 ± 4.14 <sub>ab</sub>	39.83 ± 2.45 <sub>a</sub>	47.0 ± 1.27 <sup>a</sup>	66.67 ± 3.77
Tozoan Sperm Motility (%)	47.50 ± 4.50 <sup>a</sup>	52.33 ± 4.94 <sub>a</sub>	60.08 ± 5.57 <sup>a</sup>	93.08 ± 1.28
Spermatozoa Viability (%)	46.83 ± 3.97 <sub>ab</sub>	76.74 ± 0.68 <sub>a</sub>	73.58 ± 5.89 <sup>a</sup>	92.41 ± 1.91
Spermatozoa Morphology (%)	83.33 ± 1.41 <sub>ab</sub>	93.08 ± 1.83	92.00 ± 1.18	91.66 ± 0.47

<sup>a</sup> Significant difference at  $p < 0.05$  with the control group

<sup>b</sup> Significant difference at  $p < 0.05$  with Groups II and III

Based on Table 1, the tozoa sperm count parameters show that after administering bitter melon extract on the tozoa sperm count, there was a statistically significant decrease ( $p < 0.05$ ) in the treatment group compared to the control group. Meanwhile within the groups, the number of tozoa sperm in group I given the 100 mg dose was statistically significantly different ( $p < 0.05$ ) from groups II (80 mg dose) and III (60 mg dose). From table I it can also be seen that the decrease in the number of spermatozoa is directly proportional to the increase in the dose of bitter melon fruit extract, where the higher the dose, the more the number of spermatozoa in the epididymis decreases.

Tozoan sperm motility decreased statistically significantly ( $p < 0.05$ ) in all treatment groups compared to the control group. The decrease in the percentage of motile spermatozoa is directly proportional to the increase in the dose of bitter melon extract given, where the higher the dose, the percentage of motile spermatozoa decreases.

Likewise, spermatozoa viability decreased statistically significantly compared to the control group. Apart from that, in the treatment groups, there was a statistically significant difference in spermatozoa viability in group I which was given a dose of 100 mg/kgbb and groups II and III.

In terms of spermatozoa morphology parameters, only treatment group I with a dose of 100 mg/100gbb had significant differences from the control group. Meanwhile, treatment groups II and III did not show statistically significant differences after administering bitter melon fruit extract.

## **DISCUSSION**

Spermatogenesis is a very complex process, the differentiation of spermatogonia into spermatozoa involves various components, both hormonal and cellular. This process includes proliferation of spermatogonia, differentiation of spermatogonia into spermatocytes, division of spermatocytes which produce spermatids, until finally they become mature spermatozoa ready for the fertilization process (Neto et al., 2016).

The quantity and quality of spermatozoa is influenced by various factors, both internal and external. The administration of bitter melon fruit extract in this study caused a significant decrease in the number of spermatozoa according to the dose given, where the higher the dose, the lower the number of spermatozoa. The average number of spermatozoa showed a significant decrease starting at a dose of 60 mg/100gBW and the lowest was when giving bitter melon fruit extract at a dose of 100 mg/100gbw.

The decrease in the number of tozoa sperm when given bitter melon fruit extract is associated with a decrease in testosterone production, where the active substance in bitter melon extract suppresses gonadal androgens which causes testosterone synthesis to decrease (Yama et al., 2011). This is indicated by increased cholesterol levels and accumulation of sudanophilic lipids which indicate obstruction of the steroidogenesis process (Joshi et al., 2011) which ultimately causes the spermatogenesis process to not run optimally (Osinubi et al., 2003). This is also in line with previous research conducted by (Naseem et al., 1998) which found that administration of bitter melon fruit extract showed a decrease in FSH and LH hormone levels.

Physiologically, spermatozoa are produced in the seminiferous tubules and mature in the microenvironment of the epididymis (Cooper, 2012). The decrease in the number of spermatozoa in the cauda epididymis may not only be caused by changes in the spermatogenesis process in the testes, but could also be due to changes in the microenvironment of the epididymis (Patil & Patil, 2011). *Momordica Charantia* contains alkaloids and flavonoids which are toxic, as well as tannins which can cause clumping. So that the administration of

Momordica charantia extract can cause changes in the epididymal microenvironment and increase free radicals which have a cytotoxic impact on epididymal cells and spermatozoa.

The results of this study showed a significant reduction in normal spermatozoa morphology, motility and viability after administration of Momordica charantia extract. This can be caused by changes in the epididymal microenvironment and protein disturbances as well as androgen deficiency caused by the anti-androgenic properties of Momordica charantia (Girini et al., 2005). Apart from that, the saponin content which is a surfactant can disrupt cell membranes and have an impact on spermatozoa motility.

