Antifertility Activity of Ethanolic Seed Extract of Celery (*Apium graveolens* L.) in Male Albino Rats

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ABSTRACT

Objectives: This study was aimed at evaluating the effect of ethanolic seed extract of *Apium graveolens* L. (Apiaceae) (ESEAG) on male rat fertility.

Methods: Two doses of the extract (425 and 213 mg/kg body weight) were administered by oral gavage for sixty consecutive days. Five days before the end of this period, each rat was cohabited with two female rats. Fertility indices, hematology, and organ weight and histology were then assessed.

Results: The ESEAG arrested spermatogenesis and caused a marked, dose-dependent decrease in sperm count, cauda epididymal sperm motility, blood testosterone concentration, weight of testes and seminal vesicles, testicular protein content as well as diameter and viability of seminiferous tubules. In addition, a lower number and weight of viable fetuses was obtained for female rats which were impregnated by ESEAG-treated male rats. Hematological parameters, serum liver enzyme levels, thyroid weight and liver and kidney histoarchitecture were not affected.

Conclusion: This study shows a dose-dependent antifertility effect of the ESEAG in male rats without toxic effects on other body organs.

Keywords: *Apium graveolens*, male fertility, sperm count and motility, seminiferous tubules.

INTRODUCTION

*Apium graveolens* L. (Apiaceae), also known as celery and known in Jordan as “kufs”, is an annual or biennial strongly aromatic herb. It is used as a condiment, carminative, emmenagogue, antiseptic, and diuretic and for the treatment of bronchitis, asthma, rheumatism, arthritis, constipation, testis pains, gout pain as well as liver and spleen disorders. The methanolic extract of celery seeds, containing apigenin, was found to have a potential anticarcinogenic effect in chemically-induced hepatocarcinoma through suppression of angiogenesis and cell proliferation.

In Jordan, laymen and herbalists prescribe the seeds of celery to increase male sexual activity. However, a variety of plant extracts have been shown to have antifertility effects in mice or rats, an effect that is often undesirable if it occurs in human beings undeliberately.

rhizomes, Aeglemarmelos Corr., Cassia fistula seeds, and Terminalia chebula fruits, among others.

The petroleum ether, alcoholic and aqueous extracts of Apium graveolens L showed no anti-implantation activity in female albino rats. However, no study evaluating the male antifertility effect of A. graveolens is available in the literature. Hence, the aim of this study was to investigate the effect of the ESEAG on several fertility endpoints, organ weight and architecture, and hematological parameters in male rats.

MATERIALS AND METHODS

Plant Processing and Screening of Chemical Constituents

Brown carmocarp seeds of A. graveolens were purchased from the local market (Amman). They were processed using a Soxhelt apparatus with 96% ethanol for two hours. The yield of the ethanolic extract was 6.26% (w/w). The extract was analyzed using TLC silica gel plates to identify the major active components.

Determination of the Lethal Dose of ESEAG

A total of 80 male albino mice (Mus musculus, JVI-1) weighing 20-21g were used to determine LD50 of the ESEAG. The mice were divided into ten groups, each consisting of eight mice and receiving no treatment (control), vehicle (tween 20 + normal saline, 1:1), 2500, 3000, 3500, 4000, 4250, 4500, 4750, and 5000 mg ESEAG/kg mouse body weight. The treated mice were gavaged with one milliliter of the treatment solution (or vehicle) and survival recorded for 24 hours. The animals were observed for the completion of one week to evaluate adverse effects of the ESEAG and detect any changes in their behavior.

Evaluating the Effect of A. graveolens Extract on Male Rat Fertility

Animal Grouping and Treatment

A total of 40 Sprague-Dawely male albino rats and 80 female albino rats (Rattus norvegicusJU-1) weighing 245-265 g were used. The male rats were obtained from the animal house of the Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan. The female rats were obtained from the animal house, Department of Biological Sciences, University of Jordan, Amman, Jordan. All animal procedures were conducted in accordance with Jordanian Regulations for Animal Experimentation and Care and were approved by the Institutional Animal Care and Use Committee.

