The improvement effects of *Gordonia bronchialis* on male fertility of rats with diabetes mellitus induced by Streptozotocin

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Abstract

Background: This study evaluated the possible protective effects of *Gordonia bronchialis* (Gb) on oxidative stress and some subsequent alterations on testis from rats undergoing an experimentally induced type 1 diabetes.

Methods: A total of 40 male rats were randomly divided into four groups of ten. Diabetes was induced by injection of 55 mg/kg streptozotocin in 30 rats. Oral administration of Gb at dose of $10^5$ (low dose) and $10^7$ (high dose) CFU/rat was performed in two groups continuously for 14 days. The third and fourth groups received normal saline as the diabetic and healthy control groups, respectively. The blood and testicular tissue samples were taken on the 14th and 21st days post treatment for biochemical and histopathological evaluations. Results: Significant differences were found in blood glucose level, insulin, IL-6 and TNF-α values together with catalase and superoxide dismutase activities and malondialdehyde level in the diabetic group in comparison with healthy and Gb recipient groups. Moreover, the histopathological lesions observed in the diabetic rats mainly included basement membrane thickening, decreased number of Sertoli cells, and severe reduction of spermatogenesis markedly attenuated in Gb-treated rats.

Conclusion: Taken together, it seems that oral administration of Gb could ameliorate testicular damage associated with some related parameters in the diabetic animal model.

Keywords: biochemical evaluation; histopathological lesions; spermatogenesis; reproductive system; oxidative stress

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia resulting from defects in both insulin secretion and/or insulin action. Out of 422 million people affected by
DM globally, approximately 90% of diabetic patients have type 2 DM (T2DM), which is closely related to obesity and metabolic syndrome and is known to be an adult or old age disease. By contrast, 10% of the diabetic patients is attributed to Type 1 DM (T1DM), which is occurred by injury or cellular-controlled autoimmune destruction of the pancreatic β cells. About 85% of T1DM patients are diagnosed before the age of 20 and more than 15% of this population are related to adults (30 years old).\textsuperscript{2,3} Generally, the DM can lead various organ dysfunctions such as nephropathy, neuropathy, hepatopathy, retinopathy, and sexual failures.\textsuperscript{4} One of the most important complications of diabetes is the disturbance in the male reproductive system which will impact more men ahead of and during their reproductive years.\textsuperscript{3} In this regard, it is believed that diabetes has a strong correlation with oxidative stress and subsequently, the expression levels of pro-inflammatory cytokines such as TNF-α (tumor necrosis factor), interleukin-6 (IL-6), IL-10 and IL-1β will change.\textsuperscript{5} These elevated pro-inflammatory cytokines are likely to result in insulin resistance.\textsuperscript{6}

Recently, some reports have described a number of aerobic Actinomycetales species closely related to mycobacteria including Rhodococcus coprophilus, Gordonia bronchialis, and Tsukamurella inchonensis which are noticeable because of immunomodulatory activities.\textsuperscript{7} Previously, suspensions of heat-killed Actinomycetales have been suggested as adjuvant agents for immune intervention strategies in Chagas’ disease, caused by Trypanosoma cruzi.\textsuperscript{8} Likewise, it was reported that these heat-killed bacteria can prevent inflammatory response to a balloon catheter in the arterial intima.\textsuperscript{9} Growing evidence suggested that both obesity and T2DM together with some major biochemical indicators and kidney-liver tissue damage can be improved by administration of Actinomycetales as an immune modulator.\textsuperscript{2} However, there is no data on the effects of these immune modulators on male fertility and spermatogenesis in the setting of DM. Therefore, the
present study evaluated the possible beneficial effects of *Gordonia bronchialis* (as a heat-killed Actinomycetales species) on an improvement to male reproductive disorders in T1DM (induction by Streptozotocin (STZ)) through histopathological and biochemical examinations.

**Materials and methods**

**Animals and experimental design**

A total of 40 healthy adult male Wistar rats weighing between 250–360 g, were chosen and divided randomly into four groups of 10 rats. T1DM was induced in 30 rats by an intraperitoneal (i.p) injection of 55 mg/kg STZ (Sigma Aldrich Co., USA). Blood glucose levels were tested three days later (the animals with a blood glucose greater than 250 mg/dl were considered diabetic), the time-point when treatments were initiated. Two independent groups were treated by $10^5$ (low dose) and $10^7$ (high dose) CFU/rat (Colony Forming Unit) of *G. bronchialis* (BE-G101, BioEos Ltd., Kent, UK) (according to the previous study ²⁹) consecutively for 14 days orally by stomach gavage needle. Two additional groups of rats were given normal saline under the same conditions, one of them with T1DM (the diabetic control) and the other one with no diabetes (the healthy control). All animals were inspected every day for 21 days and their body weights were recorded on the 14th and 21st days post treatment.

