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# EFFECT OF *RUELLIA TUBEROSA* L. LEAF NANOPARTICLES ON SEMINIFEROUS TUBULE DIAMETER AND GERMINAL EPITHELIUM THICKNESS IN DIABETIC RATS

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**Abstrak**

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia that can cause various pathological changes in the human body and animals. Oxidative stress due to diabetes can lead to spermatogenesis disorders and increased cell apoptosis in the testes. *Ruellia tuberosa* L. leaf contain antioxidant compounds, such as saponins, carotenoids, flavonoids, and phenols, that can be used as free radical scavengers to reduce oxidative stress levels. The manufacture of *Ruellia tuberosa* L. leaf nanoparticles using the top-down method enhances pharmacological activity, particularly by facilitating cell wall penetration and improving the absorption of active substances in the body. This study employed a proper experimental design with a post-test-only control group model using Wistar rats as experimental animals. The treatment groups in this study included K- (control), K+ (diabetic control), P1 (exogenous long-acting insulin 1.80 IU/kg BW), P2 (*Ruellia tuberosa* L. leaf nanoparticles dose of 200 mg/kg BW), and P3 (*Ruellia tuberosa* L. leaf nanoparticles dose of 400 mg/kg BW). Testicular retrieval was performed 28 days after administration of *Ruellia tuberosa* L. leaf nanoparticles. Testicular histopathology was stained using Hematoxylin-Eosin staining and examined under a trinocular microscope at 100X magnification. Data on the diameter and thickness of the germinal epithelium were analyzed using the ANOVA test, followed by Duncan's test. The results showed that administration of *Ruellia tuberosa* L. leaf nanoparticles at a dose of 400 mg/kg BW was an effective dose in increasing the diameter of the seminiferous tubules and the thickness of the germinal epithelium compared to *Ruellia tuberosa* L. leaf nanoparticles at a dose of 200 mg/kg BW and exogenous long-acting insulin 1.80 IU/kg BW.

**Keywords**: Diabetes, Nanoparticle, Ruellia Tuberosa L

## Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and glucose intolerance (Ohiagu *et al*., 2021). The prevalence of human diabetes globally reached 451 million cases in 2017 and is projected to increase to 693 million cases by 2045. Type 1 diabetes mellitus (T1DM) is one of diabetes mellitus types caused by the immune system attacking pancreatic β-cells, therefore reduced insulin production (IDF, 2017). Diabetic condition leads to an imbalance in reactive oxygen species (ROS) production, which significantly contributes to cellular damage (Rendra *et al*., 2019). Diabetes cause in various medical complications, such as stroke, neuropathy, retinopathy, cardiomyopathy, hypertension, renal failure, sexual dysfunction, and infertility due to a chain reaction


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of free radicals (Mezil *et al*., 2021). High ROS level can cause functional impairment of the testes, including reduced secretion of reproductive hormones such as testosterone, LH, and FSH, as well as structural damage to the testicular organ, including injury to seminiferous tubule germ cells, fibrosis, vascular damage, and alterations in testicular morphology (Andlib *et al*., 2023). ROS levels imbalance induces lipid peroxidation in testicular blood vessels, which triggers cell death and decreases the production of germ cells. This process leads to reduced germinal epithelium thickness and directly impacts the decrease in seminiferous tubule diameter (Dutta *et al*., 2021).

Diabetes mellitus therapy must be administered to maintain blood glucose levels within the normal range and to prevent complications. Diabetic patients with insulin deficiency often use exogenous insulin to maintain normal blood glucose levels (Soto-Mota *et al*., 2021). Exogenous insulin has long been used as a clinician's choice for pharmacological management of diabetes mellitus cases, but there is a significant concern associated with severe hypoglycemia. In patients with neuropathy, insulin degradation is reduced, which subsequently decreases insulin requirements and increases the risk of hypoglycemia (Rzepczyk *et al*., 2022). Side effect from long-term use of hypoglycemic drugs and insulin encourages the search for alternative therapies, such as exogenous antioxidants to counteract free radicals responsible for organ dysfunction associated with diabetes mellitus.

*Ruellia tuberosa* L. is used as an antidiabetic, anticancer, antidyslipidemia, antihypertensive, and antidiarrheal herb. Safitri et al. (2019) reported that *Ruellia tuberosa* L. root extract administered doses of 200 and 500 mg/kg BW indicated preventive effects on histopathological findings compared to the control group. Antioxidant activity of *Ruellia tuberosa* L. is associated with the presence of secondary metabolites such as flavonoids, vitamin E, alkaloids, steroids, and tannins (Ullah *et al*., 2016). The synergistic interactions among bioactive compounds can decrease diabetes complications and infertility by acting as antioxidants to scavenge free radicals.