The male rats were divided into 4 groups, each consisting of ten animals and receiving no treatment (control), vehicle (tween 20 + normal saline, 1:1), and two doses of the extract equal to 1/10th and 1/20th the determined LD50 (425 and 213 mg ESEAG/kg rat body weight, respectively). The treated rats were gavaged with one ml of the treatment solution (or vehicle) daily for sixty consecutive days (WHO, 1983).

Fertility Test

On day 55 of the experiment, each male rat was cohabited with two adult proven female rats in mating cages overnight. The females were removed from the cages during the day time to avoid decline of sexual behavior associated with continuous cohabitation with the males. This process was conducted for five consecutive days during which one complete estrous cycle in female rats should have elapsed. The day in which a vaginal plug appeared was considered day one of the pregnancy. Then on day 17 of the pregnancy the females were sacrificed. The uteri were examined, number of viable fetuses and resorption sites counted, and fetuses were freed from the surrounding membranes and blotted dry using filter paper to determine their weight.

Sperm Count and Motility

At the time of euthanasia, cauda epididymides were taken immediately and minced into two halves to release the epididymal content into a 35-mm Petri dish containing 2 mL phosphate buffer (0.1 M, pH 7.4). A drop of the solution was put on a Neubauer chamber to assess sperm motility. Both motile and immotile spermatozoa were counted in different fields to determine the percentage of motile sperms. Sperm count was determined in the same way, with the exception that 1% formalin solution was used instead of phosphate buffer
Antifertility Activity of...

and the count was expressed as millions of cells/mm$^3$. To maintain activity of the viable sperms, all tools, containers and surfaces used in the experiments were kept at a temperature of 37°C.

**Male Autopsy**

At the end of the treatment schedule the weights of the animals were recorded, and then blood samples were taken by heart puncture for hematological, biochemical, and hormonal studies. Then the animals were sacrificed using an overdose of ether anesthesia. The testes, seminal vesicles, kidneys, livers, thyroid glands, and adrenal glands were dissected, blotted free of blood and weighed.

**Hematological and Biochemical Studies**

Erythrocyte count, leukocyte count, packed cell volume (PCV), and liver enzymes (AST and ALT) were determined for each rat blood sample. The protein content of one testis of each rat was determined using Lowry's method.22

**Testosterone Analysis**

Serum level of testosterone was determined using a free testosterone enzyme immunoassay test kit (DiaMetra, Foligno, Italy).

**Histological Studies**

Testes, livers, and kidneys were cut into pieces, fixed in Bouin's fluid, dehydrated in graded ethanol series, cleared in xylene and infiltrated using paraffin wax. Resulting tissues were sectioned at 5 µm and stained in hematoxylin and eosin.

To evaluate the effect of the ESEAG on spermatogenesis, the percentages of normal and affected seminiferous tubules were determined for seven randomly selected tubules from seven different rats in each group. In addition, the diameter of 10 randomly selected seminiferous tubules was averaged for each rat in each group using an ocular micrometer, and then the average diameter for the group was determined.

**Statistical Analysis**

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s test for the comparison of group means. The effect of the ESEAG was evaluated by comparing the results of the ESEAG-treated groups with those of the vehicle-treated group. Differences were considered significant at p<0.05.

**RESULTS**

**Chemical Constituents of the ESEAG**

Using TLC to analyze the active constituents of the ESEAG, the main components were flavonoids and terpenoids, followed by lesser amounts of coumarins. No alkaloids were detected.

**The LD$_{50}$ of the ESEAG**

The LD$_{50}$ of the ESEAG was 4250 mg/kg, hence the choice of the two doses in the experiments to be equal to 1/10th and 1/20th of the determined LD$_{50}$ (425 and 213 mg ESEAG/kg rat body weight, respectively). No behavioral change or sign of adverse extract effect was noticed in the surviving mice for the observational week.

