**Effect of Aqueous Extract of Celery Leaves on Fertility in Diabetic Male Rats**

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**Abstract:**

The presented study delves into the potential effects of an aqueous extract of celery leaves on spermatogenesis in male rats with diabetes. **Objective**. Celery has a history of traditional use in alternative medicine for various conditions, including impotence. This study aims to explore how an aqueous extract of celery might impact spermatogenesis, lipid profiles, and liver enzyme levels in male rats with diabetes. **Methodology**: The study involved 24 male rats, divided into three groups, each comprising eight rats: Control Group: Normal rats receiving a daily oral dose of 1 ml of distilled water. Diabetic Group: Diabetic rats also receiving a daily oral dose of 1 ml of distilled water. Celery Extract Group: Diabetic rats treated with 200 mg/kg b.w. of celery extract daily for 30 consecutive days. **Results:** Spermatogenesis, The group treated with 200 mg/kg of celery extract exhibited significant increases in the diameter of seminiferous tubules and spermatid count compared to the control group. Testis Volume and Organ Weights, The treated group demonstrated significant increases in testis volume, as well as the weights of the testes, cauda epididymis, and vas deferens, Glucose and Lipid Profile, The celery extract had notable effects on glucose and triglyceride levels in the diabetic rats. Liver Enzymes, The extract also had a discernible impact on liver enzyme levels, specifically AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase). **Conclusion**: The findings suggest that the aqueous celery extract holds potential positive effects on various aspects in male rats with diabetes, including spermatogenesis, reproductive parameters, glucose levels, lipid profiles, and liver enzymes. This implies that celery might offer benefits for enhancing male fertility and addressing diabetes-related complications. Nevertheless, further comprehensive research is warranted to elucidate the underlying mechanisms responsible for these effects and to validate the findings.

**Keywords:** celery, spermatogenesis, Diabetic and liver

**Introduction**

In developed nations, traditional and herbal medicines have gained widespread use in the treatment of various ailments, including wound healing, hypertension, diabetes, and reproductive issues (Modaresi, 2012). The interest in studying the impact of different plants on the fertility of laboratory animals has grown, yielding valuable insights (Tala'a et al., 2020).Celery, an herbaceous biennial plant belonging to the parsley family (Umbelliferae), has garnered attention. It typically grows to a height of 20 to 60 cm with a branched stem (Nasri et al., 2009). The leaves of celery encompass a range of compounds, such as valerophenone (19.90%), 1-dodecanol (16.55%), and 9-octadecanoic acid, and methyl ester (4.93%) (Nagella et al., 2012). Notably, celery leaf and root juice exhibit favorable effects on various biochemical parameters. These include the reduction of glutathione content, catalase, xanthine oxidase, glutathione peroxidase, and peroxidase activities, influencing lipid peroxidation levels in both the liver and blood. Furthermore, there's evidence that the extract offers protection when co-administered with doxorubicin (Kolarovic et al., 2009).Research also indicates that celery extract plays a role in modulating serum levels of total cholesterol and low-density lipoprotein (LDL), while concurrently increasing hepatic triglyceride levels. This effect is achieved through the reduction of hepatic triacylglycerol lipase activity in individuals (Mansi et al., 2009). Given that fatty acids are recognized as integral to spermatogenesis (Taati et al., 2011), the potential implications of these extracts on this process are noteworthy. Moreover, celery extracts exhibit a range of other beneficial properties, including anti-inflammatory, anticancer, anti-hepatotoxic, anti-hypercholesterolemic, analgesic, antibacterial, and anti-spasmodic effects (Modaresi et al., 2012). Additionally, their impact on smooth muscle contractions has been documented (Gharib Naseri et al., 2007). In traditional medicine, celery is acknowledged as an appetite and libido stimulant (Khosravi, 2006), and historical accounts suggest it enhances breast milk secretion (Fluke, 2005). Notably, celery extracts display protective effects against sodium valproate in the testes (Hamza and Amin, 2007), and they have been found to promote improvements in sperm parameters, contributing to beneficial effects on the testes (Kerishchi et al., 2011). Considering these diverse properties, the primary aim of this study was to probe the potential influences of celery extract on spermatogenesis and histological alterations in the testes of male rats.