The process of changing the morphology of spermatozoa occurs in the seminiferous tubules and epididymis (Auger, 2010), while motility is fully formed in the epididymis. Morphological changes in the head and tail of spermatozoa will cause motility disorders which are directly related to their role during the conception process and are correlated with fertility and pregnancy rates both in vitro and in vivo (Liu et al., 2003).

The number, motility, morphology and viability of spermatozoa are very important in relation to the fertilization process. If the number and motility are inadequate, the ability of spermatozoa to penetrate the cervical mucus will be lost so that fertilization of the egg will most likely fail (Jerald et al., 2012). In this study, the antifertility compounds contained in Momordica charantia extract are thought to work through two mechanisms, namely providing a hormonal effect by disrupting the balance of reproductive hormones and a cytotoxic effect by changing the microenvironment of the reproductive organs.

## CONCLUSION

Based on the results of this study, it can be concluded that administration of Momordica charantia extract has the effect of reducing the number, motility, viability and morphology of spermatozoa in the epididymis. The most significant effect was obtained at a dose of 100mg/100gBW. Apart from that, it was found that the parameters of the number and motility of spermatozoa showed that the effect was directly proportional to the increase in the dose given.

## REFERRENCY

- Agrahari, P. (2015). A Review on Salient Pharmacological Features of Momordica charantia. *International Journal of Pharmacology* , 11 (5), 405–413. <https://doi.org/10.3923/ijp.2015.405.413>
- Ariel, B., Bland, M., & Sutherland, A. (2022). *Experimental Designs* . SAGE Publishing.
- Astuti, Y., Fitriana, S., & Rahayu, NS (2016). Effect of Giving Bitter Gourd Extract (Momordica charantia L) on Mice Sperm Motility and Morphology. *Mutiara Medika Journal* , 9 (1), Article 1.
- Auger, J. (2010). Assessing human sperm morphology: Top models, underdogs or biometrics? *Asian Journal of Andrology* , 12 (1), 36–46. <https://doi.org/10.1038/aja.2009.8>
- Bakare, RI, Magbagbeola, OA, Akinwande , AI, & Okunawa, OW (2010). Nutritional And Chemical Evaluation of Momordica Charantia. *Journal Of Medicinal Plants Research* , 4(21) , 2189–2193.
- Behera, T.K., Staub, J.E., Behera, S., & Simon, P.W. (2007). Bitter Gourd and Human Health. *Medicinal and Aromatic Plant Science and Biotechnology* , 2 , 224–226.
- BPS. (2018). *Statistical Yearbook Of Indonesia 2018* (p. 719). Indonesian Central Statistics Agency.