**Effect of the ESEAG on Male Rat Fertility**

There was a significant ($p<0.05$) and dose-dependent decrease in the fertility rate of female rats mated with the ESEAG-treated male rats and in the weight of their fetuses. The number of viable fetuses decreased but failed to show dose-dependence. On the other hand, the extract increased the number of resorption sites (Figure 1 and Table 1).

**Effect of the ESEAG on Animal Body and Organ Weights**

Both doses of the ESEAG decreased the weight of seminal vesicles and testes when compared with the vehicle ($p<0.05$). Neither treatment had a significant effect on the total body weight, or the weight of kidney, adrenal gland or thyroid gland (data not shown). However, only the higher dose increased liver weight (10.57± 2.77 vs. 8.10± 1.59 for ESEAG 425 mg/kg and vehicle, respectively).

**Effect of the ESEAG on Sperm Count and Motility**

The ESEAG decreased sperm count and motility dose-dependently (Table 3). The sperm motility was reduced.
more profoundly than absolute sperm count.

**Effect of the ESEAG on the Histology of Seminiferous Tubules, Liver and Kidneys**

Histology of both the liver and the renal cortical region in the ESEAG-treated groups was not different from that of the control and vehicle groups (data not shown). However, the extract caused a dose-dependent decrease in the percentage of normal seminiferous tubules as well as the tubular diameter (Table 4). Figure 2 shows the effect of the ESEAG on the histoarchitecture of representative seminiferous tubules; while the lower dose (213 mg/kg) caused reduction in the spermatogenesis process, the higher dose (425 mg/kg) caused a complete loss of germinal cells and damage in the surrounding supporting cells.

**Effect of the ESEAG on Hematological and Biochemical Markers and Serum Testosterone**

The ESEAG had no effect on red and white blood cell counts, hematocrit or liver function enzymes (GOT and GPT). However, it decreased serum testosterone concentration and protein content of testes dose-dependently. The testicular protein content of the rats treated with the high dose was about one half that of the vehicle group (Table 2).

**DISCUSSION**

Recently, there has been an increased interest in exploring the antifertility effect of plant extracts, including a male antifertility effect. This effect can be viewed in two different ways. It can be perceived as an environmental factor contributing to the deteriorating male fertility. However, it can also be viewed as a convenient and relatively safe method of contraception, especially that contraception with a ‘male pill’ remains a distant prospect due to its toxicity and inconvenience.

In this study, we have shown that the ethanolic seed extract of *A. graveolens*, administered for 60 days, had a dose-dependent antifertility effect in male albino rats. The decrease in the fertility rate caused by the ESEAG (Fig. 1 and Table 1) is in agreement with the decrease in sperm count and motility caused by the treatment (Table 3). However, it is noticeable that the number of pregnancies was affected more profoundly than sperm count and motility, especially at the lower extract concentration. This may indicate that the extract affects the fertilizing capacity of the spermatozoa through an effect that is independent of decreasing sperm motility, so that this effect contributes additively to decreased sperm count and motility, in decreasing fertility. One potential mechanism is the inhibition of hyaluronidase activity in rat spermatozoa, which decreases their ability to penetrate ova. A fact that supports this possibility is the high content of flavonoids in *A. graveolens*, as shown in our study (section 4.1 above) and in another study. This is in concordance with the note that the ability of *Terminalia chebula* fruit extract to inhibit hyaluronidase activity was attributed to its high flavonoid content.

Not only was the number of fetuses reduced by the ESEAG, but the average fetus weight was also decreased dose-dependently (Table 1). The average fetus weight for females impregnated by male rats treated with the high ESEAG dose was about 1/3rd that of the vehicle group. Such a pathological development of the embryos is usually attributed to the poor sperm quality in treated males. The alterations in sperm motility and viability might have resulted from disturbances in the function and microenvironment of the testes and epididymis, increased apoptosis, decreased gonadotropins, decreased FSH, or decreased testosterone. The decrease in testosterone concentration reached statistical significance only at the higher dose of the ESEAG (Table 2) and can be due to one or more mechanisms. The mechanisms shown for plant extracts include inhibition of gonadotropin secretion, interference with steroidogenesis at the testicular level, or decreased progesterone concentration.

