

## Daun Cincau Hijau (*Premna Oblongifolia*) Has the Potential to Improve Spermatozoa Quality in Hyperglycemic White Rats (*Rattus Norvegicus*)

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### ABSTRACT

*Diabetes mellitus is a health problem that has a wide impact on various organs, including the male reproductive system. Chronic hyperglycemia can increase oxidative stress and deteriorate the morphological quality of spermatozoa. The leaves of the green grasshopper (*Premna oblongifolia*) contain flavonoids and antioxidants that have the potential to suppress oxidative stress and repair histological damage caused by hyperglycemia. This study aims to assess the effect of green grasshopper leaf extract on blood glucose levels and spermatozoa quality by evaluating the Johnsen score and spermatozoa morphology in hyperglycemic mice. The study used an experimental design with a post-test-only control group approach. A total of twenty-four male mice were randomly divided into four groups: normal control, negative control, and two treatment groups that received green grasshopper leaf extract at doses of 200 and 400 mg per kg of body weight. Diabetes was induced using streptozotocin, while the green grasshopper leaf extract was obtained by the maceration method and administered orally for fourteen days. The results showed that a dose of 400 mg per kg of body weight significantly reduced blood glucose levels compared to the negative control. In addition, the high-dose treatment group showed a near-normal Johnsen score and a decrease in the number of abnormal spermatozoa. These findings indicate that green grasshopper leaf extract has potential as a phytotherapeutic agent that not only lowers blood glucose levels but also improves the reproductive quality of males impaired by diabetes mellitus.*

**KEYWORDS** *Diabetes mellitus, Spermatozoa, Green Grasshopper, Johnsen Scale, Spermatozoa Morphology*



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## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease with an increasing prevalence globally and in Indonesia. In 2019, there were 463 million cases of DM worldwide, with predictions of an increase to 700 million cases by 2022. Indonesia ranks seventh in the number of DM sufferers worldwide (Safitri, 2018). Most cases in Indonesia are type 2 DM, accounting for up to 90% of total cases (Husain, 2022).

Unhealthy lifestyle factors, such as poor diet, lack of physical activity, and inadequate monitoring of blood glucose levels, are the main contributors to the rising prevalence of DM and its complications (Irwansyah, 2021; Murtiningsih, 2021). Uncontrolled diabetes mellitus can lead to serious complications, including impaired male reproductive function characterized by decreased spermatogonium count, reduced diameter of seminiferous tubules, lower sperm motility, and damage to testicular structures (Adelati et al., 2016; Warson, 2017; Arundani, 2021). In addition, DM can also trigger erectile dysfunction and decreased testosterone levels, which further contribute to infertility (Barkabi-Zanjani et al., 2020; He et al., 2021; Huang et al., 2024; Kamrul-Hasan et al., 2023; Shi et al., 2017).

The standard therapy for DM currently involves the use of metformin, which is effective in lowering blood glucose levels and preventing complications (Indarto, 2023). However, long-

term use of metformin often causes side effects that can decrease patients' adherence to medication (Mierza, 2023; Rosalinda, 2023). This situation encourages the search for alternative plant-based herbal therapies that are considered safer, more economical, and more effective for long-term treatment (Gumantara, 2017; Mierza, 2023).

One potential plant is the green grasshopper (*Premna oblongifolia*), which contains flavonoids, polyphenols, and other antioxidants with antidiabetic activity (Santoso, 2017; Febrianto, 2022). These compounds can lower blood glucose levels, protect organs from damage, and prevent DM-related complications (Prawitasari, 2019).

Previous research has explored various herbal alternatives for managing diabetes and its complications (Chen et al., 2015). For instance, studies have shown the efficacy of *Biophytum petersianum* (kebar grass) extract in improving spermatozoa viability in diabetic mice (Arundani et al., 2021). Similarly, research on *Cyclea barbata* and *Manilkara zapota* (sawo manila) leaves has demonstrated their phytochemical potential and protective effects on testicular cells under hyperglycemic conditions (Febrianto et al., 2022; Octavyani et al., 2022). Furthermore, investigations into herbal plants such as *Pleurotus ostreatus* and moringa leaves have highlighted the role of flavonoids and antioxidants in lowering blood glucose and reducing oxidative stress (Dewi et al., 2018; Safitri, 2018). However, specific research focusing on the effect of green grasshopper (*Premna oblongifolia*) leaves on spermatozoa quality—encompassing Johnsen scoring and detailed morphological analysis—in hyperglycemic animal models remains limited. Most prior studies emphasize general antidiabetic or antioxidant effects without addressing the compound's direct impact on reproductive histopathology and gamete morphology.