- Cholifah, S., Arsyad, A., & Salni, S. (2014). Effect of Giving Bitter Gourd Extract (Momordica Charantia, L) on the Histological Structure of the Testes and Epididymis of Male Rats (Rattus Norvegicus) Sprague Dawley®. *Sriwijaya Medical Magazine* , 46 (2), Article 2.
- Cooper, T. G. (2012). *The Epididymis, Sperm Maturation and Fertilization* . Springer Science & Business Media.
- Costa, GMJ, Lacerda, SMSN, Figueiredo, AFA, Leal, MC, Rezende-Neto, JV, & França, LR (2018). Higher environmental temperatures promote acceleration of spermatogenesis in vivo in mice (Mus musculus). *Journal of Thermal Biology* , 77 , 14–23. <https://doi.org/10.1016/j.jtherbio.2018.07.010>
- Girini, M.M., Ahamed, R.N., & Aladakatti, R.H. (2005). Effect Of Graded Doses Of Momordica Charantia Seed Extract On Rat Sperm: Scanning Electron Microscope Study. *Journal of Basic and Clinical Physiology and Pharmacology* , 16 (1). <https://doi.org/10.1515/JBCPP.2005.16.1.53>
- Harlis, WO, Malik, N., & Nelpiani. (2015). Effect of Giving Pariah Fruit Juice (momordica Charantia, L.) on the Morphology of Mouse Epididymis Spermatozoa (mus Musculus, L.). *Biowallacea* , 2 (1), Article 1.
- Hernawati. (2015). *Potential of Bitter Gourd Fruit (Momordica Charantia L.) as an Antifertility Herbal.pdf* . FPMIPA Indonesian Education University. [file.upi.edu/Directory/FPMIPA/...Hernawati/File\\_16.pdf](file.upi.edu/Directory/FPMIPA/...Hernawati/File_16.pdf)
- Ilyas, S. (2014). Effect of Methanolic Momordica charantia seed extract and Depot medroxyprogesterone acetate (DMPA) on quantity and quality of rat sperm. *International Journal of PharmTech Research* , 6 (6), Article 6.
- Ilyas, S. (2018). Histological changes in the testes if the guinea pig during administration of methanol extract of bitter melon ( Momordica charantia L.) seed and DMPA. *Journal of Physics: Conference Series* , 1116 , 052031. <https://doi.org/10.1088/1742-6596/1116/5/052031>
- Jerald, S. E., Pandey, A., Bigoniya, P., & Singh, S. (2012). Antifertility Activity of Momordica Charantia Descourt Pulp and Seed Hydroalcoholic Extract. *Journal of Applied Pharmacy* , 4 (4), Article 4.
- Joshi, S. C., Sharma, A., & Chaturvedi, M. (2011). Antifertility potential of some medicinal plants in menace: An overview. *International Journal Of Pharmacy and Pharmaceutical Science* , 3 (5), 14.
- Indonesian Ministry of Health. (2014). *Family Planning Situation and Analysis* . Indonesian Ministry of Health Data and Information Center.
- Kumar, D.S., Sharathnath, K.V., Yogeswaran, P., Harani, A., Sudhakar, K., Sudha, P., & Banji, D. (2010). A Medicinal Potency of Momordica Charantia. *International Journal of Pharmaceutical Sciences Reviews and Research* , 1 (2), Article 2.
- Liu, D.Y., Garrett, C., & Baker, H.W.G. (2003). Low proportions of sperm can bind to the zona pellucida of human oocytes. *Human Reproduction* , 18 (11), 2382–2389. <https://doi.org/10.1093/humrep/deg456>
- Naseem, M. Z., Patil, S. R., Patil, S. R., Ravindra, & Patil, S. B. (1998). Antispermatic and androgenic activities of Momordica charantia (Karela) in albino rats. *Journal of Ethnopharmacology* , 61 (1), Article 1. [https://doi.org/10.1016/S0378-8741\(98\)00006-3](https://doi.org/10.1016/S0378-8741(98)00006-3)
- Neto, FTL, Bach, PV, Najari, B.B., Li, P.S., & Goldstein, M. (2016). Spermatogenesis in humans and its influencing factors. *Seminars in Cell & Developmental Biology* , 59 , 10–26. <https://doi.org/10.1016/j.semcdb.2016.04.009>

- Osinubi, AA, Prof.dr.med. Ajayi, G., Adeyemi, & Banmeke. (2003). The Relationship between testosterone concentration Sperm Count and Motility In Fertile Nigerian Male. *Journal of Endocrinology and Metabolism* , 4 , 43–45.
- Patil, S.A., & Patil, S.B. (2011). Toxicological studies of Momordica charantia Linn. Seed extracts in male mice. *Int J Morphol* , 29 (4), Article 4.
- Putri, FD, Wurlina, W., & Tehupuring, BC (2019). Potential of Bitter Melon (Momordica charantia L.) Fruit Extract on the Number of Leydig Cells and Testosterone Hormones in Male Mice (Mus musculus). *Ovozoa Journal of Animal Reproduction* , 8 (1), Article 1. <https://doi.org/10.20473/ovz.v8i1.2019.32-35>
- Sudarma, IM (2014). *Chemistry of Natural Products ( Extraction , Isolation, and Transformation)* (1st ed.). FMIPA Press, Mataram University.
- Tumkiratiwong, P., & Thong-asa, W. (2014). Reproductive toxicity of Momordica charantia ethanol seed extracts in male rats. *Iranian Journal Of Reproductive Medicine* , 12 (10), Article 10.
- Villarreal-La Torre, V.E., Guarniz, W.S., Silva-Correa, C., Cruzado-Razco, L., & Siche, R. (2020). Antimicrobial Activity and Chemical Composition of Momordica Charantia: A Review. *Pharmacognosy Journal* , 12 (1), 213–222. <https://doi.org/10.5530/pj.2020.12.32>
- World Health Organization. (2010). *WHO laboratory manual for the examination and processing of human semen* . World Health Organization. <https://apps.who.int/iris/handle/10665/44261>
- Yama, OE, Duru, FI, Oremosu, AA, Osinubi, AA, Noronha, CC, & Okanlawon, AO (2011). Sperm quotient in Sprague–Dawley rats fed graded doses of seed extract of Momordica charantia. *Middle East Fertility Society Journal* , 16 (2), Article 2. <https://doi.org/10.1016/j.mefs.2011.02.001>