**Method**

This study uses healthy male Wistar rats (*Rattus norvegicus*), aged 10-13 weeks, with body weights of approximately 200-250 grams. Rats' cages have good ventilation and a humidity level of 40-60%, with a temperature range of 22-25 °C. Rats were given feed and drinking water twice a day. In this study, 25 male rats were used as experimental animals, which were randomly divided into five groups, each group consisting of 5 rats.

*Ruellia tubesa* L. leaf obtained from Kedungkandang, Malang City. *Ruellia tubesa* L. fresh leaf were dried in the oven at 60°C. Dried leaves were made into powder with a grinder. Leaves powder was sieved using a 200-mesh to obtain a powder with particle sizes below 74 microns. Nanoparticle synthesis of *Ruellia tuberosa* L. was performed using the top-down method. Simplicia powder was placed into a high speed milling machine at 5000 rpm for 5 minutes. The milling process was carried out continuously, with 60 minutes of milling followed by a 120-minute pause, over a period of 3 days to minimize heat generation on the material. After milling, size distribution of *Ruellia tuberosa* L. leaf nanoparticles were analyzed using a particle size analyzer (PSA).

All experimental animals were adapted for seven days before treatment. All of the treatment groups, except the normal control group, were subcutan injected with a single dose of alloxan (Sigma-Aldrich®) 150 mg/kg BW dissolved in NaCl 0.9%. The blood glucose level in rats was assessed using a glucometer with test strips (Accu Chek Instant) seven days after injection. Rats with fasting glucose levels ≥ 200 mg/dL were categorized as a diabetes mellitus model. Nondiabetic rats (negative control) and diabetic rats (positive control) were each administered 1.5 mL of physiological NaCl solution. Additional diabetic groups received long-acting insulin (Levemir®) 1,80 IU/kg BW, *Ruellia tuberosa* L. leaf nanoparticles at doses of 200 mg/kg BW and 400 mg/kg BW for 28 days.

Neck dislocations were performed on all groups of rats on the 28th day after *Ruellia tuberosa* L. leaf nanoparticles administration to retrieve testicular tissue. Testes fixed in a 10% formalin buffer solution, dehydrated in 70%, 90%, and 95% alcohol, followed by absolute ethanol and xylol. The infiltration process was then completed by applying paraffin before embedding. The slides were stained with hematoxylin and eosin (HE). The diameter of the seminiferous tubules was measured with microscope (Nikon Eclipse E100, Japan) with Image Raster and Optilab (OptiLab Advance V2) in five fields, categorized as round or nearly round, at 100× magnification by drawing the longest distance between two mutually perpendicular lines and subsequently averaging the results (Wang *et al*., 2022). Morphometric measurement of germinal epithelium thickness was performed from the spermatogonium layer to the spermatid layer in five fields of view from five seminiferous tubules, and the results were averaged (Behmanesh *et al*., 2018). The data on seminiferous tubule diameter and germinal epithelium thickness were analyzed using ANOVA and followed by Duncan's test to examine the interaction among the treatments.

**Result and Discussion**

Table 1 presents the mean values of seminiferous tubule diameter and germinal epithelium thickness of rat testes in each treatment group. The results show that group P2 did not differ significantly ($P > 0.05$) from group P3, but was significantly different ($P < 0.05$) from groups P1, K-, and K+. The mean diameter of the seminiferous tubules, from lowest to highest, was as follows diabetic control, long-acting exogenous insulin 1.80 IU/kg BW, normal control, *Ruellia tuberosa* L. leaf nanoparticles at 200 mg/kg BW, and *Ruellia tuberosa* L. leaf nanoparticles at 400 mg/kg BW. According to Duncan's test for germinal epithelium thickness, group P2 did not differ significantly ($P > 0.05$) from group P3, but was significantly different ($P < 0.05$) from groups P1, K-, and K+. The highest mean germinal epithelium thickness was observed in the group given *Ruellia tuberosa* L. leaf nanoparticles at 400 mg/kg BW, and the lowest mean was found in the diabetic control group.

The administration of *Ruellia tuberosa* L. leaf nanoparticles resulted in an increase in seminiferous tubule diameter and germinal epithelium thickness compared to diabetic group. In the normal control group, spermatogenic cell structure beginning from the lamina basalis layer was well organized, with a distinct basal lamina boundary and a substantial number of spermatozoa observed in the lumen of seminiferous tubules. In the contrast, in diabetic group, spermatogenic cells forming seminiferous tubule epithelium were sparse, and germ cell cycle was not clearly visible due to structural alterations of spermatogenic cells, along with evident luminal vacuolization of seminiferous tubules. Diabetic rats receiving treatment showed improved testicular morphology compared to untreated diabetic group. In group P3, the mean germinal epithelium thickness was higher than that in group P2, although the difference was not statistically significant.