**Materials and Methods:**

**Plant Material:**

Celery (Apium graveolens L.) leaves were obtained from a local market in Mafraq, Jordan.

**Preparation of Aqueous Extract:**

The celery leaves were washed, dried, and then ground into a fine powder. Aqueous extraction was performed by adding 200 g of the powder to 1 L of distilled water, followed by heating at 60°C for 2 hours. The extract was filtered, concentrated, and then freeze-dried to obtain a powder.

**Induction of Diabetes**

Diabetes was induced in the rats by a single intraperitoneal injection of streptozotocin (STZ) dissolved in citrate buffer (pH 4.5) at a dose of 60 mg/kg body weight (b.w.).

**Experimental Design:**

Twenty-four adult male Wistar rats, aged 8 weeks and weighing between 170-220 g, were used for the study. The rats were obtained from the animal reproduction and breeding center of Applied Sciences Private University. Ethical guidelines of the Al al-Bayt University Research Ethics Committee were followed for all procedures involving animals and their care. The rats were housed in standard cages at a temperature of 22±2°C with a 12-hour light/12-hour dark cycle. They had free access to water and standard food throughout the experiment. The rats were acclimated to the housing conditions for 24 hours before the experiment began.

The rats were randomly divided into three groups, each comprising eight rats:

1. Control Group: Normal rats receiving a daily oral dose of 1 ml of distilled water.

2. Diabetic Group: Diabetic rats also receiving a daily oral dose of 1 ml of distilled water.

3. Celery Extract Group Diabetic rats treated with 200 mg/kg b.w. of celery extract daily for 30 consecutive days.

The rats received their respective treatments orally for 30 consecutive days. The celery extract was prepared by diluting the calculated weight of the extract with distilled water to achieve a final volume of 1 ml per rat. After the final administration of the celery extract, the rats were anesthetized on the following day. A longitudinal incision was carefully made across the abdomen, scrotum, and testis. This incision allowed access to the reproductive structures. The ducts of the attached epididymis were then surgically detached. Precise measurements of the testicular dimensions including length, width, and volume were taken to assess potential effects of the celery extract. Moving on to the epididymis sperm count, the cauda epididymis, a part of the male reproductive system, was meticulously isolated. From this isolated portion, spermatozoa (sperm cells) were extracted from the tubules and then homogenized to ensure an even distribution. The resulting sperm suspension was suitably diluted using physiological saline solution. Subsequently, a technique called sperm counting was performed. This involved placing a diluted sample of the sperm suspension within a specialized chamber called a hemocytometer. The hemocytometer, when viewed under a light microscope, enabled the accurate enumeration of sperm cells present in the sample. This count provides valuable information about the quantity of sperm and potential reproductive health.

**Assessment of Spermatogenesis:**

At the end of the treatment period, rats from each group were euthanized under anesthesia using ketamine/xylazine, and their testes were removed and weighed. Spermatogenesis was assessed by measuring the diameters of seminiferous tubules, spermatocytes, spermatids, and spermatozoids using a calibrated eyepiece graticule under a light microscope. The diameters of seminiferous tubules and the count of spermatids were determined.

**Assessment of Testis Volume and Organ Weights:**

Testis volume was measured using the water displacement method. The weights of the testes, cauda epididymis, and vas deferens were recorded.

**Assessment of Glucose and Lipid Profiles:**

Blood samples were collected from the rats at the end of the experimental period to measure glucose, cholesterol, and triglyceride levels. Fasting blood glucose levels were determined using a standard glucometer. Serum cholesterol and triglyceride levels were assessed using enzymatic colorimetric methods.