This decrease in testosterone concentration, however, seems contradictory with the traditional use of celery seeds for increasing male sexual performance, since decreased testosterone synthesis below normal values is generally correlated with decreased sexual performance. This contradiction may be cleared by...
assuming that the effect of the extract is dose-dependent, with the lower concentration applied traditionally being augmentory for testosterone concentration and, therefore, sexual performance, whereas the higher dose of 425 mg/kg body weight tested in our study mitigates testosterone synthesis. We can find support for this presumption in a study of the aqueous extract of *Bulbinenatalensis* stem, where doses of 25 and 50 mg/kg body weight enhanced the mating, fertility rate and testosterone concentration in male rats, whereas the extract dose of 100 mg/kg body weight decreased these fertility outputs.34

The ESEAG also decreased testicular weight dose-dependently (Table 2). This decrease is due to the ability of the ESEAG to decrease testicular protein content (Table 2), germ cell number, and secretory components in the treatment group.

Histopathologically, the ESEAG caused a drastic and dose-dependent decrease in the number of spermatozoa, arrest of spermatogenesis, reduction in the diameter of seminiferous tubules, reduction in the percentage of normal seminiferous tubules and loosening or destruction of germinal epithelium (Fig. 2, Table 4). These results are in concordance with the decreased testicular weight since the tubules and germinal elements account for approximately 90% of the wet weight of the normal rat testis.35 The changes, however, were not uniform as both affected and normal seminiferous tubules were observed in the same section of the testis. The detection of flavonoids and terpenoids as the active constituents of ESEAG in this study is in agreement with the results of a phytochemical study in which triterpenoids and flavonoids were isolated and elucidated the structures of the whole plant of fresh *A. graveolens*.26

The effects of the ESEAG appear to be specific to the reproductive system and not accompanied with systemic toxicity in the rats. This is evident from the lack of change in the whole body and organ weights (Table 2) and kidney and liver histology, as well as hematologic and serologic parameters (Table 4). One study showed that D-Limonene, a monocyclic monoterpenes found in the celery seeds induced hyaline nephropathy.36 However, we did not find such an effect in our study. The reason for this contradiction could be that the mentioned study used a concentration of the compound much higher than that found in our extract. We have also noticed no change in rat behavior or activity during the experimental period.

Finally, future studies are required to evaluate the antifertility effects of singular constituents of the ESEAG, the mechanism of their antifertility action, dose-dependency of their anti-testosterone action and the reversibility of the antifertility effect of the ESEAG.

**ACKNOWLEDGMENTS**

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Figure 1: Uteri of female rats impregnated by vehicle and ESEAG-treated male rats
(A) Uterus of a female rat impregnated by a vehicle-treated male rat. The uterus contains nine viable fetuses. (B) Uterus of a female rat impregnated by a male rat treated with 213 mg/kg ESEAG. The uterus contains five viable fetuses and several resorption sites, one of which is pointed at with the arrow. (C) Uterus of a female rat impregnated by a male rat treated with 425 mg/kg ESEAG. The uterus contains three viable fetuses and many resorption sites.

