The effectiveness of *Aloe vera* peel extract on the reproductive status of streptozotocin-induced diabetic rats

W Christijanti¹*, A Z Juniarto² and L Suromo³

¹Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Semarang, Indonesia
²Faculty of Medicine, Diponegoro University, Semarang, Indonesia

*Corresponding author: wulan.christijanti@mail.unnes.ac.id

**Abstract.** Diabetes mellitus is a disorder in glucose metabolism that can cause complications in the reproductive organs. *Aloe vera* is a medicinal plant that has been widely used as an antidiabetic. The research objective was to analyze *Aloe vera* peel extract's effect on motility, sperm concentration, and testosterone levels in diabetic rats. The study design was a post-test-only randomized control group design consisting of a diabetes control group (DM) and a group with peel extract at a dose of 200 mg (PE1) and 400 mg/kg body weight (PE2) for 4 weeks. Rats were diabetic with glucose levels > 200 mg/dl after 72 hours of induction of streptozotocin 65 mg/kg BW. Data in the form of motility, sperm concentration, and testosterone levels were analyzed using ANOVA and LSD tests. The results showed a significant increase in the motility of the PE1 and PE2 groups compared to DM. Testosterone concentrations and levels were significantly increased in the PE1 and PE2 groups compared to controls. The conclusion that can be stated is that the peel extract of *Aloe vera* can increase motility, sperm concentration, and testosterone levels of diabetic rats.

1. **Introduction**

Sperm is produced by a series of spermatogenesis stages involving various components such as Leydig cells as a testosterone producer, spermatogenic cells, and Sertoli cells in the seminiferous tubules [1]. Sertoli cells on the basement membrane and extend into the seminiferous tubules' lumen provide structural, functional, metabolic, and endocrine support to germ cells [2]. Sertoli cells have androgen receptors and secrete androgen binding protein (ABP), which allows testosterone from Leydig cells to affect meiosis, spermatid elongation, and spermiogenesis [3-4].

Sperm released from the testes undergoes a breakdown of proteins and membranes in the epididymal lumen with low pH and higher osmolality [5]. During the process of sperm maturation in the lumen of the epididymis, changes in morphology, biochemistry, physiology, and fertilization ability occur due to interactions with epididymal secretory proteins [6-7]. High intraluminal epididymal osmotic pressure and the increase in nuclear condensation cause a change in the size of the sperm head [8]. Approximately 90% of water is reabsorbed by the movement of sodium, chloride, and HCO3 in the proximal part of the epididymis to increase sperm concentration and shrinkage of cytoplasmic droplets [9].

Physiological regulation of the number of sperm produced depends on the success of spermatogenesis, the nature of the extracellular environment, intracellular metabolic status, and apoptosis [10]. Sperm have a membrane composed of unsaturated fatty acid components, which are very...
susceptible to lipid peroxidation. This property makes cell membranes susceptible to lipid peroxidation, which can cause reduced motility and oxidative damage to sperm DNA [11].

Diabetes can be caused/induced pharmacologically, surgically, or by genetic manipulation in some animal species. Streptozotocin (STZ) is a chemical substance that is widely used to induce experimental animals with its ability to damage pancreatic β cells [12]. One of the points in diabetes management is the treatment to maintain blood glucose levels within normal limits or near normal values and prevent further complications in reproductive organs, DNA damage, and testicular dysfunction due to free radicals [13-14]. Aloe vera has been used as an antioxidant that increases enzymatic antioxidant levels [15-16]. This is important because the epididymis' total antioxidant status is necessary to protect spermatozoa from the effects of oxidative stress [17].

2. Materials and methods

2.1. Preparation of extract
Aloe vera plants were obtained from the Sleman area of Yogyakarta Special Region and identified in the Animal Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, UNNES. Fresh leaves more than 25 cm long are washed with water, cut into small pieces, and separated between the skin and the center/gel. The skin is homogenized and extracted with ethanol solvent. The peel extract is given according to the dose once a day.