**Sampling**

Blood samples were collected after deep anesthesia (by i.p injection of 50 mg/kg BW of ketamine and 8 mg/kg BW of xylazine) on the 14th and 21st days and sera were separated for biochemical tests at $750 \times g$ for 15 min. In addition, five rats in each group were sacrificed by cervical dislocation on the same days. After necropsy, samples from the testis were taken for biochemical
assays and stored in -70 °C. Moreover, additional testicular tissue specimens which were placed and fixed in 10% buffered formalin were taken for histopathological evaluation.

**Biochemical assays**

**Testicular antioxidant system evaluation**

At first, testicular tissue was homogenized and prepared as previously described. Then, assays for superoxide dismutase (SOD) activity, catalase (CAT) activity and malondialdehyde (MDA) levels were performed using commercial kits (Pars Azmoon, Tehran, Iran) according to the manufacturer’s instructions by using a spectrophotometer (Photometer 5010, Robert Riele GmbH & Co KG, Berlin, Germany) at 560nm, 240nm, 430nm and 535nm, respectively.

**Blood glucose and serum insulin levels**

These parameters were also measured by commercial kits (Pars Azmoon, Tehran, Iran) by using a spectrophotometer (Photometer 5010, Robert Riele GmbH & Co KG, Berlin, Germany) at 546 nm and 450 nm, respectively.

**IL-6 and TNF-α measurement**

The concentrations of IL-6 and TNF-α were measured in the obtained samples using Rat IL-6 and TNF-α ELISA kits (Koma Biotech, Korea) according to manufacturer’s instructions.

**Histopathological examination**

Tissues were fixed in 10% buffered formalin, processed routinely, sectioned, stained with hematoxylin and eosin (H&E), and finally studied microscopically using a light microscope. The tissue sections were examined for the presence of the pathological changes including atrophy, necrosis, hemorrhage, hyperemia and/or congestion. Moreover, Sertoli cells vacuolization, Leydig cells degeneration, basement membrane thickening, and interstitial fibrosis were evaluated. Seminiferous tubules (STs) diameters, germinal cell layer thicknesses and number of spermatogonia were measured using an ocular micrometer in a light microscope as previously
described.\textsuperscript{13} Additionally, spermatogenesis in the tissue samples of the testes was evaluated according to the Johnsen scoring system\textsuperscript{14} as shown in Table 1.

**Statistics**

Statistical analysis of the data was performed using SPSS software version 16 for windows (SPSS, USA). The ANOVA and non-parametric tests (Kruskal-Wallis H and Mann-Whitney U) were used for comparison of serum biochemical indicators and histopathological changes between the different groups, respectively. Differences were considered significant with $p < 0.05$.

<table>
<thead>
<tr>
<th>score</th>
<th>Microscopic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No germ cells and no Sertoli cells present</td>
</tr>
<tr>
<td>2</td>
<td>No germ cells, but only Sertoli cells present</td>
</tr>
<tr>
<td>3</td>
<td>Only spermatogonia present</td>
</tr>
<tr>
<td>4</td>
<td>Only a few spermatocytes present</td>
</tr>
<tr>
<td>5</td>
<td>No spermatozoa or spermatids, but numerous spermatocytes present</td>
</tr>
<tr>
<td>6</td>
<td>Only a few spermatids present</td>
</tr>
<tr>
<td>7</td>
<td>No spermatozoa, but numerous spermatids present</td>
</tr>
<tr>
<td>8</td>
<td>Only a few spermatozoa present in the section</td>
</tr>
<tr>
<td>9</td>
<td>Numerous spermatozoa present, but the germinal epithelium is disorganized</td>
</tr>
<tr>
<td>10</td>
<td>Complete spermatogenesis and normally organized tubules</td>
</tr>
</tbody>
</table>

**Results**

**Body weight**

As depicted in Figure 1, rats undergoing T1DM had a lower body weight with respect to healthy rats on the days 14 and 21. Body weight values did not differ between the low dose and high dose groups.
**Biochemical findings**

**Blood glucose and plasma insulin levels**

Higher blood glucose values together with lower insulin levels were seen in the third group of diabetic rats, with respect to control rats, on the days 14 and 21 (Figure 2). The increase in blood glucose level was observed as early as 72 h after the STZ injection and was maintained during the study. There were no significant differences between the low dose and high dose groups.

