The Oleo-Gum-Resin of *Commiphora myrrha* Ameliorates Male Reproductive Dysfunctions in Streptozotocin-Induced Hyperglycemic Rats

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**Article Info**

**Article History:**
- Received: 14 April 2019
- Revised: 4 June 2019
- Accepted: 28 June 2019
- ePublished: 20 December 2019

**Keywords:**
- Apoptosis
- *Commiphora myrrha*
- Diabetes
- Sperm
- Testis

**ABSTRACT**

**Background:** The oleo-gum-resin of *Commiphora myrrha* (myrrh) has a long history of therapeutic use in traditional medicine. The aim of this study was to seek for the scientific evidence to determine whether the ethanolic extract of myrrh (EEM) has any beneficial effects on Streptozotocin (STZ) induced testicular impairments, and explore the possible mechanisms underlying such actions.

**Methods:** Forty-eight severe and complicated diabetic rats (fasting blood glucose above 350 mg/dL), randomly were divided into six equal groups (n=8). Besides, eight healthy rats allocated as a normal control group and only treated with vehicle solution. The diabetic animals orally received the extract (100, 200, 300, and 500 mg/kg), metformin (500 mg/kg) or vehicle solution for 28 days. As a final point, plasma glucose and insulin, circulatory sex hormones, sperm parameters including sperm concentration, motility and viability and also testicular malondialdehyde (MDA) levels were assessed. Furthermore, quantitative histological evaluation of seminiferous tubules area and determination of germinal cells apoptosis were performed.

**Results:** None of the studied doses of EEM showed anti-diabetic effects. However, the extract mainly at the maximum dose (500 mg/kg) exhibited beneficial effects on reproductive impairments. The EEM treated rats mainly at 500 mg/kg had significantly higher sperm concentration, sperm motility, sperm viability, sex hormones and lower testicular MDA and germ cell apoptosis index than untreated diabetic rats.

**Conclusion:** These results indicated that EEM may have beneficial effects against reproductive dysfunction induced by diabetes. The mechanisms behind the effects might be associated with the EEM sex hormone booster potential, antioxidant and anti-apoptotic properties.

**Introduction**

In diabetic patients, sexual dysfunction along with severe and common complications such as nephropathy, retinopathy and neuropathy has frequently been reported. It is well established that diabetes can develop sexual problems in both male and female. The sexual dysfunction in diabetic patients has been recognized since antiquity. In the tenth century, persian physician Avicenna described “collapse of sexual function” as a complication of diabetes in men. Currently, several studies have proven a wide spectrum of structural and functional effects of diabetes on male reproductive system. Diabetes can cause male infertility by reducing sperm concentration, sperm motility, serum gonadotropins and testosterone, and making structural alterations in the testes. Despite the fact that diabetes-associated reproductive disorders have widely been studied, the exact responsible mechanisms for male reproductive impairment are not yet completely understood. In the last years, giving antioxidant, testosterone and insulin replacement therapies have been investigated to control the development of diabetes-associated reproductive damages. Nevertheless, there is still no ideal treatment for diabetic reproductive injury and searching for therapeutic agents with multi-targets efficacy appear vital. The use of medicinal plants and complementary medicine to enhance reproductive function and to stimulate sexual desire is almost as old as the human rise itself. Medicinal plants have a long history of usage and nowadays, global interest in herbal medicine is increasing. Numerous natural products have been employed in the management of diabetes mellitus and its complications. Among them, some natural products are more popular and widely used.
in different traditional medicine systems. The myrrh is a resin made from *Commiphora myrrha* (Nees) Engl., (Burseraceae family) dried sap. It is one of the most researched natural products in the world and appears to be one of the oldest medicines. Traditional practice and evidence-based research have supported the medicinal properties of myrrh such as anti-inflammatory, wound healing, antiseptic, menstrual stimulant, expectorant, antitumour, and anti-parasitic. There is evidence showing that myrrh exhibited hypoglycemic and hypolipidemic activities in diabetic animals. Moreover, myrrh gum showed potent antioxidant activity in different oxidative stress circumstances. Despite these interests, no scientific literature has been performed regarding its effects on male reproductive function. Therefore, the present study was designed to investigate the ameliorative effects of different oral doses of myrrh on reproductive dysfunctions in diabetic rats.

**Methods**

**Chemicals and reagents**

Streptozotocin (STZ), Horseradish peroxidase (HRP)-conjugated peanut agglutinin (PNA) lectin, 3, 3'-diaminobenzidine (DAB), bovine serum albumin (BSA), eosin Y and nigrosin were purchased from Sigma Chemicals Company (St. Louis, MO, USA). Ham's F-10 nutrient mix was procured from Caisson Laboratories (North Logan, UT, USA) and metformin tablet obtained from Merck Sante S.A.S. (Lyon, France). Alcian blue 8GX dye was procured from Bio Basic (Canada Inc, Canada). The STZ was freshly dissolved in citrate buffer, pH 4.5. Also, the solvent solution containing 0.1% dimethyl sulfoxide (DMSO) (Carlo ERBA, France) in saline was used for dissolving myrrh extracts as well as metformin.

