Full Length Research Paper

Efficacy of *Tribulus terrestris* extract and metformin on fertility indices and oxidative stress of testicular tissue in streptozotocin-induced diabetic male rats

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The present study aimed at evaluating the effect of *Tribulus terrestris* on different parameters of oxidative stress and enzymatic/non-enzymatic antioxidant as well as the number, viability and abnormalities of sperm in testis tissues of male rats after induction of diabetes. The animals were divided into six groups; group I (control) was administered vehicle only, group II was treated with metformin (MET) and those in group III were given *T. terrestris* plant extract (TT extract). Group IV acted as positive diabetic control, group V and VI were diabetic animals treated with MET and TT-extract, respectively. The treatments were continued for 5 days/week for 60 days. Various oxidative stress parameters such as lipid peroxidation and activity of antioxidant enzymes were used to confirm the per-oxidant state of animals as an effect of different treatments. *T. terrestris* was noticed to reduce the oxidative stress levels, and restore antioxidant enzyme activity in testis tissues as well as to improve the lipid profile content in serum. Histological analysis showed that *T. terrestris* treatment decreased testis tubular damage, and restored it to normal morphology. It can be concluded that TT extract as compared to metformin has potential effect against spermatotoxic and testicular toxicity and it can improve redox state in diabetic male rats.

**Key words:** *Tribulus terrestris*, oxidative/antioxidant, fertility indices, diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (Thévenod, 2008; Bai et al., 2011; Wankeu-Nya et al., 2014). Sustained higher levels of blood glucose cause damage to nerves and blood vessels, leading to complications such as erectile dysfunction (ED) (Thorve, 2011; Cao et al., 2012). DM is one of the predominant risk factors of ED and also one of the most difficult to treat (Chitaley et al., 2009). DM may cause ED through a number of pathophysiologic changes, including neuropathy, endothelial dysfunction and hormonal changes (Konstantinos and Dimitrios, 2012).

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2009). Although pathophysiologic changes may be more pronounced in type 1 diabetes than in type 2, they are mainly due to oxidative stress, through the formation of oxygen free radicals and advanced glycation end-products (AGEs) (Giacco and Brownlee, 2010; Ramesh et al., 2012).

Streptozotocin (STZ) induces diabetes mellitus by destroying pancreatic β-cells, possibly through generating excess reactive oxygen species (ROS) (Yamagishi et al., 2001). STZ generated lipid peroxidation (LPO) and DNA breaks in pancreatic islets cells have been demonstrated (Lenzen, 2008). Exaggerated production of these reactive species in diabetes can lead to very serious problems including cardiovascular disease, liver and kidneys failure, blindness, and nerve injury (Neyenwe et al., 2011; El-Shenawy et al., 2013). Due to multiple action of streptozotocin intoxication, understanding how uncontrolled hyperglycemia impacts the sexual function and seeking for efficient drugs able to alleviate diabetes-induced complications are yet important areas of inquiry. However, despite the increasing availability of effective conventional medical treatments for erectile dysfunction, understanding how uncontrolled hyperglycemia impacts the sexual function and seeking for efficient drugs able to alleviate diabetes-induced complications are yet important areas of inquiry. However, despite the increasing availability of effective conventional medical treatments for erectile dysfunction, plant-derived and herbal remedies continue to provide a popular alternative for diabetic men seeking to improve their sexual life (Watcho et al., 2007; Yakubu and Afolayan, 2009).

Thus, antioxidant therapy is one of the strategies for diabetes treatment. Many herbal extracts or derivatives with high antioxidant activity are useful for treatment of diabetes and other metabolic syndrome (Samad et al., 2009). Several plant extracts are known to have antidiabetic properties and a large number of compounds from plant extracts have been reported to have beneficial effects for the treatment of DM (Ramesh et al., 2010). Diabetes can be managed by diet, exercise and chemotherapy. However, the pharmacological drugs are either too expensive or have undesirable side effects or contraindications (Maiti et al., 2008). Throughout the world, many traditional plant treatments for diabetes exist, and therein lies a hidden wealth of potentially useful natural products for the control of diabetes. Natural plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones (Ramesh et al., 2012; Sunil et al., 2009).

*Tribus terrestris* (TT) is a member of the Zygophyllaceae family and a natural herb. It is widely distributed in Africa, Mediterranean region western Asia, China, Japan, Korea and Europe (Mohammed et al., 2013). TT is used in the folk medicine against sexual impotence, edemas, abdominal distention and cardiovascular diseases (Chhatre et al., 2014). It has been shown to increase the free serum testosterone (Brown et al., 2001) and it possesses aphrodisiac activity probably due to androgen increasing property of TT (Mohammed et al., 2013). *Tribus* has no significant side effects if used at the safe range of 250 to 750 mg/day (Heidari et al., 2007). TT extract contains many compounds such as alkaloids, flavonoids oil, saponins, resins and nitrates (Adaay and Mosa, 2012), possesses antihypertensive activity (Braca et al., 2001) and hypolipidemic effect (Chu et al., 2003). Amin et al. (2006) found that the ethanolic extract of TT exhibits a significant antioxidant activity against STZ-induced diabetes in liver tissues without explaining the mechanism of protection.