**IL-6 and TNF-α levels**

As expected, we investigated a notable higher level of IL-6 and TNF-α cytokines in the diabetic rats as compared to healthy animals which attenuated in both Gb treated groups (Figure 3). These pro-inflammatory cytokines values did not differ between the low dose and high dose groups.

**Evaluation of testicular biochemical oxidative stress markers**

Changes in tissue levels of MDA, SOD, and CAT were observed on the 14th and 21st days of sampling which are presented in Table 2. The present results demonstrated that STZ-induced diabetes significantly decreased testicular SOD and CAT activities and significantly increased testicular MDA levels (p < 0.05). Interestingly, the increased MDA level due to diabetes was significantly decreased in both low dose and high dose treatment groups. CAT and SOD enzyme levels were also significantly increased compared to the diabetic group.
Table 2 lipid peroxidation (MDA level) and antioxidant enzyme activities (SOD and CAT) in the four experimental groups on the days 14 and 21. Data represent means ± SEM of ten rats/group.

<table>
<thead>
<tr>
<th>Day</th>
<th>groups</th>
<th>MDA (µM)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>healthy</td>
<td>178.58 ± 12.72 a</td>
<td>1275.41 ± 122.6 a</td>
<td>32.74 ± 13.21 a</td>
</tr>
<tr>
<td></td>
<td>diabetic</td>
<td>305.11 ± 14.94</td>
<td>739.28 ± 16.97</td>
<td>17.46 ± 4.32</td>
</tr>
<tr>
<td></td>
<td>low dose</td>
<td>219.66 ± 13.76 ab</td>
<td>952.72 ± 19.89 ab</td>
<td>25.23 ± 5.5 ab</td>
</tr>
<tr>
<td></td>
<td>high dose</td>
<td>237.24 ± 9.86 ab</td>
<td>991.06 ± 27.56 ab</td>
<td>26.16 ± 3.92 ab</td>
</tr>
<tr>
<td>21</td>
<td>healthy</td>
<td>184.35 ± 16.72 a</td>
<td>1305.63 ± 36.5 a</td>
<td>29.69 ± 8.98 a</td>
</tr>
<tr>
<td></td>
<td>diabetic</td>
<td>337.82 ± 27.36</td>
<td>616.85 ± 28.39</td>
<td>13.22 ± 3.09</td>
</tr>
<tr>
<td></td>
<td>low dose</td>
<td>232.69 ± 15.54 ab</td>
<td>997.27 ± 31.23 ab</td>
<td>25.84 ± 5.35 ab</td>
</tr>
<tr>
<td></td>
<td>high dose</td>
<td>241.11 ± 35.73 ab</td>
<td>1103.19 ± 42.38 ab</td>
<td>27.63 ± 6.82</td>
</tr>
</tbody>
</table>

CAT: catalase; MDA: malondialdehyde; SOD: superoxidase dismutase. Low dose and high dose treated groups were received 10⁵ and 10⁷ CFU/rat *G. bronchialis*, respectively. a: significant difference respect the diabetic control group (p<0.05); b: significant difference respect the healthy control group (p<0.05).