**Plant Materials**

Dried myrrh gum-resin was purchased from traditional plant market at Birjand (Iran). It was tested (carbon disulfide and nitric acid colour reaction) by comparison with a genuine sample and identified as gum-resin derived from *Commiphora myrrha* (Nees) Engl. A specimen was kept in the herbarium of Agriculture Faculty at the University of Birjand (voucher number: 527).

To prepare the ethanolic extract, the pulverized myrrh was macerated in 80% ethanol 1:10 (w/v) on a magnetic stirrer for 2 days at room temperature. Afterwards, the mixture was passed through the filter paper (Whatman No. 4, England), concentrated under vacuum evaporator, and lyophilized using a freeze-dryer (Dena Vacuum Industry, model FD-5005-BT, Iran). The extraction yield was about 9.5%.

**Animals**

Adult male Wistar rats weighing 180-200g (8 weeks old) were obtained from animal facility of Research Centre of Experimental Medicine at Birjand University of Medical Sciences, Birjand, Iran. The animals were housed in polypropylene cages, temperature-controlled room (22 ± 2 °C) with a 12 h light/dark cycle and free access to commercial animal chow (Behparvar Co, Iran) and tap water during the study. All animal procedures were conducted in accordance with the guide for the care and use of laboratory animals approved by the Ethics Committee of the Birjand University of Medical Sciences (permit code: Ir.bums.REC1396.16). All efforts were made to minimize animal suffering and to reduce the number of used animals.

**Induction of diabetes and experimental design**

Diabetes was induced by a single intraperitoneal (i.p) injection of STZ (55 mg/kg) into the overnight fasted rats. Age-matched control animals only received an i.p injection of vehicle (citrate buffer). After 72h of the injection, rats with plasma glucose concentration above than 350 mg/dL were considered as severe diabetic. It is worth be noted that, in order to prevent failure in diabetes induction and also animals death before starting the experiment, 18 rats more than the required number (n=48) were subjected to STZ injection. All diabetic animals left untreated for 4 weeks to develop a complicated form of diabetes. Forty-eight diabetic rats randomly divided into six equal groups (n=8). Group 1 received only vehicle solution (0.1 % DMSO in saline) as diabetic model group (DM), groups 2-5 treated with the ethanolic extracts of myrrh (EEM) at the doses of 100 mg/kg (EEM100), 200 mg/kg (EEM200), 300 mg/kg (EEM300) and 500 mg/kg (EEM500) respectively, and group 6 received metformin at the dose of 500 mg/kg (MET500). The selection of the studied doses of EEM was based on previous studies reported hypoglycemic activity of myrrh in rats. Moreover, eight healthy rats allocated as normal control (NC) group and received just vehicle solution (0.1% DMSO in saline). All the samples were administrated orally to the animals using an intragastric tube for a period of four weeks.

**Sample collection**

At 29th day and after 24 h of the last treatment, the fasted rats were weighed and their blood glucose levels were measured using a glucometer (Accue-Chek, Roche). Subsequently, animals were anaesthetized by an i.p. injection of ketamine: xylazine (65:10 mg/kg) and immediately their blood specimens were collected from the heart. The blood samples were centrifuged for 10 min at 3000 g and the obtained plasma collected. The reproductive organs including testis, epididymides, and prostate were dissected, weighed and fixed in the fixative solution containing 4% paraformaldehyde and 0.1 M glutaraldehyde in 0.1 M phosphate buffer (PBS) (pH 7.4). Besides, a part of the left testis (100 mg) was homogenized in cold PBS (900 µL), and centrifuged (5000 g for 10 min) at 4º C. The collected supernatants were used for lipid peroxidation assay.

**Plasma sex hormones, plasma insulin and testicular oxidative stress**

Plasma testosterone (T), follicular stimulating hormone (FSH), and luteinizing hormone (LH) levels were measured using a commercially available radioimmunoassay kit (Aurotam, Iran). Plasma insulin levels were determined using a commercially available kit (MCE, Iran). The oxidative stress was measured using the level of thiobarbituric acid reactive substances (TBARS) as the end product of lipoperoxidation of lipid membrane in testicular homogenates using the spectrophotometer at 532 nm (Beckman, USA). The level of TBARS was calculated using malondialdehyde (MDA) as standard. The contents of TBARS were expressed as nmol/mg protein.