**Assessment of Liver Enzymes:**

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined using commercial assay kits.

**Statistical Analysis:**

Data were analyzed using SPSS version 20.0. Results were expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. p-values less than 0.05 were considered statistically significant.

**Results:**

The study unveiled significant impacts of a 200 mg/kg dose of aqueous celery extract on spermatogenesis in male diabetic rats. As detailed in Table 1, substantial increases were observed in the diameter of seminiferous tubules, spermatocyte counts, spermatogonia counts, and spermatozoa (cauda epididymis) counts when compared to the control group (p≤0.001). Additionally, the group treated with 200 mg/kg of celery extract exhibited a significant boost in spermatid counts compared to the control group (p≤0.05). Moreover, a statistically significant increase in the mean diameter of seminiferous tubules was noted (p≤0.05).

Table 2 illustrates the effects on testis volume and organ weights after 30 days of treatment with the same celery extract. It was observed that testis volume significantly increased in the group treated with 200 mg/kg of celery extract compared to the control group (p≤0.001). Furthermore, the weights of the testes, cauda epididymis, and vas deferens showed increments. Notably, the weight of the epididymis in the group treated with 200 mg/kg of celery extract demonstrated a statistically significant increase compared to the control group (p≤0.05).

In summary, these results suggest that the aqueous extract of celery leaves exerts positive effects on spermatogenesis, and reproductive parameters in male diabetic rats. Additionally, Table 3 highlights significant reductions in glucose levels and triglycerides at p≤0.001, while Table 4 demonstrates significant decreases in liver enzymes, ASL, and ALT at p≤0.001. These findings provide valuable insights into the potential benefits of celery in improving male diabetic fertility and call for further exploration and research in this area.

**Discussion:**

Our study offers significant insights into the potential effects of celery extract on spermatogenesis and reproductive parameters in diabetic male rats. Medicinal herbs have gained popularity as alternatives to chemical drugs due to their accessibility, reduced side effects, lower toxicity, and cost-effectiveness (Shamsa et al., 2009). Consistent with this perspective, our study reveals substantial changes in experimental groups following the administration of celery extract when compared to the control group. Our findings demonstrate that administering celery extract at a dose of 200 mg/kg orally to diabetic male rats over a 30-day period resulted in noteworthy increases in the diameter of seminiferous tubules, spermatogonia, spermatocytes, spermatozoa counts, and testis volume. These results align with traditional beliefs regarding the potential of celery extract to enhance male sexual performance. The observed increase in testis weight and size suggests an increase in cell numbers within the testis, possibly linked to the modulation of sex hormone levels, as sexual organ weights are known to be influenced by sex hormones (Juan et al., 2005; Khan et al., 2004, Soliman et al., 2020). Celery extract may influence the pituitary gland, leading to heightened sex hormone secretion and subsequent effects on testicular and epididymal functions (Parandin et al., 2009; McLachlan et al., 2002). The increased number of spermatozoa in the cauda epididymis, along with the elevated epididymal weight at the higher extract dose, further supports the potential impact of celery extract on reproductive functions.

Divergent findings in the literature regarding celery extract's effects on male reproductive hormones could be attributed to dose-dependent effects and variations in the solubility profiles of its compounds. The complex composition of celery extracts may result in differing impacts on the reproductive system, depending on administration routes and specific compound concentrations (Modaresi et al., 2012). The observed protection of testicular tissue against the toxic effects of sodium valproate, attributed to celery's antioxidant properties and apigenin content, highlights the potential of celery extract (Hamza and Amin, 2007).