**Table 1. Mean diameter and germinal epithelium thickness of seminiferous tubules in rat testes**

| Group | Diameter of Seminiferous Tubule (µm) | Germinal Epithelium Thickness of Seminiferous Tubules (µm) |
|---|---|---|
| K- | $256{,}58 \pm 19{,}68^{c}$ | $78{,}32 \pm 9{,}24^{c}$ |
| K+ | $223{,}28 \pm 22{,}61^{a}$ | $60{,}54 \pm 6{,}44^{a}$ |
| P1 | $242{,}28 \pm 22{,}24^{b}$ | $69{,}72 \pm 6{,}42^{b}$ |
| P2 | $283{,}41 \pm 21{,}92^{d}$ | $86{,}50 \pm 7{,}96^{d}$ |
| P3 | $285{,}75 \pm 20{,}85^{d}$ | $88{,}54 \pm 7{,}67^{d}$ |

Description: K-: normal control; K+: diabetic rats + normal saline; P1: diabetic rats + insulin 1.80 IU/kg BW; P2: diabetic rats + *Ruellia tuberosa* L. leaf nanoparticles at 200 mg/kg BW; P3: diabetic rats + *Ruellia tuberosa* L. leaf nanoparticles at 400 mg/kg BW.
Different superscripts a,b,c,d indicates significant difference (P<0.05)

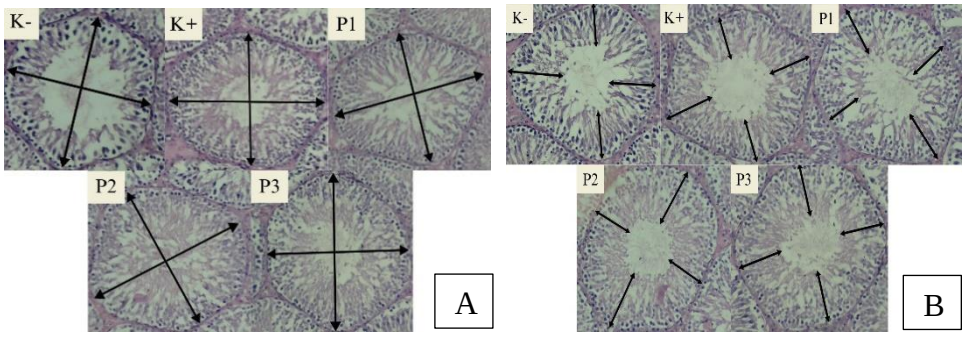


**Figure 1. (a) Diameter of seminiferous tubule; (b) Germinal epithelium thickness of seminiferous tubules of rats (H&E, ×100)**

Diabetes mellitus (DM) leads to increased production of reactive oxygen species (ROS). Oxidative stress causes a reduction in gonadotropin releasing hormone (GnRH) levels, so stimulation of the anterior pituitary to secrete luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH) and follicle-stimulating hormone (FSH) decreased, which consequently leads to reduced testosterone production (Minas *et al*., 2024). The decline in testosterone disrupts Sertoli cell stimulation, impacting germ cells during spermatogenesis (Griswold *et al*., 2018). Germ cells that are deprived of nutrients and continuously exposed to ROS will undergo apoptosis and fail to develop. A reduction in the number of germ cells is directly proportional to the decrease in seminiferous tubule diameter and germinal epithelium thickness (Rojas *et al*., 2017). In this study, the diabetic control group without treatment exhibited a significant decrease in the mean diameter of the seminiferous tubules and the mean thickness of the germinal epithelium compared to the normal control group.

Groups P2 and P3 received *Ruellia tuberosa* L. leaf nanoparticles at 200 mg/kg BW and 400 mg/kg BW demonstrated significant increases in seminiferous tubule diameter and germinal epithelium thickness compared with diabetic control. The antioxidants present in *Ruellia tuberosa* L. leaf, including alkaloids, polyphenols, ascorbic acid, lycopene, tocopherol, carotene, steroids, and terpenoids are capable of reducing ROS levels and preventing the oxidation of cellular molecules, therefore decreasing oxidative stress (Tuong Vi *et al*., 2022). Balanced ROS levels can restore hormonal regulation, normal spermatogenesis and effective stimulation of Sertoli and Leydig cells, which directly affect the diameter of the seminiferous tubules and the thickness of the germinal epithelium.

## Conclusion

Based on the results of this study, it can be concluded that the administration of Ruellia tuberosa L. leaf nanoparticles effectively increases the diameter seminiferous tubule and germinal epithelium thickness of diabetic rats induced with alloxan. Administration of Ruellia tuberosa L. leaf nanoparticles at 200 mg/kg BW and 400 mg/kg BW showed effectiveness as exogenous antioxidants, evidenced by increased seminiferous tubule diameter and germinal epithelium thickness compared to the diabetic control group and long-acting exogenous insulin group (1.80 IU/kg BW).

Further studies are required to comprehensively evaluate the potential toxicity of Ruellia tuberosa L. leaf. Expansion of research is necessary to specifically investigate the effects of every bioactive compound on the improvement of infertility caused by metabolic diseases such as diabetes mellitus.

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