**Histological evaluation**

Mature epididymides were dissected for histological evaluation. After fixation for 3 days in a 4% paraformaldehyde solution, the epididymides were washed in 0.1 M phosphate buffer (pH 7.4), and embedded in paraffin. Five-micrometer-thick sections were cut and stained with haematoxylin and eosin. The epididymal sections were studied under a light microscope (Leica, Germany). The seminal vesicles were examined for size, tubule morphology, and smooth muscle sheath.

**Statistical analysis**

All the data were presented as mean ± SD. Comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test for multiple comparisons. Differences were considered statistically significant at p < 0.05 (Statistical Package for Social Sciences, version 21).
measured using enzyme-linked immunosorbent assay (ELISA) kits (Pishtazteb, Iran) according to the manufacturer’s instructions. Plasma insulin levels were determined using an insulin ELISA kit (Mercodia, Sweden). Lipid peroxidation in testicular tissue was evaluated by determination of malondialdehyde (MDA), the main end-products of lipid peroxidation using Thiobarbituric Acid Reactive Substances (TBARS). Briefly, based on reaction with thiobarbituric acid at 90–100 °C, the absorbance of the resulting pink product was measured spectrophotometrically at 535 nm. MDA has been considered equivalent to TBARS.\(^{19}\)

**Sperm evaluation**

The spermatozoa were collected by mincing cauda epididymis using two NO.11 sterile scalp el blades in 2 mL Ham’s F10 medium supplemented with 0.5% BSA, and pre-warmed at 37°C. Following incubation for 30 min, for sperm count, 10 µL of the suspension was diluted in 190 µL cold saline, and then 10 µL of the diluted sample was transferred into each chamber of Neubauer cell counting spaces under 100X magnification. The sperms which lied in the five secondary squares were counted.\(^{20}\)

The percentage of motile sperms was evaluated individually as follow: 10 µL of sperm suspension (in medium) were transferred into a pre-warmed microscope slide and covered with a coverslip. The percentage of motile sperms was assessed under 400X magnification in at least 10 microscopic fields and being 200 sperms per rat were evaluated.\(^{21}\) Sperm viability evaluated using eosin-nigrosin staining method. Briefly, 10µL of sperm suspension gently mixed with 10µL eosin-nigrosin solution (containing an equal volume of 1.67% eosin Y and 10% nigrosin) and a smear of the suspension was performed and dried at room temperature. The slides were examined by light microscope under 1000X magnification. 200 spermatozoa per rat (10-20 microscopic fields) were evaluated in order to determine viable or non-viable sperms. Stained sperms (pink) were considered as non-viable, while unstained sperms (white) or poorly stained sperm heads evaluated as viable sperms.\(^{22}\)

**Testicular histology and lectin histo-chemistry**

Tissue samples of the testis were processed for paraaffin-embedding and serial sections prepared for staining with hematoxylin and eosin. For each testis, three random slides (9 sections) were analyzed under a light microscope (UPLAN FL, Japan) and for each section; 4–6 unbiased counting frames were sampled. Seminiferous tubule area (um\(^2\)), germinal layer area (um\(^2\)), and the number of spermatogonia cells were measured using Image J Software (1.44p; National Institute of Health, USA).\(^{23}\) The ratio of germinal layer area to seminiferous tubule area was calculated for each seminiferous tubule.

To determine in-situ spermatogenic cell apoptosis, the classical lectin histochemistry was performed.\(^{24}\) Briefly, the testicular sections were deparaffinized in xylene and rehydrated through a descending ethanol series, rinsed in PBS (pH: 7.4) for 5 min and then 30 min incubated in 0.3% H\(_2\)O\(_2\) in methanol solution for blocking the endogenous peroxidase activity at a dark chamber. Then, the sections were washed in PBS and incubated in HRP-conjugated peanut agglutinin lectin (PNA) in a moisture chamber at room temperature for 1.5 h. After washing in PBS, the sections were reacted in PBS containing 0.05% DAB and 0.3% H\(_2\)O\(_2\) for 5 min at room temperature. Finally, the sections were counterstained with 1% solution of Alcian blue dye in 0.1 M HCl (pH: 2.5), dehydrated, cleared and mounted. For negative control, the next serial section of each specimen was exposed to HRP, DAB, and H\(_2\)O\(_2\) without lectin. Germ cells with marked reaction and diffuse stained cytoplasm or nuclear were considered as apoptotic.\(^{25}\) The quantitative assessment of testicular apoptosis was done as follows: the incidence of apoptosis was assessed in 100 seminiferous tubules. The tubule with three or more apoptotic germ cells considered as positive. The apoptosis percentage was calculated by the ratio of the positive tubules to the total number of seminiferous tubules in a cross-section.\(^{25}\)

**Statistical analyses**

Results are expressed as mean ± SD for all parameters. Data distributions were examined for normality and homogeneity of variance by Kolmogorov-Smirnov test. Statistical differences between groups were detected by analysis of variance (ANOVA) followed by Dunnett’s test. Statistical significance was inferred at \(p<0.05\). The SPSS software, version 22 was used for all analysis.