2.2. The experimental animals
Eighteen adult male albino rats were from the Laboratory of Food and Nutrition, Inter-University Food and Nutrition Studies Center (PAU), Gadjah Mada University, Yogyakarta. They were given pellet feed twice / day and access to drinking water ad libitum. Rats were induced with streptozotocin 65 mg/kg previously injected with nicotinamide acid 230 mg/kg intra peritoneal. There are three groups, the control group (D), the group given the skin extract 200 mg/kg (PE1), and 400 mg/kg (PE2). The use of the animals was in accordance and has been approved by The Health Research Ethics Committee of the Faculty of Medicine Diponegoro University / RSUP Dr. Kariadi Semarang.

2.3. Variable analysis
The variables observed included testosterone levels, the percentage of motility, and sperm concentration. Blood from the orbital vein is collected in an ependorphous tube and centrifuged to obtain serum. Testosterone levels were analyzed from serum with the Elisa kit (DRG, Catalog # EIA1559). Stock solution for motility and concentration examination was obtained from 0.1 ml of epididymal fluid diluted by adding 0.9 ml of normal saline (modification from Ogbomade et al., 2014). The stock solution is dropped onto a glass object and examined under a binocular microscope.

Sperm that is not moving is called nonmotile, while cells that show some movement are considered motile. Sperm concentration was calculated with a hemocytometer (Neubauer's count chamber). The counting-room is closed with glass and filled with a stock solution that has been diluted 200 x. This Neubauer's count chamber is placed under a microscope at 400x magnification. The sperm count was counted in five out of 16 boxes. The sperm concentration is then calculated and multiplied by 10⁶ [18].

2.4. Statistic
Data in the form of testosterone levels, motility, and sperm concentration were analyzed by ANOVA and further test with the LSD.

3. Results and discussion
The results of the three observed variables are presented in Table 1. Testosterone levels, motility, and sperm concentration between the three groups showed significantly different p < 0.05.


Table 1. Mean ± SD from testosterone levels, motility and concentration sperm in control and treatment groups

| Variable                        | D         | PE1       | PE2         | P      |
|---------------------------------|-----------|-----------|-------------|--------|
| Testosterone Levels (ng/mL)     | 0.28 ± 0.03 | 2.49 ± 1.70 | 1.76 ± 1.37 | 0.00   |
| Motility (%)                    | 5.83 ± 12.01 | 49.17 ± 9.17 | 26.67 ± 16.33 | 0.005  |
| Concentration (10^6/ml)         | 568.75 ± 825.98 | 8750.00 ± 6196.77 | 8166.67 ± 3141.13 | 0.025  |

Figure 1. Testosterone levels of three groups after 4 weeks of treatment.

Testosterone levels in the control group (0.28) significantly lower than the treatment group (2.49 and 1.76) (Figure 1). Hyperglycemia increased oxidative stress, which affects reduces the number of Leydig cells [19]. Vitamin E as an antioxidant minimizes the effect of lipid peroxidase on the seminiferous tubules and Leydig cells. Flavonoids in the Aloe vera peel play a role in blocking the work of enzymes that contribute to testosterone metabolisms such as aromatase and 5-α reductase [20].

Sperm motility was significantly increased in treated diabetic rats (49.17 and 26.67) while the control group was 5.83 % (Gambar 2). The sperm membrane was very susceptible to lipid peroxidase, which affects the membrane's structure and fluidity and results in damage to the contractile protein in the tail [21]. Aloe vera peel is rich in antioxidant components, both enzymatic and non-enzymatic, can contribute to the extracellular defense system. Limited antioxidants epididymal fluid requires extracellular antioxidants to reduce free radicals' influence on the sperm membrane [22].

Figure 2. Percentage of sperm motility in control group and treatment with peel extract

Sperm concentration was significantly decreased in non-treated diabetic rats (568.75) compared to the group PE1 (8750) and PE2 (8166.67) (Figure 3).
Figure 3. Sperm concentration in the control group and treatment with peel extract.

*Aloe vera* peel components such as folic acid and zinc contribute to increasing the concentration of sperm in the epididymal fluid. Foods containing folic acid and zinc have increased about 74% of sperm [23]. Increased sperm concentration in the treatment group was associated with an increase in reproductive hormones, as the results of the study of [19] which states that *Aloe vera* extract at a dose of 400 mg/kg can increase follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. Testosterone is needed to support meiosis, adhesion of Sertoli cells and spermatids, and sperm release [24]. Follicular hormones are associated with Sertoli cell activity and spermatogenic cells' proliferation in the seminiferous tubules [25].