This study aims to explore the potential of green grasshopper leaves in repairing testicular histopathological damage in diabetic rats, focusing on their effect on blood glucose levels and spermatozoa quality. The results of this study are expected to support the development of more effective and safe herbal therapies for managing DM, particularly in preventing reproductive complications.

## **METHOD**

### **Types of Research**

This study is an experimental research with a post-only control group design.

### **Place and Time of Research**

The research was carried out at the Herbal Laboratory of Yarsi University, the Histology Laboratory of Yarsi University, the Integrated Research and Testing Laboratory of the Experimental Animal Development Sub-Unit of Gadjah Mada University, and the Integrated Laboratory of the Faculty of Dentistry, Gadjah Mada University. The duration of the study was 12 weeks.

### **Subjects and Samples**

The study population was a white rat (*Rattus norvegicus*) of the male Wistar strain aged 2–3 months, healthy, and weighing 300–400 grams. The animals were obtained from the Faculty of Veterinary Medicine, Gadjah Mada University.

The number of samples was determined based on Federer's formula, a minimum of 6 were obtained per group, so that the total test animals were 24, randomly divided into 4 treatment groups.

## Research Procedure

### 1. Manufacture of Green Grasshopper Leaf Extract (*Premna oblongifolia*)

The leaves of green grasshoppers are dried, cut into small pieces ( $\pm 69$  g), then extracted by the maceration method using 1400 mL of aqueducts for  $3 \times 24$  hours. The filtrate is filtered every 24 hours, then compacted using a rotary evaporator with vacuum pressure at a temperature of  $\pm 40^\circ\text{C}$  until a concentrated extract of grasshopper leaf water is obtained.

### 2. Test Animal Maintenance

The rats were raised to experimental animal laboratory standards, fed standard pellet feed and drinking water ad libitum.

### 3. Weight Measurement

The weight of the mice was measured to determine the dose of the treatment.

### 4. Diabetes Induction

Diabetes is induced by intraperitoneal streptozotocin injection at a dose of 15 mg/kgBB. Blood glucose levels were checked 3 days after injection using a glucose meter.

### 5. Treatment Delivery

Green grasshopper leaf extract is administered orally with a sonde once every 2 days for 14 days.

### 6. Blood Glucose Level Measurement

Blood sampling was carried out from the orbital vein using microhematocrit capillaries in the morning. Glucose levels are analyzed using a glucometer.

### 7. Preparation of Histology Preparations

Rats were anesthetized by intraperitoneal injection of ketamine–xylazine combination (0.1 mg/200 g BB). After cervical dislocation, the testicular organs are removed, cleaned, and put in a pot containing 10% Neutral Buffer Formalin. Furthermore, the process of dehydration, clearing, paraffin infiltration, and Hematoxylin–Eosin (HE) staining was carried out. The preparation was observed using a light microscope with a magnification of 400 times.

### 8. Data analysis was carried out using SPSS version 29.

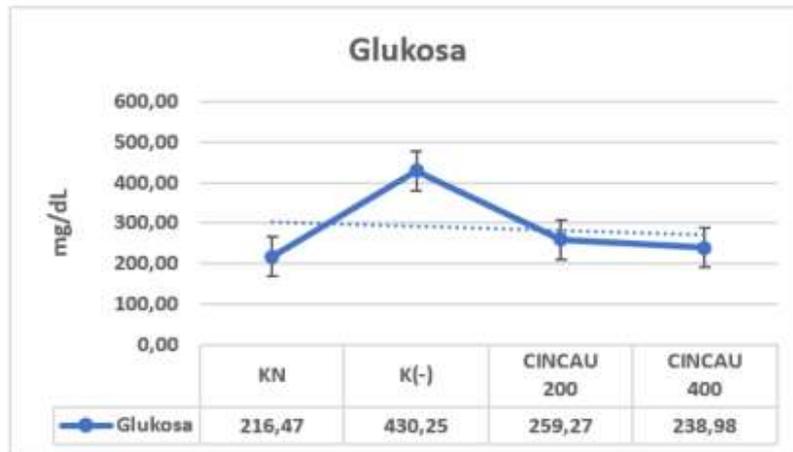
For data with normal distribution, a one-way ANOVA test is used, while data that are not normally distributed are analyzed with the Kruskal-Wallis test. The results of the analysis are presented in the form of text, tables, and graphs.