**Results**

**Fasting blood glucose, plasma insulin, body and organ weights**

The results of the final FBG, plasma insulin, body weight and reproductive organs weight are presented in Table 1. Compared to the NC group, a marked increase in the FBG was observed in diabetic animals (\(p<0.0001\)). The elevation only normalized in metformin-treated rats (MET500 mg/kg) efficiently (\(p=0.21\) compared to NC), while the EEM in all doses (EEM 100-500 mg/kg) could not exhibit any hypoglycemic activity. In comparison with NC group, diabetes caused a significant decrease in insulin level (\(p<0.0001\)). There was no significant difference between the EEM-treated rats (at all doses) and DM group. Metformin treated diabetic rats exhibited slightly higher plasma insulin than DM group (\(p=0.001\)), but the level was still significantly lower than normal level (\(p<0.0001\)). In diabetic animals, body weight, weight of testis, epididymis and prostate were significantly lower than NC group (\(p<0.0001\)). The EEM treatment at the all doses significantly prevented testis weight loss (\(p<0.0001\)). In epididymis weight, both metformin and EEM (100-500mg/kg) significantly improved and normalized it in diabetic animals. There was no significant difference between EEM doses in testes and epididymis weights.
Metformin not only did not affect prostate weight in diabetic animals but also significantly decreased it even more than the DM group \((p<0.04)\). On the other hand, EEM at the doses of 300 mg/kg \((p<0.001)\) and 500 mg/kg \((p<0.001)\) could increase and normalize prostate weight in comparison with DM group.

### Plasma sex hormones and testicular MDA concentration

Table 2 illustrates the effects of EEM (100-500 mg/kg) on the STZ-induced changes in the plasma concentrations of T, LH, FSH and testicular tissue MDA levels. Compared to the NC group, the plasma levels of T, LH and FSH significantly decreased \((p<0.0001)\) in DM group. The EEM at the doses of 300 mg/kg \((p<0.0001)\) and 500 mg/kg \((p<0.0001)\) as well as metformin \((p<0.0001)\) significantly increased T concentration in diabetic rats; however, in comparison with the NC group the level was still low \((p<0.001)\). The EEM at all doses \((100-500\ mg/kg)\) and metformin could significantly increase and normalize LH levels in diabetic animals. There was no significant difference between the EEM doses and metformin. Like LH, the EEM at all doses restored FSH levels in diabetic rats while metformin treated animals had significantly lower FSH level than NC group \((p<0.002)\).

### Sperm count and quality

The results of sperm analysis are presented in Table 3. Diabetic rats had significantly lower sperm count \((p<0.0001)\), sperm motility \((p<0.0001)\) and sperm viability \((p<0.0001)\) than non-diabetic rats. Metformin and EEM at the doses of 300-500 mg/kg increased sperm concentration, and improved sperm motility as well as its viability in diabetic rats, however; the EEM only at maximum dose (500 mg/kg) could normalize the alterations.

### Histopathological evaluation

In the NC group, light microscopic examination of testis sections revealed normal histological structure with rounded seminiferous tubules in most of which different phases of spermatogenic cells were clearly observed.

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### Table 1. Effects of ethanolic extract of myrrh (EEM) on fasting blood glucose (FBG), insulin, body weight, and reproductive organ weights in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>FBG (mg/dL)</th>
<th>Insulin (ng/µL)</th>
<th>Body weight (g)</th>
<th>Testis</th>
<th>Epididymis</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>98.33±8.84</td>
<td>2.55±0.44</td>
<td>263.33±34.33</td>
<td>1.62±0.23</td>
<td>0.65±0.05</td>
<td>0.46±0.06</td>
</tr>
<tr>
<td>Diabetic model</td>
<td>447.33±74.03 *</td>
<td>0.68±0.12 *</td>
<td>176.66±31.34</td>
<td>0.58±0.28</td>
<td>0.27±0.10</td>
<td>0.21±0.01 *</td>
</tr>
<tr>
<td>Metformin (500 mg/kg)</td>
<td>163.16±21.59 *</td>
<td>1.29±0.08 *</td>
<td>168.50±35.54</td>
<td>1.15±0.28</td>
<td>0.49±0.07</td>
<td>0.10±0.08 *</td>
</tr>
<tr>
<td>EEM (100 mg/kg)</td>
<td>437.00±50.34 *</td>
<td>0.44±0.20 *</td>
<td>174.15±17.57</td>
<td>1.45±0.20</td>
<td>0.42±0.06</td>
<td>0.25±0.03 *</td>
</tr>
<tr>
<td>EEM (200 mg/kg)</td>
<td>513.66±52.89 *</td>
<td>0.36±0.18 *</td>
<td>164.33±15.21</td>
<td>1.32±0.24</td>
<td>0.41±0.06</td>
<td>0.32±0.03 *</td>
</tr>
<tr>
<td>EEM (300 mg/kg)</td>
<td>457.50±16.86 *</td>
<td>0.59±0.09 *</td>
<td>163.66±19.27</td>
<td>1.34±0.27</td>
<td>0.46±0.03</td>
<td>0.38±0.10 *</td>
</tr>
<tr>
<td>EEM (500 mg/kg)</td>
<td>486.33±15.08 *</td>
<td>0.78±0.16 *</td>
<td>178.33±29.15</td>
<td>1.28±0.24</td>
<td>0.49±0.008 *</td>
<td>0.39±0.04 *</td>
</tr>
</tbody>
</table>

