The Inhibitory Effect of Aqueous Extract of Coriander (Coriandrum sativum L.) Leaves on the Activity of Male Reproductive System of Albino Mice

Rana Hanan Khudhair Al-Rubaye*
Department of Biology, College of Education for Pure Sciences/ Ibn Al-Haitham, University of Baghdad, Iraq

Abstract
The aim of present study to investigate the effect of Coriandrum sativum leaves extract on reproductive activity of male albino mice. Thirty male mice with age of 80-100 day and weight between 25-30 g were divided into three groups: group 1 (untreated), group 2 and 3 were administrated orally for 30 days with aqueous extract of Coriandrum sativum leaves at dose 125 and 250mg/kg.b.w. respectively.

The following parameters were evaluated: serum testosterone levels, testes weights, sperm characteristics [motility, viability, spermatozoa, morphology and concentration] and histology changes of the testis. The results showed that the treatment caused highly significant decrease (P<0.01) in testosterone levels and the weight of testes associated with highly significant decrease (P<0.01) in sperm progressive motility, viability and concentration while the percentage of abnormal sperms was increased significantly. Testicular histology showed gradual degeneration in the population of germ cells lining seminiferous tubules and also degeneration in the interstitial tissue of Leydig cells.

In conclusion, the results indicate that Coriandrum sativum leaves extract at dose 125 and 250 mg/kg.b.w. has antifertility effect in male albino mice.

Keywords: Coriandrum sativum, Testosterone, Leaves extract, Testes, Sperms.

The inhibitory effect of aqueous extract of coriander leaves on the activity of male reproductive system of albino mice

Rana Hanan Khudhair Al-Rubaye*

Abstract

The aim of present study to investigate the effect of Coriandrum sativum leaves extract on reproductive activity of male albino mice. Thirty male mice with age of 80-100 day and weight between 25-30 g were divided into three groups: group 1 (untreated), group 2 and 3 were administrated orally for 30 days with aqueous extract of Coriandrum sativum leaves at dose 125 and 250mg/kg.b.w. respectively.

The following parameters were evaluated: serum testosterone levels, testes weights, sperm characteristics [motility, viability, spermatozoa, morphology and concentration] and histology changes of the testis. The results showed that the treatment caused highly significant decrease (P<0.01) in testosterone levels and the weight of testes associated with highly significant decrease (P<0.01) in sperm progressive motility, viability and concentration while the percentage of abnormal sperms was increased significantly. Testicular histology showed gradual degeneration in the population of germ cells lining seminiferous tubules and also degeneration in the interstitial tissue of Leydig cells.

In conclusion, the results indicate that Coriandrum sativum leaves extract at dose 125 and 250 mg/kg.b.w. has antifertility effect in male albino mice.

Keywords: Coriandrum sativum, Testosterone, Leaves extract, Testes, Sperms.

Email: ranahananmsc@yahoo.com
Introduction

The use of complementary traditional medicine which includes herbal medicines as a remedies for human diseases has expanded rapidly attributable to affordability, accessibility and efficacy [1]. Various medical plants are used by the population in nature or in the form of pharmaceutical preparation and their use has grown worldwide [2]. World health organization (WHO) estimates that approximately 80% of the developing world's population is using traditional medicine for primary healthcare [3]. However, there is a prevalent misunderstanding that herbal medicines are devoid of toxic effects [4]. Bioactive compounds derived from medicinal plants can be useful but might have serious dose related side effects [5].

*Coraindrum sativum* L. (Apiaceae) is an annual herb, the fresh leaves and dried seeds of which a common component of middle eastern, Mediterranean, Indian, Latin American, African and Southeast Asian cuisines [6]. It's commonly known as coriander in English [7] and famous as cilantro [8].

Experimental studies using different methodologies demonstrated that *Coraindrum sativum* leaves contain essential oil, flavonoids (quercitrin, isoquercitrin), polyphenols (rutin, caffeic acid, ferulic acid, galic acid and chlorogenic acid). Other molecules are found like linalool (Monoterpene alcohol) linoleic acid, sugars and proteins [9,10]. The methanolic and aqueous extracts of coriander leaves have been assessed for total phenolic content [11], [12] demonstrated that monoterpenoid, monoterpenoid glucose sulfate and other glycosides are water soluble compounds.

Alphatic compounds (90%) mainly comprised of aldehydes and alcohol predominate in the steam-volatile oil extracted from leaves of *Coraindrum sativum* are responsible for its peculiar ftd-like aroma [13]. In addition to its culinary value coriander is known for its wide range of healing properties. *Coraindrum sativum* leaves have been used as antispasmodies, anorexia, dyspeptic and appetizer [14]. Moreover it has been shown to possess antioxidant [15], antimicrobial [16], antihyperlipidemic [17], antidiabetes [18] and memory enhancing effect as reported by [19].

Earlier study revealed that the aqueous extract of fresh coriander seed has a toxic effect on female reproductive system [20]. Oral supplementation of 250 mg and 500 mg/kg in rats resulted in dose dependent decrease in implantation due to decreased progesterone level.