Figure 2: Histopathological changes in seminiferous tubules due to ethanolic seed extract of A. graveolens (Magnification: 200X) (A) and (B) Cross sections of seminiferous tubules from control and vehicle-treated rats, respectively. The sections contain germinal cells of all stages of maturation and spermatozoa. (C) Tubular section from a rat treated with 213 mg/kg ESEAG showing seminiferous tubules with germinal epithelium containing different stages of spermatogenesis but with lower density than the control or vehicle-treated rats. Note loosening of germinal epithelium. (D) Tubular section from a rat treated with 425 mg/kg ESEAG showing severe damage in the tubules and loss of germinal cells. Note appearance of intraepithelial vacuoles (arrows) in the seminiferous tubules.
Table 1: Fertility parameters for control, vehicle, and ESEAG-treated rats (Mean± SD for 20 mated female animals)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fertility rate in mated females</th>
<th>Average no. of viable fetuses/rat</th>
<th>Average fetus weight</th>
<th>Number of resorption sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17/20 (85.0%)</td>
<td>7.2± 3.4</td>
<td>4.45± 0.84</td>
<td>3.8± 3.2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>17/20 (85.0%)</td>
<td>8.4±3.9</td>
<td>4.44± 0.75</td>
<td>3.5± 3.0</td>
</tr>
<tr>
<td>ESEAG 213 mg/kg</td>
<td>7/20 (35.0%) *</td>
<td>2.2 ± 3.9 *</td>
<td>2.65± 0.64 *</td>
<td>7.3± 4.1 *</td>
</tr>
<tr>
<td>ESEAG 425 mg/kg</td>
<td>5/20 (25.0%) *</td>
<td>2.2 ± 4.2 *</td>
<td>1.48±0.85 *</td>
<td>9.1± 3.9 *</td>
</tr>
</tbody>
</table>

* Significantly different from the vehicle group (p<0.05).

Table 2: The effect of ESEAG treatment on reproductive organs and testosterone concentration (Mean± SD for ten animals)

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight of testes (g)</th>
<th>Weight of seminal vesicles (g)</th>
<th>Testosterone conc. (pg/ml serum)</th>
<th>Protein content of testes (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.54± 0.20</td>
<td>2.28± 0.67</td>
<td>8.4 ± 5.9</td>
<td>291.3± 54</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.39± 0.13</td>
<td>1.98± 0.30</td>
<td>7.0 ± 6.8</td>
<td>288.3± 59</td>
</tr>
<tr>
<td>ESEAG 213 mg/kg</td>
<td>1.06± 0.37*</td>
<td>1.61± 0.48</td>
<td>3.0 ± 3.0</td>
<td>180.5± 58*</td>
</tr>
<tr>
<td>ESEAG 425 mg/kg</td>
<td>0.89± 0.28*</td>
<td>1.46± 0.59*</td>
<td>2.1± 2.1*</td>
<td>148.2± 69*</td>
</tr>
</tbody>
</table>

* Significantly different from the vehicle group (p<0.05).

Table 3: Sperm count and motility for untreated, vehicle, and ESEAG-treated rats (Mean± SD for ten rats)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm count (x 10^6)</th>
<th>Sperm motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>36.2 ± 2.2</td>
<td>88.4 ± 5.6</td>
</tr>
<tr>
<td>Vehicle</td>
<td>37.0 ± 2.2</td>
<td>83.8 ± 4.3</td>
</tr>
<tr>
<td>ESEAG 213 mg/kg</td>
<td>29.6 ± 1.5*</td>
<td>46.5± 11.3*</td>
</tr>
<tr>
<td>ESEAG 425 mg/kg</td>
<td>27.2 ± 3.5*</td>
<td>32 .4 ± 10.7*</td>
</tr>
</tbody>
</table>

* Significantly different from the vehicle group (p<0.05).

Table 4: Percentage of normal seminiferous tubules and the tubular diameter for untreated, vehicle, and ESEAG-treated rats (Mean± SD for ten rats)

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage of normal seminiferous tubules</th>
<th>Diameter of seminiferous tubules (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>57.4 ±13.5</td>
<td>304.9 ± 12.7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>53.7 ±12.7</td>
<td>298.8 ± 12.2</td>
</tr>
<tr>
<td>ESEAG 213 mg/kg</td>
<td>19.9 ± 14.5*</td>
<td>229.4 ± 20.2*</td>
</tr>
<tr>
<td>ESEAG 425 mg/kg</td>
<td>11.3 ± 11.7*</td>
<td>204.8 ± 21.1*</td>
</tr>
</tbody>
</table>

*Significantly different from the vehicle group (p<0.05).
REFERENCES

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Apium graveolens L.) The effect of some plants on the epididymal sperm count in rats

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