Values are expressed as mean± standard deviation \((n=8)\). *Represented as \(p<0.05\) in comparison with the normal control group. **Represented as \(p<0.01\) in comparison with the diabetic model group.

### Table 2. Effects of ethanolic extract of myrrh (EEM) on plasma levels of testosterone (T), follicular stimulating hormone (FSH), luteinizing hormone (LH), and testicular malondialdehyde (MDA) concentration in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T (ng/mL)</th>
<th>FSH (mIU/mL)</th>
<th>LH (mIU/mL)</th>
<th>MDA (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.80±0.36</td>
<td>2.02±0.11</td>
<td>3.33±0.31</td>
<td>27.12±3.07</td>
</tr>
<tr>
<td>Diabetic model</td>
<td>0.05±0.01 *</td>
<td>1.01±0.55 *</td>
<td>1.13±0.14 *</td>
<td>58.15±5.12 *</td>
</tr>
<tr>
<td>Metformin (500 mg/kg)</td>
<td>0.47±0.07 *</td>
<td>1.02±0.42 *</td>
<td>3.39±0.37 *</td>
<td>33.19±2.21 *</td>
</tr>
<tr>
<td>EEM (100 mg/kg)</td>
<td>0.33±0.06 *</td>
<td>2.01±0.20</td>
<td>3.38±0.10 *</td>
<td>38.71±4.19 *</td>
</tr>
<tr>
<td>EEM (200 mg/kg)</td>
<td>0.42±0.04 *</td>
<td>2.01±0.41</td>
<td>4.22±0.91</td>
<td>37.42±5.41 *</td>
</tr>
<tr>
<td>EEM (300 mg/kg)</td>
<td>0.47±0.36 *</td>
<td>2.03±0.22 *</td>
<td>3.35±0.67</td>
<td>29.08±5.11 *</td>
</tr>
<tr>
<td>EEM (500 mg/kg)</td>
<td>0.80±0.25 *</td>
<td>2.11±0.10 *</td>
<td>3.33±0.22 *</td>
<td>25.42±4.02 *</td>
</tr>
</tbody>
</table>

Values are expressed as mean± standard deviation \((n=8)\). *Represented as \(p<0.05\) in comparison with the normal control group. **Represented as \(p<0.01\) in comparison with the diabetic model group.

### Table 3. Effects of ethanolic extract of myrrh (EEM) on epididymal sperm count, motility and viability in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count ((×10^9/\text{mL}))</th>
<th>Motility (%)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>20.76±1.52</td>
<td>80.80±3.27</td>
<td>89.20±2.28</td>
</tr>
<tr>
<td>Diabetic model</td>
<td>8.63±1.31 *</td>
<td>46.40±10.13 *</td>
<td>55.40±8.59 *</td>
</tr>
<tr>
<td>Metformin (500 mg/kg)</td>
<td>17.80±2.83 *</td>
<td>74.50±3.44 *</td>
<td>78.83±5.30 *</td>
</tr>
<tr>
<td>EEM (100 mg/kg)</td>
<td>8.16±2.22 *</td>
<td>40.76±5.58 *</td>
<td>45.20±3.98 *</td>
</tr>
<tr>
<td>EEM (200 mg/kg)</td>
<td>9.33±1.03 *</td>
<td>61.04±10.36 *</td>
<td>64.00±10.93 *</td>
</tr>
<tr>
<td>EEM (300 mg/kg)</td>
<td>17.16±2.40 *</td>
<td>62.38±1.78 *</td>
<td>65.20±6.94 *</td>
</tr>
<tr>
<td>EEM (500 mg/kg)</td>
<td>19.16±1.72 *</td>
<td>70.80±7.66 *</td>
<td>78.60±8.08 *</td>
</tr>
</tbody>
</table>

Values are expressed as mean± standard deviation \((n=8)\). *Represented as \(p<0.05\) in comparison with the normal control group. **Represented as \(p<0.05\) in comparison with the diabetic model group.
The epithelium of germinal layer was stratified with the spermatogonia (small rounded cells resting on basement membrane), sertoli cells (column cells with euchromatin nuclei), primary spermatocytes (large rounded nuclei and greater than spermatogonia) and spermatids (small rounded cells with paler nuclei) radially arranged about 5-7 layers toward the lumen (Figure 1). On the other hand, testicular histopathology of diabetic rats showed extensive shrinkage of seminiferous tubules and massive sloughing of the germinal layer (Figure 1).