**Histopathological studies**

The microscopic examination of the healthy control group on the 14th and 21st days of study showed normal testicular structure with properly arranged, normal germinal cells and tubules and no pathological changes. By contrast, the diabetic group left untreated displayed moderate to severe pathological changes in their testicular tissue on the day 14. This included vascular hyperemia and congestion, interstitial edema, thickening of basement membrane, Sertoli cells vacuolization, Leydig cells degeneration, atrophy of Seminiferous tubules (with various sizes and shapes) and noticeable decrease of spermatogenesis (with 3.5 and 4.5 mean scores on the days 14 and 21, respectively; Figure 4). Such pathological changes were even more severe when studying 21-day samples. Surprisingly, Gb recipients showed quite ameliorated pathological lesions at both time-point evaluations, with day 21 studies revealing a nearly normal testicular structure. This group also showed a marked spermatogenesis improvement (mean scores: 9 and 9.25 on the days 14 and 21, respectively) yielding a significant difference when compared to the diabetic control.
In most sections from the treated groups, there were numerous spermatids and spermatozoa with a slight disorganized germinal epithelium, in addition to a lower number of germ cells, Sertoli cells, spermatocytes and spermatids (Figure 5). Additionally, comparison of testicular histologic parameters presented in Table 3 demonstrated statistically significant differences in evaluated histomorphometrical parameters between the diabetic control with the healthy and the treatment groups. Indeed, the treatment group showed marked increased in the thickness of germinal epithelium, Seminiferous tubules diameters and number of spermatogonia compared to the diabetic untreated rats. Importantly, there was notably mild to moderate basement membrane thickening associated with mild to moderate vascular congestion in the high dose Gb treated group on the 21\textsuperscript{st} day.

**Table 3.** Comparison of testicular histologic parameters on 14\textsuperscript{th} and 21\textsuperscript{st} days of study in different experimental groups. There were significant differences ($p<0.05$) in all of the parameters in both days of sampling between diabetic control with negative control and also treatment group. However, there was no significant difference between negative control and treatment group.

<table>
<thead>
<tr>
<th>Days of sampling</th>
<th>groups</th>
<th>Thickness of germinal epithelium (µm)</th>
<th>Seminiferous Tubules diameter (µm)</th>
<th>Number of spermatogonia (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>healthy</td>
<td>$3.02 \pm 0.22$ \textsuperscript{a}</td>
<td>$3.87 \pm 0.23$ \textsuperscript{a}</td>
<td>$54.74 \pm 2.84$ \textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>diabetic</td>
<td>$1.48 \pm 0.15$</td>
<td>$3.06 \pm 0.14$</td>
<td>$33.62 \pm 1.74$</td>
</tr>
<tr>
<td></td>
<td>low dose</td>
<td>$2.73 \pm 0.23$ \textsuperscript{a}</td>
<td>$3.45 \pm 0.30$ \textsuperscript{a}</td>
<td>$47.45 \pm 2.14$ \textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>high dose</td>
<td>$2.91 \pm 0.17$ \textsuperscript{a}</td>
<td>$3.38 \pm 0.21$ \textsuperscript{a}</td>
<td>$44.15 \pm 2.56$ \textsuperscript{a}</td>
</tr>
<tr>
<td>21</td>
<td>healthy</td>
<td>$3.16 \pm 0.47$ \textsuperscript{a}</td>
<td>$4.3 \pm 0.35$ \textsuperscript{a}</td>
<td>$52.89 \pm 9.56$ \textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>diabetic</td>
<td>$1.2 \pm 0.07$</td>
<td>$2.78 \pm 0.11$</td>
<td>$26.36 \pm 5.14$</td>
</tr>
<tr>
<td></td>
<td>low dose</td>
<td>$3.14 \pm 0.33$ \textsuperscript{a}</td>
<td>$3.72 \pm 0.38$ \textsuperscript{a}</td>
<td>$43.08 \pm 8.85$ \textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>high dose</td>
<td>$3.32 \pm 0.36$ \textsuperscript{a}</td>
<td>$3.69 \pm 0.19$ \textsuperscript{a}</td>
<td>$45.42 \pm 7.61$ \textsuperscript{a}</td>
</tr>
</tbody>
</table>

Low dose and high dose treated groups were received $10^5$ and $10^7$ CFU/rat *G. bronchialis*, respectively. a: significant difference respect the diabetic control group ($p<0.05$); b: significant difference respect the negative control group ($p<0.05$). There was only a significant difference between days 14 and 21 in the diabetic rats.
**Discussion**