The aim of this study is to evaluate the effect of aqueous extract of *Coraindrum sativum* leaves in male reproductive function in mice such as sperm characteristics, testosterone secretion and histopathological of testes.

Materials and methods

Plant preparation and extraction

*Coraindrum sativum* leaves were collected from local markets in Baghdad city washed with distilled water, leaves were sliced in to small pieces and ground with pestle to produce a fine paste. Coriander leaves aqueous extract were prepared by mixing limited weight of the paste in 10 ml of distilled water and then the mixture was filtered by cleaning cloth to prepare the extract at dose level 125, 250 mg/kg.b.w. Fresh extract were administrated to mice immediately [21, 22].

Animals

Thirty males of Swiss albino mice (*Mus musculus*) their age between 80-100 day and their weight between 25-30g were used in the investigation. Mice were maintained under hygienic condition in well ventilated room and had free access to maintenance food and water ad libitum.

Treatment and Dosage

The animals were randomly devided in to three groups of ten animals in each group and treated as follows.

Group-1: Control mice that received orally by gavage needle 0.1ml of tap water daily for 30 days.

Group-2: Male mice treated orally by gavage needle with 0.1 ml of coriander leaves aqueous extract at dose 125 mg/kg of body's weight daily for 30 days.

Group-3: Male mice treated orally by gavage needle with 0.1 ml of coriander leaves aqueous extract at dose 250mg/kg.b.w. daily for 30 days.

Blood sample collection and organ

On day 31 of the experiment all animals were sacrificed. Blood samples from each mouse was collected via cardiac puncture in to a sterilized sample tube and was allowed to clot at room
temperature, the samples were centrifuged at 3000 xg for 15 min and sera were collected and stored at 20°C. Both tests and epididymis immediately dissected out and cleared of their adhering tissue. Testes weight were measured and fixed in formalin 10% for histological analysis.

**Hormonal study**

Testosterone level in serum samples collection was measured by radio immunoassay system.

**Sperm collection and analyses**

The epididymis was carefully separated from the testis and the cauda severed from its remaining part. Cauda was finely minced by anatomical scissors in 1 ml of isotonic saline at 37°C in a petridish, it was completely squashed by tweezers for 1 min to expel the sperms to the petridish. Sperms parameters were assessed according to World Health Organization [23], progressive motility was tested at once. Using a 100-point scale for linear movement the data were collected from 5 different fields in each sample and expressed in percentage of total cells.

A viability study was done using eosin/nigrosin stain and the average percentage of vital (unstained) sperms was calculated. Sperms morphology were done by the same stain and air-dried the slides were examined under the microscope using 100x objectives under oil immersion, the abnormal sperm cells were counted and the percentage calculated. Sperm concentration was determined using standard hemocytometric method. The dilution rate was 1:200 and the concentration was expressed per ml.

**Histological examination**

For each testis two slides of 10 μm sections were prepared according to [24] and stained with hematoxylin-eosin.

**Statistical analysis**

Data were expressed as mean ± standard error of mean (SEM). Statistical significance between the various groups was determined using ANOVA [25].

**Results**

**Serum testosterone levels**

Means serum testosterone levels of mice treated with 125 and 250mg/k.g.b.w for the extract of *C. sativum* significantly decrease (P<0.01) as compared with control Table-1.

**Testes weights**

The administration of aqueous extract of *C. sativum* leaves for 30 days at dose 125 and 250mg/kg/b.w. caused highly significant decrease (P<0.01) in testes weight compared with control Table-2.

**Sperm parameters**

Oral administration of the extract for 30 days caused highly significant decrease (P<0.01) in the mean epididymal sperm counts compared with control Table-3.

**Table 1**- Effect of *C. sativum* Leaves extract on serum testosterone levels ng/ml.

<table>
<thead>
<tr>
<th>Control</th>
<th><em>C. sativum</em> 125 mg/kg.b.w</th>
<th><em>C. sativum</em> 250 mg/kg.b.w</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.52 ± 0.04</td>
<td>1.95 ± 0.2</td>
<td>1.79 ± 0.2</td>
</tr>
</tbody>
</table>

Data expressed as Means ± SEA.

* B significantly different from the control at (P<0.01). n=10

**Table 2**- Effect of *C. sativum* leaves extract on testes weights (mg/100g b.w.).

<table>
<thead>
<tr>
<th>Control</th>
<th><em>C. sativum</em> 125mg/kg.b.w</th>
<th><em>C. sativum</em> 250kg/b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.32 ± 0.04</td>
<td>0.27 ± 0.03</td>
<td>0.23 ± 0.04</td>
</tr>
</tbody>
</table>

Data expressed as Means ± SEA.

* B significantly different from the control at (P<0.01). n=10
Table 3- Effect of *C. sativum* Leaves extract on sperms parameters.