The quantitative histopathological assessment clearly showed marked reduction \( (p<0.0001) \) in the number of spermatogonia, seminiferous area, germinal layer area and germinal layer to seminiferous area coefficient in diabetic animals with respect to the NC group (Table 4). The number of spermatogonia cells in the EEM treated rats at doses of 200 mg/kg \( (p=0.012) \), 300 mg/kg \( (p<0.0001) \) and 500 mg/kg \( (p<0.0001) \) were significantly higher than DM group. However, EEM at the doses of 300 mg/kg and 500 mg/kg could efficiently restore the spermatogonia number towards the normal level. Furthermore, EEM at the doses of 200 mg/kg \( (p=0.033) \), 300 mg/kg \( (p<0.0001) \) and 500 mg/kg \( (p<0.0001) \) efficiently improved the germinal epithelium area in comparison with DM group. Nevertheless, only at the EEM300 and EEM500 groups the germinal layer area was same as NC group.

**Germ cell apoptosis**

There were a few PNA-positive germ cells in NC group (Figure 2a), whereas in DM group the number of apoptotic cells was markedly increased (Figure 2b). Compared to NC group, the apoptotic index was significantly higher in DM group \( (p<0.0001) \) (Figure 3). The index significantly mitigated in EEM treated rats at the doses of 200-500 mg/kg \( (p<0.0001) \). Only in EEM300 and EEM500 groups, the apoptotic index had no significant difference in respect to NC group \( (p>0.05) \).

**Discussion**

In the present study, we provide evidence that EEM might mitigate reproductive dysfunction induced by diabetes in male rats through antioxidant potential and apoptosis inhibition. The results showed that compared to control group, diabetic animals had markedly higher levels of blood glucose, testicular MDA concentration and germinal cells apoptosis, whereas lower plasma insulin, body and reproductive organ weights, plasma T and LH levels, sperm count, sperm motility, sperm viability, and also seminiferous tubules area. These findings clearly show that animal model of diabetes-induced reproductive impairment was well established.\(^\text{15}\) Our findings demonstrated that EEM had no hypoglycemic effect. However, diabetic rats treated with EEM, had significantly higher reproductive organ weights, plasma T and LH, and sperm parameters, and lower germinal cells apoptosis and testicular lipid peroxidation than untreated diabetic rats. In the present study diabetic animals were left untreated for 4 weeks to develop a complicated form of the disease. Accumulating evidence suggests that uncomplicated type 1 diabetes is not associated with the hallmarks of male infertility or hypogonadism.\(^\text{15}\)

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**Figure 1.** Photomicrographs of the testicular sections of rats belong to the normal control group (NC), diabetic model group (DM), diabetic treated with metformin at the dose of 500mg/kg (MET500) and diabetic groups treated with ethanolic extract of myrrha gum at the doses of 100-500mg/kg (EEM100-500). Hematoxylin and eosin staining with two magnifications 100X (left column, bars=100 um) and 400X (right column, bars=50 um). In NC group, the epithelium clearly can stratify with spermatogonia (yellow arrow), Sertoli cells (black arrow), primary spermatocytes (blue arrow), spermatids (red arrow), spermatozoa (green star) and active Leydig cells (green arrow).
tic model
dy
and also slightly increased plasma insulin
sterologenic
direct STZ cytotoxicity to Sertoli cells, whereas
the early
group.
as
(EEM200),
(EEM)
 diabetic
treated
normal
treated
Figure
histochemistry,
treated
Figure
2.
Effects of ethanolic extract of myrrh (EEM) on the number of spermatogonia per seminiferous tubule, areas of seminiferous tubule (SA), seminiferous epithelium (EA) and EA to SA coefficient in diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Spermatogonia (number/tubule)</th>
<th>SA (um²)</th>
<th>EA area (um²)</th>
<th>EA/SA×100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>34.3±2.95</td>
<td>68304.5±7561.64</td>
<td>56931.69±6042.03</td>
<td>83.63±7.22</td>
</tr>
<tr>
<td>Diabetic model</td>
<td>21.1±3.55 *</td>
<td>12838.37±1303.97</td>
<td>6347.50±1451.16</td>
<td>49.16±6.70</td>
</tr>
<tr>
<td>Metformin (500 mg/kg)</td>
<td>30.3±4.37 *</td>
<td>29043.92±1070.95</td>
<td>10919.10±2796.14</td>
<td>67.72±10.70</td>
</tr>
<tr>
<td>EEM (100 mg/kg)</td>
<td>18.5±4.48 *</td>
<td>10930.86±1143.91</td>
<td>4457.52±1728.70</td>
<td>39.87±10.99</td>
</tr>
<tr>
<td>EEM (200 mg/kg)</td>
<td>26.6±6.74 *</td>
<td>22245.29±3108.69</td>
<td>14690.27±3191.15</td>
<td>66.06±11.62</td>
</tr>
<tr>
<td>EEM (300 mg/kg)</td>
<td>31.8±2.13 *</td>
<td>48403.91±3978.68</td>
<td>37868.16±4280.03</td>
<td>78.18±4.76</td>
</tr>
<tr>
<td>EEM (500 mg/kg)</td>
<td>32.3±1.74 *</td>
<td>52879.90±1689.54</td>
<td>44256.25±2882.310</td>
<td>83.67±4.27</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (n=8). *Represented as p<0.05 in comparison with the normal control group. # Represented as p<0.05 in comparison with the diabetic model group.