It is well known that DM has an adverse effect on male infertility in animal models.\(^1\) Earlier reports showed that the STZ induced rat model of T1DM revealed male reproductive disorders resembling human diabetic infertility.\(^1\)\(^6\) In this regard, a previous study indicated that experimental STZ diabetes presented a significant increase in teratozoospermia and a decrease in serum testosterone, tubular diameter, and testicular and epididymal weight. STZ-resistant animals showed significantly decreased serum testosterone, epididymal weight, and sperm count.\(^1\)\(^7\) In line with this, present findings indicate that STZ-induced diabetes significantly reduced body weight, serum insulin, testicular SOD and CAT activities while augmenting serum glucose and testicular MDA levels. What is more, the current results presented that both cytokines IL-6 and TNF-\(\alpha\) become elevated in STZ-exposed groups which thereby attenuated in both recipient Gb groups and anti-inflammatory potential of Gb may be concluded. The latter findings were associated with testicular pathological lesions consisting of Seminiferous tubules atrophy, basement membrane thickening, germinal cells degeneration and apoptosis, decreased number of Sertoli cells and Leydig cells, interstitial edema and fibrosis, capillary congestion, along with presence of atypical cells and marked reduction of spermatogenesis. Several studies reported STZ-induced DM changes in testicular tissue.\(^1\)\(^8\)-\(^2\)\(^1\) Expanding these studies, we also demonstrate that STZ-induced diabetes resulted in a significant increase of the testicular MDA values accompanied by a marked decrease of testicular antioxidant enzymatic activities (CAT and SOD). Notably, most of these alterations were significantly reverted upon oral administration of Gb to the diabetic rats, probably to an antioxidant property acting to protect testicular germ cells against the adverse effects of DM. Remarkably, oral administration of Gb to these diabetic rats resulted in a significant recovery of these biochemical and histological
disturbances. Notably, the improvement of some evaluated parameters due to the Gb treatment was not associated with dose-dependent manner. Similarly, in the recent research studies the beneficial effects of Gb on some biochemical parameters (like triglyceride, cholesterol, uric acid and glucose), enzyme activity (ALP and AST, lysozyme and peroxidase) in rainbow trout in independent-dose manner have been presented.\textsuperscript{22,23} Considering the important role of oxidative stress in pathogenesis of DM induced male reproductive disorders,\textsuperscript{24} it may be speculated that Gb improves diabetic injury of testicular tissue through antioxidant properties and pro-inflammatory cytokines.

Convincing evidences demonstrated beneficial effects of Gb in rats either by limiting parasitemia and chronic myocarditis in a model of infection with \textit{Trypanosoma cruzi} or improving obesity and T2DM.\textsuperscript{2,8} Mainly because heat-killed preparations of the \textit{Actinomycetales} species including \textit{G. bronchialis} and \textit{T. inchonensis} ameliorated the progress of rat T2DM and obesity in terms of pancreas, lung, liver and kidney involvement along with improved ability to regulate blood glucose, serum insulin, TNF-\(\alpha\) and IL-6 levels.\textsuperscript{5} Extending this finding, we now asked whether heat-killed \textit{Actinomycetales} was also beneficial on T1DM and its complications, particularly in the male reproductive tract. In line with the previous study we now demonstrate that Gb was also effective to reduce body weight, along with serum glucose and insulin levels. In the same sense, other studies reported the protective effects of ghrelin, royal jelly, gum Arabic and gallic acid on antioxidant defense system in DM male infertility.\textsuperscript{19-21,25} Elevated oxidative stress accompanied by an antioxidant deficit is proposed to be a major factor leading in the pathogenesis of diabetic complications such as male reproductive disorders.\textsuperscript{26} Indeed, hyperglycemia during diabetes causes an increased production of free radicals, especially reactive oxygen species (ROS) in all tissues.\textsuperscript{27} Subsequently, production of high levels of free radicals and decreased efficiency of
antioxidant enzyme defense can injure cellular organelles and enzymes in addition to increasing lipid peroxidation. Since the male reproductive compromise during DM can be mediated through ROS, antioxidant therapy may be useful for treating DM related infertility in males. While our studies revealed no great differences in antioxidant enzymes activities and lipid peroxidation between days 14 and 21, data from the latter time point evaluation showed a slight improvement in antioxidant defense systems. Moreover, present results suggest that usage of Gb can be one of the more suitable choices for ameliorating oxidative stress on testis during DM.

It is well known that hyperglycemia in DM enhances the inflammatory markers such as TNF-α, IL-1 and IL-6 which may be mediated indirectly by ROS. As previously described, these elevated proinflammatory mediators reluctant insulin function due to their potential to supplement insulin receptor substrate phosphorylation, resulting in insulin resistance. It was recently presented that the administration of T. inchonensis showed an improvement effect on TNF-α and IL-6 alterations in activated murine peritoneal macrophages. On the other hand, it is believed that the serum content of IL-6 in patients with T1DM was significantly greater than healthy controls which are involved in the inflammation and immune responses and were associated with development of T1DM. Our results were consistent with several previous studies that showed reduction levels of TNF-α, IL-6 in the diabetic rats after administration of curcumin, gallic acid, quercetin, and lutein and consequently, the remarkable improvement of testicular structure and spermatogenesis grading were found. Interestingly, it was recently clarified that STZ exposure leads to enhancing the expression of TNF-α receptors in testicular tissue which promotes caspase-8 and extrinsic pathway of apoptosis in spermatogonia cells series.