4. Conclusion

*Aloe vera* peel extract increased motility, sperm concentration, and testosterone levels in streptozotocin-induced diabetic rats.

Acknowledgment

This research was funded by the Directorate of Research and Community Service, Directorate General of Research and Development Strengthening Ministry of Research, Technology and Higher Education Number: 64.2.4/UN37/PPK.3.1/2018

References

[1] Sharma A and Agarwal A 2011 *Sperm Chromatin: Biological and Clinical Applications in Male Infertility and Assisted Reproduction* (New York: Springer)

[2] Sharma R K, Bhat R A, Goyal A K and Bhardwaj J K 2015 *J. Entomol. Zool. Stud.* 3 506

[3] Walker W H 2011 *Spermatogenesis* 1 116

[4] Sharma R & Agarwal A 2014 *Spermatogenesis: An Overview* 2 in *Sperm Chromatin: Biological and Clinical Applications in Male Infertility and Assisted Reproduction* (New York: Springer)

[5] Liu Y, Wang D K and Chen L M 2012 *Biol. reprod.* 86 99.

[6] Gawecka J E, Boaz S, Kasperson K, Nguyen H, Evenson DP & Ward WS 2015 *Hum. reprod.* 30 2725

[7] Xie S W, Li G T, Qu L J, Cao Y, Wang Q, Zhou J Y, Zhong R H, Guo X J, Zhu Y 2016 *Molecules* 21 602

[8] Cooper TG & Ching-Hei Y 2010 *Andrology: Male Reproductive Health and Dysfunction.* 3rd Edition (New York: Springer)

[9] Björkgren I 2014 *Novel Genes and Regulatory Systems In Epididymal Differentiation And Sperm Maturation Of The Mouse* (Finland: University of Turku)

[10] Aitken R J, Findlay J K, Hutt K J and Kerr J B 2011 *Reproduction* 141 139

[11] Zini A and Al-Hathal N 2011 *Asian J. Androl.* 13 374
[12] He-Lin T, Li-Shun W, Zhong-Xin X, Ru-Tong Z, Dong-Ling J & Jin-Sheng G 2010 *Glob. J. Pharmacol.* **4** 111

[13] American Diabetes Association 2012 *Diabetes Care* **35** 564

[14] Alves M G, Martins A D, Rato L, Moreira P I, Socorro S & Oliveira P F 2013 *Biochim. Biophys. Acta (BBA)-Mol. Basis Dis.* **1832** 626

[15] Enas A K M 2011 *Aust. J. Basic Appl. Sci.* **5** 1321

[16] Mohapatra S, Pradhan S, Rath B & Tripathy S 2013 *Int. J. Pharm Bio Sci.* **4** 187

[17] Christijanti W, Juniarto A Z, & Suromo LB 2019 *Pharmacogn. J.* **11** 1

[18] World Health Organization 2010 *Laboratory manual for the examination and processing of human semen 5th ed.* (Switzerland: WHO)

[19] Behmanesh M A, Erfani Majd N, Shahriri A & Najafzadeh H 2017 *Iran. J. Vet. Med.*, **11** 165

[20] Modaresi M and Khodadadi A 2014 *Res. J. Biol. Sci.* **9** 165

[21] Zribi N, Chakroun N F, Elleuch H, Abdallah F B, Hamida A S B, Gargouri J, Fakhfakh F, and Keskes L A 2011 *Reprod. Biol. Endocrinol.* **9** 47

[22] Oluwakemi O and Olufeyisipe A 2016 *Iran J Basic Med. Sci.* **19** 511

[23] Shahraki A, Mojahed A S & Afshar-Goli J 2014 *Int. J. Biosci.* **5** 158

[24] Smith L B and Walker W H 2014 *Semin. Cell Dev Biol.* **30** 2

[25] Ramaswamy S & Weinbauer GF 2014 *Spermatogenesis* **4** e996025-1