Figure 2. Photomicrographs of the testicular sections of rats belong to the normal control group (a), diabetic model group (b), diabetic groups treated with ethanolic extract of myrrh gum at the doses of 100 mg/kg (c), 200 mg/kg (d), 300 mg/kg (e) and 500 mg/kg (f). PNA lectin histochemistry, 400X magnification, bar=50um. The yellow arrows show apoptotic cell.

Figure 3. The apoptotic index of seminiferous tubules of the normal control group (NC), diabetic model group (DM), diabetic treated with metformin at the dose of 500 mg/kg (MET500), and diabetic groups treated with ethanolic extract of myrrh gum (EEM) at the doses of 100 mg/kg (EEM100), 200 mg/kg (EEM200), 300 mg/kg (EEM300), and 500 mg/kg (EEM500). Each bar indicates mean standard deviation. *Represented as p<0.05 in comparison with the normal control group. # Represented as p<0.05 in comparison with the diabetic model group.

Evidence also showing that spermatogenic dysfunction in the early stage of STZ-induced diabetic rats is due to direct STZ cytotoxicity to Sertoli cells, whereas steroidogenic dysfunction is because of insulin deficiency. Therefore, STZ-induced diabetic model, in the early stage, is not a suitable model to study the diabetes-related reproductive dysfunction. The EEM had no effect on glucose and plasma insulin levels while metformin effectively reduced blood glucose level and also slightly increased plasma insulin concentration in diabetic animals. These findings have not confirmed previous research on hypoglycemic activity of myrrh. Helal et al. reported that aqueous extract of Commiphora myrrha at the dose of 50 mg/100g body weight significantly reduced blood glucose levels (from 200 mg/dL to 110 mg/dL) after 30 days of administration in rats. Moreover, they reported that the C. myrrha extract significantly increased plasma insulin levels from 20 µU/mL to 40 µU/mL. In the above-mentioned study, rats with FBG around 250 mg/dL were considered as diabetic. Also, the plant part that was used and its route of administration did not mention. The possible reason for these differences may be due to diabetes severity. In the present study, the mean of FBG level of diabetic animals was around 450 mg/dL while in the Helal et al. study it was around 250 mg/dL. Generally, in a rat model of diabetes, animals with glycemia between 120 and 300 mg/dL, characterizing moderate or mild diabetes and rats with glycemia above 300 mg/dL consider as severe diabetic.
There is evidence indicating that these phenotypes have different responses to antidiabetic agents. As expected, in the present study reproductive organ weights including testis, epididymis, and prostate markedly decreased in diabetic rats. The EEM treatment in a dose dependent manner could effectively ameliorate weight reduction in all organs. It has previously been shown that chronic oral administration of *C. molmol* oleo-gum–resin, a synonym of *C. myrrha*, at dose of 100 mg/kg for 90 days caused markedly increase in weight of testis, cauda epididymis and seminal vesicles in normal mice. Like EEM, metformin prevented testicle and epididymis weight loss, but the prostate weight of metformin-treated rats was significantly even lower than diabetic untreated animals. This is in good agreement with a study conducted by Mosli *et al.* in which oral treatment with 500–1000 mg/kg metformin for 14 days significantly inhibited the testosterone-mediated increase in the prostate weight of rats. Previous studies indicated that diabetic rats represented a reduction in Leydig cells number and impairment in their function. Therefore, testosterone secretion decreases in diabetic patients or animal models. Results of the present study showed that both of EEM and metformin could improve these parameters. However, it worth to be noted that the T levels failed to revert to normal in both EEM and metformin-treated diabetic animals. The reason for this result are not yet completely understood, but it could be attributed to the chemical composition of EEM and the study period. 