## RESULT AND DISCUSSION

### 1. Blood Glucose Levels During

The results showed that there was a difference in blood glucose levels between the treatment group with green grasshopper leaf extract compared to the negative control group (diabetic rats). The average highest blood glucose level was found in the negative control group of 430.25 mg/dL, while the normal group had the lowest level of 216.47 mg/dL. In the treatment group, a dose of 200 mg/kgBB lowered blood glucose levels to 259.27 mg/dL, while a dose of 400 mg/kgBB resulted in a more optimal reduction of 238.98 mg/dL. The Kruskal-Wallis test showed significant differences between groups ( $p = 0.01$ ).

Daun Cincau Hijau (*Premna Oblongifolia*) Has The Potential To Improve Spermatozoa Quality In Hyperglycemic White Rats (*Rattus Norvegicus*)



**Figure 1.** Average Blood Glucose in Mice

**Description:** KN (Normal Control), K(-) (Negative Control), Grasshopper 200 (Control with a dose of Grasshopper 200 mg/KgBB, Grasshopper 400 (Control with a dose of Grasshopper 400 mg/KgBB).

Source: Primary Data Analysis by Researcher, 2025

These results are in line with the research of Amandasari *et al* (2021) which stated that the content of bioactive compounds such as flavonoids, saponins, and alkaloids in green grasshopper extract plays an important role in lowering glucose levels through the mechanism of increasing insulin sensitivity and suppressing oxidative stress. Flavonoids are known to support pancreatic cell regeneration, increase insulin secretion, and lower blood glucose by stimulating glucose uptake into peripheral tissues (Sumartini *et al*, 2022).

This study also corroborates the findings of Dewi (2018) and Arbilla (2021) that herbal plants with flavonoid content have significant hypoglycemic activity. In general, the mechanism of lowering blood glucose by flavonoids occurs through antioxidant activity, improvement of pancreatic  $\beta$  cell function, and increased expression of GLUT4 (Jaiswal, 2009; Safitri, 2018).

## 2. Skoring Johnsen

Johnsen scoring is used to assess spermatogenesis growth and overall sperm quality, including sperm morphology, motility, and concentration. The results showed a decrease in Johnsen scoring in the negative group compared to the normal group.

**Table 1. Descriptive Results of Johnsen Scoring**

Groups	Total	Mean $\pm$ SD
Control	6	9.66 $\pm$ 0.63
Negatives	6	8.5 $\pm$ 1.76
Cincau 200	6	9 $\pm$ 1.09
Cincau 400	6	9.33 $\pm$ 0.51

Source: Primary Data Analysis by Researcher, 2025

Description: Normal group = normal rat; Negative group = diabetic rats; Grasshopper 200 = Rats treated with green grasshopper leaf extract at a dose of 200 mg/kgBB, Grasshopper 400 = Rats treated with green grasshopper leaf extract at a dose of 400 mg/kgBB.