Similarly, in the present study, the diabetic rats undergoing no treatment showed several pathological changes on testis together with significant decrease in spermatogenic series cells
(especially in spermatogonia and spermatocytes) and also Sertoli cells. The exact mechanism of spermatogenic cells apoptosis on DM is not fully understood. Some researchers proposed various mechanisms for testicular apoptosis and spermatogenic cell death. One of them is related to hyperglycemia which can increase the production and accumulation of ROS causing cell apoptosis in different tissues such as testis.\textsuperscript{35} Present findings along with data from other previous studies, suggest that the respective increased and decreased circulating levels of glucose and insulin result in excessive production of ROS, further promoting apoptosis and the ensuing decreased number of both Leydig and Sertoli cells, and appearance of sexual dysfunctions. Particularly, the healthy and Gb recipient groups exhibited healthy histological structure of the seminiferous tubules and nearly normal spermatogenesis. The testicular sections of the Gb-treated diabetic rats demonstrated significant improvement in Seminiferous tubules structure as well as noticeable increase in spermatogenic cells number.

**Conclusion**

In essence, Gb is able to improve most biochemical and histological surrogates of DM-related gonadal damage, providing a stimulating background for further analyzing its potential usefulness in diabetic patients. Meanwhile, further studies are still required on other different doses of Gb, other administration routes of Gb, and evaluation of other pro- and anti-inflammatory cytokines involved in DM is recommended.

**Animal ethics**

The experiments were designed and performed according to the ethical guidelines of Animal Research Ethics Committee of Tabriz University of Medical Sciences (ethical approval code: 5-4-1171, date: 4 May 2013).
Abbreviations

Diabetes mellitus: DM; type 1 diabetes mellitus: T1DM; type 2 diabetes mellitus: T2DM; TNF-α: tumor necrosis factor; Streptozotocin: STZ; superoxide dismutase: SOD; catalase: CAT; malondialdehyde: MDA; hematoxylin and eosin: H&E; Seminiferous tubules: STs.

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References


Figure 1. Body weight values (mean ± SEM) in the experimental groups on the days 14 and 21. a: significant difference with the diabetic group on the day 14; a’: significant difference with the diabetic group on the day 21 (p<0.05).
Figure 2. Serum glucose and insulin levels (mean ± SEM) in the experimental groups on the days 14 and 21; a: significant difference with the diabetic groups on the day 14; b: significant difference with the healthy group on the day 14; a’: significant difference with the diabetic group on the day 21; b’: significant difference with the healthy group on the day 21 (p<0.05).
Figure 3. TNF-α and IL-6 levels (mean ± SEM) in the experimental groups on the days 14 and 21; a: significant difference with the diabetic group on the day 14; b: significant difference with the healthy group on the day 14; a’: significant difference with the diabetic group on the day 21; b’: significant difference with the healthy group on the day 21 (p<0.05).
Figure 4. A significant improvement of spermatogenesis (mean ± SEM) in the treatment groups on both days of sampling according to Johnsen’s scoring system. a: significant difference with the diabetic group on the day 14; a’: significant difference with the diabetic group on the day 21 (p<0.05).
Figure 5. histioarchitecture of rat testis. a: the healthy control group with normal Seminiferous tubules structure and complete spermatogenesis. b, c, d: the diabetic rats testis displayed pathological changes including Seminiferous tubules atrophy, thickening of basement membrane (bm), vascular congestion (cg) and interstitial edema (ie). Moreover, there were a significant reduction of spermatogenesis together with degeneration and apoptosis of spermatogonia (sp), primary spermatocytes (ps), spermatids (sd) and Sertoli cells; e: the diabetic rats treated by low dose of G. bronchialis; f: the diabetic rats treated by high dose of G. bronchialis. The latter groups showed nearly normal testicular structure, particularly on the 21st day. H&E.