In summary, our study provides evidence of the potential positive influence of celery extract on spermatogenesis and reproductive parameters in diabetic male rats. However, deeper mechanistic insights and optimal dosages for potential therapeutic applications in human fertility and reproductive health require further investigation. Furthermore, our findings in Table 3 underscore celery extract's potential benefits for diabetic rats, evident by the reduction in glucose and triglyceride levels, which are often elevated in diabetes. The extract's less pronounced impact on cholesterol levels aligns with previous research, highlighting the antidiabetic and lipid-lowering effects of celery components due to their antioxidative and anti-inflammatory properties (Zhao et al., 2021; Ingallina et al., 2020).

Moreover, the outcomes presented in Table 4 indicate celery extract's hepatoprotective potential, as evidenced by the reduction of elevated liver enzyme levels (AST, ALT, ALP) in diabetic rats. These observations are consistent with prior studies showcasing celery's ability to protect the liver from oxidative stress and inflammation (Jun Yan et al., 2022; Turner et al., 2021).

In conclusion, our study demonstrates the potential of celery extract to positively affect reproductive parameters and metabolic health in diabetic rats. These observed effects underscore celery's multifaceted beneficial attributes, warranting further exploration into the underlying mechanisms and its potential applications in human health and therapeutics.

**Conflict of Interest:**

The authors declare no conflicts of interest.

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**Table 1: Effects of Aqueous Extract of Celery on Spermatogenesis in Diabetic Rats**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Groups | Spermatids(mm 2 ) | Spermatocytes(mm 2 ) | Spermatogonia(mm 2 ) | Spermatozoids(10 7 ) | Seminiferous tubules diameter (µm) |
| (control) | 14.66±0.03 | 13.51±0.03 | 12.57±0.02 | 4.12±0.03 | 0.34±0.004 |
| Diabetic rats | 12.6±0.17 | \*12.28±0.28 | \*\*10.72±0.2 | \*\*2±0.17 | \*\*0.21±0.001 |
| Diabeticrats fed Celery (200mg/k g)  | \*15.1±0.02 | \*14.23±0.05 | \*\*13.68±0.22 | \*\*4.55±0.17 | \*\*0.38±0.006 |

\*p≤0.05, \*\*p <0.001.

Table 2: Effects of Aqueous Extract of Celery on Testis Volume and Organ Weights in Diabetic Rats. (Mean ±SEM).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Groups | Testis volume (mm 3 10 -3 ) | Testis (mg) | Cauda epididymis (mg) | Vas deferens (mg) |
| (control) | 12.86±1.9 | 1502.4±4.98 | 459.4±4.64 | 92±0.29 |
| Diabetic rats | \*\*9.89±1.6 | 1210±5 | 298.4±4.64 | 75.5±55.17 |
| Diabetic Rats fed Celery(200mg/k g)  | \*\*13.77±7.5 | 1610±3.79 | \*548.2±6.28 | 96.7±2.9 |

\*p≤0.05, \*\*p <0.001.

**Table (3): Levels of lipid profiles after consumption of aqueous extract of celery on diabetic rats presented as mean ± SE.**

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | Glucose(mg/dl) | Cholesterol (mg/dl) | Triglycerides (mg/dl) |
| (control) | 104.7 ±2.92a | 62.23 ±2.7 | 76.56 ±0.62 |
| Diabetic rats | 211.13 | 90.77 | 166.4 |
| Diabetic rats fed Celery (200mg/k g) | \*\*140.6±0.134 | \*\*87.6±2.55 | \*\*102.3±0.21 |

\*p≤0.05, \*\*p <0.001.

**Table (4): Levels of Liver Enzyme after consumption of aqueous extract of celery on diabetic rats presented as mean ± SE.**

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | AST(U/L) | ALT(U/L) | ALP.(U/L) |
| (control) | 25.26±0.24 | 12.5± 0.596 | 40.38±0.618 |
| Diabetic rats | 49.4 | 25.65 | 80.49 |
| Diabetic rats fed Celery (200mg/k g)  | \*\*33.268±0.219 | \*\*15.4±0.416 | \*\*45.58±0.163 |

\*p≤0.05, \*\*p <0.001.

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