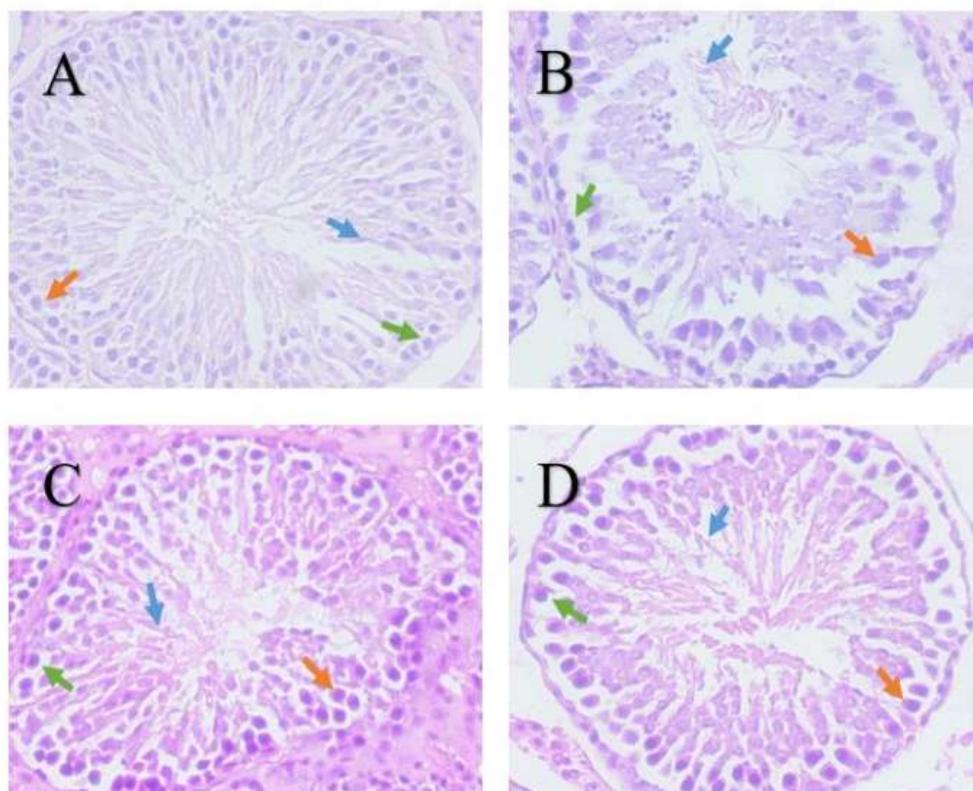
The results of the study showed that by being given a high dose of green grasshopper leaf extract treatment, which is 400 mg/KgBB, it can increase Johnsen scoring even though it has not reached the normal limit, but the results will be more optimal if done for a longer time, in this case more than 1 month so that the results obtained show a difference. Another possibility that can occur is that the process of spermatogenesis is not too affected by the hyperglycemic conditions that mice experience.

**Table 2. Scoring Johnsen**

<b>Score</b>	<b>Rating</b>
10	Epitel tubulus normal. Spermatogenesis lengkap, lumen tubulus terbuka, sel spermatozoa >10
9	Epitel tubulus rusak, lumen tubulus tertutup, sel spermatozoa >10
8	Sel spermatozoa <10
7	Sel spermatozoa 0, sel spermatid >10
6	Sel spermatozoa 0, sel spermatid <10
5	Sel spermatozoa dan sel spermatid 0, sel spermatosit >5
4	Sel spermatozoa dan sel spermatid 0, sel spermatosit <5
3	Sel spermatogenik hanya terdiri atas sel spermatogonium
2	Sel spermatogenik 0, hanya ada sel sertoli
1	Tidak ada sel sama sekali dalam tubulus

Source: Adapted from Johnsen, 1970

Johnsen's average score shows a difference between groups. The normal mice had a score of 9.66 which reflected a healthy physiological condition, while the diabetic group decreased to 8.5 due to oxidative stress. The administration of green grasshopper leaf extract at a dose of 200 mg/kgBB increased the score to 9, while the dose of 400 mg/kgBB gave the best result of 9.33, close to normal conditions. Histology, a dose of 400 mg/kgBB is more effective in normalizing gamete cell count and reducing inflammatory signs in the testicles of diabetic rats.



**Figure 2. Skoring Johnsen Tubulus Seminiferus (HE, 400x)**

Source: Primary Data Analysis by Researcher, 2025

**Remarks:** (A) Normal group: it appears that spermatogenesis is still complete among spermatogonium (green arrow), primary spermatocyte (orange arrow), and spermatozoa (blue arrow). (B) Negative group/diabetic mice: appears to be a more roomy lumen with fewer germ cells. (C) The group given a dose of grasshoppers of 200 mg/KgBB: it appears that the germ cells are starting to improve. (D) The group given a dose of grasshoppers of 400 mg/KgBB: it appears that spermatogenesis has improved again as normal group.

Green grasshopper leaf extract at doses of 200 mg/kgBW and 400 mg/kgBW showed a significant improvement in Johnsen scoring, with the 400 mg/kgBW dose providing the best results, close to the normal control. Bioactive compounds, especially flavonoids, play a major role in neutralizing free radicals, reducing oxidative stress, and protecting spermatogenic cells from damage caused by Reactive Oxygen Species (ROS) (Santoso, 2017). Flavonoids act by preventing apoptosis, supporting tissue regeneration, and increasing the production of Leydig cells as well as spermatogenic cells, which are essential for improving reproductive function. By reducing ROS levels and enhancing hormonal signals such as LH, herbal plant extracts not only aid in tissue recovery but also prevent the adverse effects of hyperglycemia on the reproductive system, including oligozoospermia and infertility. The cytoprotective effects of these flavonoids make herbal plant extracts a potential therapeutic agent for repairing reproductive damage caused by diabetes mellitus (Octavyani et al., 2022). Thus, green grasshopper extract has the potential to serve as a phytotherapeutic agent for repairing reproductive damage due to diabetes mellitus.