<table>
<thead>
<tr>
<th>Sperm parameters</th>
<th>Control</th>
<th><em>C. sativum 125 mg/k.g.b.w</em></th>
<th><em>C. sativum 250 mg/k.g.b.w</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>S.motility %</td>
<td>70 ± 3.6 A</td>
<td>60 ± 4.8 B*</td>
<td>54 ± 4.9 B*</td>
</tr>
<tr>
<td>S.viability %</td>
<td>82 ± 4.5 A</td>
<td>75 ± 4.8 B*</td>
<td>71 ± 5.1 B*</td>
</tr>
<tr>
<td>Abnormal sperms %</td>
<td>26 ± 2.1 A</td>
<td>35 ± 3.1 B*</td>
<td>40 ± 5.1 B*</td>
</tr>
<tr>
<td>S.concentration X10⁶/ml</td>
<td>46 ± 3.6 A</td>
<td>38 ± 5.4 B*</td>
<td>33 ± 2.7 B*</td>
</tr>
</tbody>
</table>

Data expressed as Means ± SEA.
*B significantly different from the control at (P<0.01). n=10

**Histologic evaluation**

Administration of 125 and 250 mg/kg/b.w of *C. sativum* leaves extract caused damages to the seminiferous epithelium characterized by varying degrees of degeneration and disorganization within the tubules and the interstitial cells, reduced spermatogenesis (Maturation arrest) Figures-2, 3 compared to normal testicular structure in the control group Figure-1.

![Histological image](image)

**Figure 1**- Cross section in testes of control group showing normal layers of germ cells within seminiferous tubules(a), normal interstitial cells of Leydig(b) (H&E 100x).
Figure 2- Cross section in testes of mouse treated with 125 mg of *C. sativum* extract showing degree of vacuolation in germinal epithelium lining seminiferous tubules(a), separation of basement membrane from germ cells(b). (H&E100x).

Figure 3- Cross section in testes of mouse treated with 250 mg of *C. sativum* extract show marked degeneration in the seminiferous epithelium (a), interstitial of Leydig cells (b) and reduce in sperm numbers(c) (H&E100x).
Discussion

Although Coriandrum sativum is a widely used plant in traditional medicine little is known about its safety. In traditional medicine plant extracts are prepared as aqueous suspensions such as infusions, decoctions and poultices [26, 27] in view of the above we conducted this work to examine the effects of aqueous extract of coriander Leaves on some reproductive parameters in male albino mice.

The results of the present study suggested that the aqueous extract of Coriandrum sativum leaves extract at dose 125 and 250 have a deleterious effect on male reproductive functions, sufficient to cause, reversible infertility in male mice.

Al-Suhaimi [28] reported that C. sativum did not appear to exert negative effect on testosterone levels and reproductive functions after treated rabbits with C. sativum seed extract for 7 days. One reason for this discrepancy in the studies that coriander leaves and seeds did not contain the same active compounds. The phytochemicals of seed and leaves are completely different [13]. Mono and poly unsaturated fatty aldehydes although minor components in seed oil coriander leaf oil contains these aldehydes as main constituents [29] another reason could be related to the type of preparations used, dose and method of administration, type of animals and duration of experiment. Serval abnormal histological alteration seen in the testes of the treatment groups, ranges from degeneration and disruption of germ cells lining seminiferous tubules and also the degeneration of the Leydig cells compared with control group. This histopathological result suggesting that the administration of C. sativum leaves extract for a long period were capable of permeating the blood-testis barrier and loss it biological control [30].

The daily treatment of coriander extract for 30 days resulted in reduction in serum testosterone levels. Coriander leaves showed cholesterol-lowering effect [31], cholesterol is most important precursor in the synthesis of steroid hormones like testosterone and its level is related to fertility [32]. Moreover the deformation of Leydig cells further indicates the inefficiency of these cells to synthesize testosterone [33].Significant decrease in the mean testicular weight in animals treated with C. sativum extract at dose 125 and 250 were observed. These results were corroborated by various degrees of degeneration in the histologic sections of testes and decreased in testosterone levels. Testosterone was necessary for development, growth and normal function of the testes and male accessory reproductive glands [34].

The present study show reduction in the progressive epididymal sperm motility of treated groups. Sperm motility depends on the coordinated propagated flagella wave. Fructose utilization and glucose oxidation are important means by which spermatozoa derive energy for their motility [29, 35] C. sativum Leaves had been shown to possess hypoglycemic and antihyperglycemic activity [36]. The reduction in motility recorded in this study could be due to the glucose lowering properties of this plant.

Epididymis normally provided a favorable milieu for acquisition of fertilizing ability and viability [37]. The decrease in sperm viability and the increase in abnormal sperm count in treated mice in this study suggested that the action of the extract could target the internal milieu of the epididymis [38]. Reduction in total number of epididymal sperm count in treated mice could also a result of description of seminiferous tubules as observed in the histological section of testes.

The arrest of spermatogenesis possibly occurred as a consequence of reduction in serum level of testosterone which had been shown to be essential for the completion of meiotic division during spermatogenesis [39].

References