Oxidative stress in the testis is a key hallmark for hyperglycemia-induced cell apoptosis. A growing body of evidence suggests that cell death mechanism plays an important role in spermatogenesis dysfunction induced by diabetes. Histological evaluation of testis revealed that in diabetic animals, thickness of germinal epithelium, as well as seminiferous area was declined and germ-cell apoptosis significantly increased. With respect to histological alterations, functional parameters including sperm count, sperm motility and sperm viability significantly decreased in diabetic rats. Furthermore, the testicular lipid peroxidation marker (MDA) significantly increased in diabetic rats. Interestingly, EEM in a dose-dependent manner could mitigate these alterations and in the maximum dose (500mg/kg) could keep histological appearance, germ-cell apoptosis, sperm parameters and MDA concentration to normal. Functional outcome of the histological improvement clearly was demonstrated by significantly increased sperm count, sperm motility and sperm viability in EEM treated diabetic rats. The anti-apoptotic effects of EEM might be attributed with guggulsterone, a steroid obtained from myrrh. There is evidence showing that the use of guggulsterone at the doses of 10-30 µM significantly reduced the formation of MDA and suppressed caspase-3 activity to reference levels in doxorubicin-induced cytotoxicity and cell death in H9C2 cell line. The beneficial effects of EEM found in the present study might be attributed to its phytochemical compounds. 

There is evidence showing that the ethanolic extract of *C. myrrha* resin contains 27 organic compounds. Among its organic compounds, limonene, curzerene, germacrene B, isocericenine, myrcenol, beta selinene, and spathulenol are more abundant. Limonene is a cyclic monoterpen, and it has two isomers: D and L. which the main active form of limonene is d-limonene. In good agreement with our findings, Bacanli *et al.* reported that 28 days oral administration of d-limonene (50 mg/kg) to diabetic rats exhibited significantly improvement in the liver, kidney and plasma MDA levels while no considerable effects on FBG and insulin levels. The sesquiterpenes such as germacrene and curzerene are responsible for antimicrobial and insecticidal properties of myrrh. The EEM lipid peroxidation inhibitory effect may be due to presence of spathulenol in this gum. Recent evidence demonstrated that spathulenol exhibited the highest antioxidant and inhibitory effect against spontaneous lipid peroxidation formation in rat brain homogenate, with IC50 value of 26.17 µg/mL, which was comparable to a commercial antioxidant butylated hydroxytoluene (34.41 µg/mL). Among the involved mechanisms behind the development of reproductive dysfunction in diabetes, oxidative stress contributes as the main causative factor for testicular alterations which consequently led to male hypogonadism. Because of germ cells have considerable amount of polyunsaturated fatty acids on their plasma membrane; they are prone to oxidative stress damages. Previous studies show that most of plant extracts with beneficial effects on reproductive dysfunction induced by diabetes exert their effects through reducing or inhibiting MDA formation as well as increasing catalase activity in testis. In line with our findings, previous studies demonstrated that myrrh extracts dose-dependently reduced hepatic as well as colonic tissues MDA formation in diabetic and ulcerative colitis rats, respectively.

**Conclusion**

Taken together, the findings suggested that the mechanisms behind the beneficial effects of EEM might be associated with its sex hormone booster potential, antioxidant and anti-apoptotic properties. The antioxidant and anti-apoptotic activities of EEM at the highest dose (500 mg/kg) were significantly greater than metformin (500 mg/kg).

**Acknowledgements**

This paper is part of Mr. Mojtaba Salimi M.Sc. thesis which was fully financially supported by Birjand University of Medical Sciences (No.455159). We thank our students; Mr. Ghasem Ivar, Ms. Asma Moghaddam, Ms. Faezeh Rezaei and Ms. Faezeh Vahdati for their support and help.

**Conflict of interests**

The authors claim that there is no conflict of interest.
References


the seminiferous epithelium of syrian hamster
(Mesocricetus auratus) subjected to short
photoperiod. Reprod Domest Anim. 2013;48(6):974-
83. doi:10.1111/rd.a.12196
25. Kanter M, Aktas C, Erboga M. Curcumin attenuates
testicular damage, apoptotic germ cell death, and
oxidative stress in streptozotocin-induced diabetic
doi:10.1002/mnfr.201200170
Studies on the mechanism of testicular dysfunction in
the early stage of a streptozotocin induced diabetic rat
11.051
35. Rashid K, Sil PC. Curcumin ameliorates testicular
damage in diabetic rats by suppressing cellular stress-
mediated mitochondria and endoplasmic reticulum-
39. do Nascimento KF, Moreira FM, Santos JA, Kassuya
CA, Croda JH, Cardoso CA, et al. Antioxidant, anti-