### 3. Spermatozoa Morphology

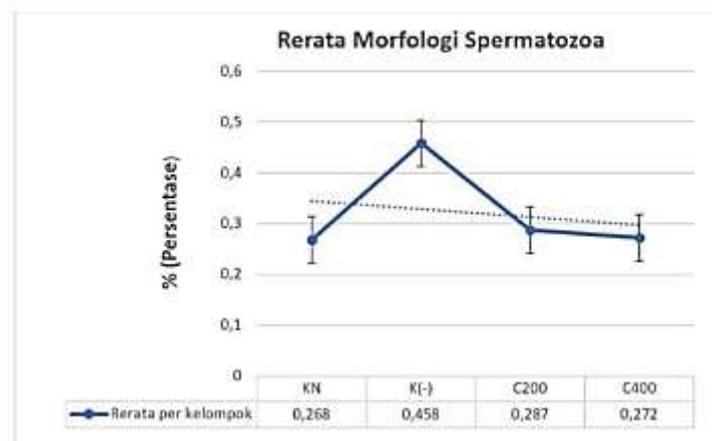
The results of the morphological analysis of spermatozoa showed a difference between the treatment group and the negative control. In diabetic mice, the proportion of spermatozoa morphological abnormalities was higher (0.458%) than in the normal group (0.268%). The administration of green grasshopper extract at a dose of 200 mg/kgBB reduced the abnormality to 0.287%, while the dose of 400 mg/kgBB was more effective with a value of 0.295%.

**Table 3. Descriptive Results of Spermatozoa Morphology**

Groups	Total	MEAN ± SD
Control Normal	6	0,268 ± 0,090
Diabetic Rats	6	0,458 ± 0,155
Cincau 200	6	0,287 ± 0,221
Cincau 400	6	0,272 ± 0,140

Source: Primary Data Analysis by Researcher, 2025

Description: Normal Control = Normal Rat, Negative Control = Diabetic Rat,  
Grasshopper 200 = Rats with a dose of Grasshopper 200 mg/KgBB,  
Grasshopper 400 = Rats with a dose of Grasshopper 400 mg/KgBB.



**Figure 3. Average Morphology of Spermatozoa**

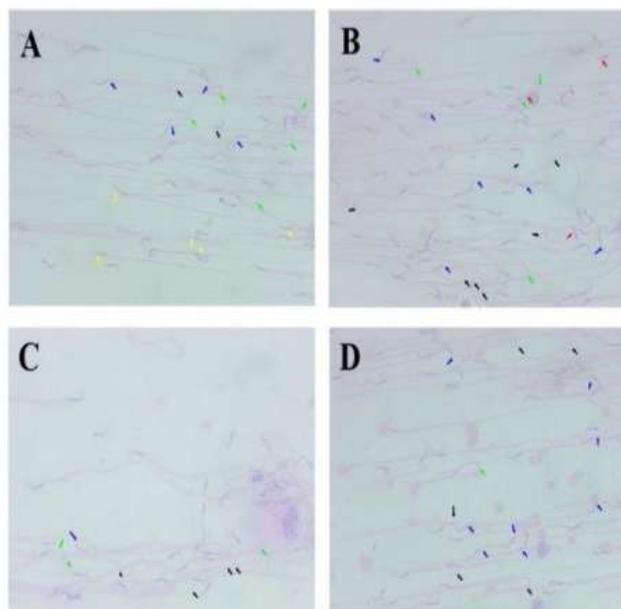
Source: Primary Data Analysis by Researcher, 2025

Description: Normal Control = Normal Rat, Negative Control = Diabetic Rat,  
Grasshopper 200 = Rats with a dose of Grasshopper 200 mg/KgBB,  
Grasshopper 400 = Rats with a dose of Grasshopper 400 mg/KgBB.

These results indicate that chronic hyperglycemia conditions in diabetic mice can increase spermatozoa morphological abnormalities through oxidative stress mechanisms, germ cell damage, and spermatogenesis disorders. The content of flavonoids, polyphenols, and antioxidants in green grasshopper leaf extract plays an important role in suppressing oxidative damage and stabilizing spermatozoa quality. Overall, the administration of green grasshopper leaf extract, both at a dose of 200 mg/kgBB and 400 mg/kgBB, was proven to be able to repair the morphological damage of spermatozoa in diabetic rats. Although the difference between

Daun Cincau Hijau (*Premna Oblongifolia*) Has The Potential To Improve Spermatozoa Quality In Hyperglycemic White Rats (*Rattus Norvegicus*)

the two doses is not too far, the 400 mg/kgBB dose tends to provide more consistent results close to the normal group.



**Figure 4. Spermatozoa Morphology  
(HE, 400x)**

Source: Primary Data Analysis by Researcher, 2025

Description: A = Normal control group or normal rats, B = Negative control group or diabetic rats, C = Mice with a grasshopper dose of 200 mg/KgBB, D = Rats with a grasshopper dose of 400 mg/KgBB. Black Arrow = Abnormal spermatozoa characterized by a circular tail, Blue Arrow = Abnormal spermatozoa characterized by neck forming an angle, Green Arrow = Abnormal spermatozoa characterized by body forming an angle, Yellow Arrow = Abnormal spermatozoa marked with a head like a banana, Red Arrow = Abnormal spermatozoa that characterized by an unrelated head.

Improvement in spermatozoa morphology in hyperglycemic mice is closely related to the flavonoid and antioxidant content of green grasshopper leaves (*Premna oblongifolia*). These bioactive compounds play a crucial role in suppressing the formation of Reactive Oxygen Species (ROS), which are known to damage DNA, disrupt cell membranes, and interfere with spermatogenesis. In addition, flavonoids can enhance the activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, which contribute to maintaining spermatozoa viability (Octavyani et al., 2022). Previous research has shown that flavonoids exert cytoprotective effects by suppressing apoptosis and promoting testicular tissue regeneration (Santoso, 2017). This supports the finding that administration of green grasshopper extract, particularly at a dose of 400 mg/kgBW, is effective in reducing morphological abnormalities of spermatozoa to near-normal conditions.

Phytochemically, green grasshopper leaves contain flavonoids that act not only as antioxidants but also as antidiabetic agents. Flavonoids can modulate glucose metabolism,

protect pancreatic  $\beta$  cells, promote proliferation, and stimulate insulin secretion. This role is significant because chronic hyperglycemia increases ROS production, damages seminiferous tubules, and triggers germ cell apoptosis, thereby causing abnormal spermatozoa morphology. Furthermore, diabetes often lowers testosterone, FSH, and LH levels, which impairs Sertoli and Leydig cell function, decreases spermatogenesis, and reduces libido (Arundani et al., 2021). Thus, green grasshopper extract has the potential to serve as a phytotherapeutic agent that not only lowers blood glucose levels but also improves spermatozoa morphology and mitigates diabetes-related complications in the male reproductive system through antioxidant, cellular protection, and hormonal regulation mechanisms.

## CONCLUSION

Green grasshopper leaf extract has the potential to serve as a phytotherapeutic agent in reducing the impact of diabetes mellitus, particularly in relation to glucose metabolism and male reproductive health. Based on the results of the study, it can be concluded that green grasshopper leaf extract (*Premna oblongifolia*) has a positive effect on hyperglycemic white rats (*Rattus norvegicus*). This extract has been proven to lower blood glucose levels during GDS, reduce the number of morphologically abnormal spermatozoa—with the best effectiveness observed at a dose of 400 mg/kgBW—and provide a protective effect by restoring the Johnsen score to near-normal conditions.

Based on these findings, several suggestions are proposed for future research and application. First, it is recommended to conduct further studies with longer treatment durations and a broader range of dosages to determine the optimal therapeutic dose and long-term effects of green grasshopper leaf extract. Second, advanced research should aim to isolate and identify the specific bioactive compounds (such as particular flavonoids or polyphenols) responsible for the observed antidiabetic and spermatoprotective effects. Third, investigations into the molecular mechanisms of action—including the extract's influence on specific antioxidant pathways (e.g., superoxide dismutase [SOD], catalase activity), hormonal regulation (FSH, LH, testosterone), and apoptosis signaling in testicular cells—would provide deeper insight. For practical application, preclinical toxicological studies should be carried out to ensure the extract's safety profile before developing it into a standardized herbal supplement. Finally, the results of this study may be shared with the community as preliminary scientific information about the potential of local plants, thereby supporting further exploration of natural ingredients for managing diabetes and its complications.

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