Moreover, Kamboj et al. (2011) reported that TT is an extraneous antioxidant, reduced oxidative stress, maintained proper renal functioning and reduced renal injury. Metformin, glucose-lowering agent, is commonly used for the treatment of type 2 diabetes. Decreasing hepatic glucose production through gluconeogenesis suppression and activating peripheral glucose utilization in muscle, intestine and liver have been reported to be contributors to the glucose-lowering effect of metformin (Yoshida et al., 2009), but the primary effect of metformin on glucose-lowering remains unknown. Therefore, the present study focused on a comparison between the chronic effect of metformin and aqueous extract of TT as a direct gluconeogenesis inhibitor and subsequently, its effects on lipid profile, serum testosterone and follicle stimulating hormones levels, semen quality and oxidative parameters of testicular tissues of streptozotocin-induced diabetic male rats. This could facilitate further understanding of the implications of the difference in mechanisms between metformin and TT extract in terms of clinical usage for the treatment of diabetes. Moreover, the histological examination of testis was evaluated.

**MATERIALS AND METHODS**

**Chemicals**

STZ was purchased from Sigma Co. (St. Louis, MO). Thiobarbituric acid aqueous solution (TBA), n-butanol, pyridine, 1,1,3,3-tetramethoxypropane standard, trichloroacetic acid (TCA), phosphate buffer, 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) and reduced glutathione (GSH) standard were obtained from Fluka (Tauffkirchen, Germany). All the chemicals used were analytical grade. The assay kits for cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were obtained from BioDiagnostic Company, Giza, Egypt.

**Plant materials collection and preparation of extracts**

*T. terrestris* fruits were obtained from the local market of herbs, Ismailia, Egypt. The plant was identified and authenticated by Botany Department, Faculty of Science, Suez Canal University, Ismailia Egypt. Fruits were ground into a fine powder. For the aqueous extraction, hundred grams (100 g) of the powdered fruit were extracted using 200 mL of distilled water in Soxhlet extraction system for 12 h. The extract was evaporated using rotary evaporator at 40°C under reduced pressure close to dryness (gummy residue). The yield was found to be 12%. The gummy residue was dissolved in appropriate volume of distilled water and stored at -20°C until use (Eagappan et al., 2015).

**Phytochemical screening**

The phytochemical profile was performed as described by Costa...
Preparation of standard drug (MET)

Metformin hydrochloride (500 mg/tablet) was purchased from a local pharmacy. Three tablets of drug were ground to fine powder and dissolved in 100 mL distilled water. Rat dose of metformin was calculated from the standard clinical human dose on the basis of surface area [rat dose = (human dose/average body weight of rats) \times 7] (Freireich et al., 1966).

Animals

Healthy adult male albino rats of the Wistar strain, 4–5 months of age and weighing 170–190 g, were supplied from the Animal Breeding House in Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. They were maintained at room temperature with a natural light : dark cycle (12:12 h) and provided with standard diet and water ad libitum. The experiments were performed in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 (European Communities (EC, 1986). Rats were acclimatized to the laboratory environment for a week prior to the start of experiments.

Induction of diabetes

Diabetes was induced in 16 h fasted male rats by a single intraperitoneal injection of a buffered solution (0.1 M citrate, pH 4.5) of streptozotocin at the dose 55 mg/kg. To prevent hypoglycemia, animals were given a 10% glucose solution for the next 48 h. Blood glucose level was measured 3 days after diabetes induction using reagent strips (Accu-Chek®, Roche). Blood was collected from tail surface area [rat dose = (human dose/average body weight of rats) \times 7] according to the methods described by Pant and Srivastava (2003) and total number of the sperm head counted at 40x magnification. Each sample was counted twice and means value was taken for calculation. Sperm count was expressed as number of sperm per ml of solution.

Sperm viability and abnormalities

Sperm suspension was pipetted very gently 20 times and placed in a hemocytometer. Sperm were stained with eosin-nigrosine staining method to evaluate the viability and abnormalities (NAFA, 2002). Sperm morphology was viewed under a light microscope (Nikon, H600L, Tokyo, Japan) under 400\times magnifications. Percentages of sperm head and acrosome abnormalities were evaluated on air-dried eosin-nigrosine stained slide and were expressed as a percentage for abnormalities (Evans and Maxwell, 1987). Data was expressed as percentage of morphologically abnormal sperm to total sperm count.

Body and genital organ weight

At the end of experiment, the testes and accessory sex organs (semenal vesicles, prostates and epididymis) were dissected out, trimmed off the attached tissues and weighed individually. Then, the organ/body weight ratio was calculated.

Biochemical assays

Blood samples were collected after 60 days of treatment from anaesthetized animals groups from retro-orbital venous plexus (Itziar et al., 2010) with a fine sterilized glass capillary tube into heparin-coated and dry tube. The gathered blood were left for 20 min at room temperature, then centrifuged at 3000 rpm (600 g) for 10 min for the separation of sera. The sera were kept in a deep freezer (at -20°C) until analyses of certain biochemical parameters. The biochemical measurements were performed according to the details given in the kit’s instructions.

Hormonal determination

Serum testosterone concentrations were determined using a solid phase enzyme-linked immunosorbsent assay (ALPCO Diagnostics, Cat No. 55-TESMS-E01, USA) based on the principle of competitive binding. An unknown amount of testosterone present in the sample and a defined amount of testosterone conjugated to horseradish peroxidase compete for the binding sites of testosterone antiserum coated to the wells of a microplate. After one-hour incubation on a shaker the microplate was washed four times. The concentration of testosterone is inversely proportional to the optical density measured (Darney et al., 1996). Follicle-stimulating hormone (FSH) was determined using a kit (ALPCO Diagnostics, Cat No. MBSB10666, USA), that depend on double-antibody sandwich enzyme-linked immunosorbsent assay (Knobil, 1980).

Determination of serum lipid profile

The serum total cholesterol (TC) and triglycerides (TG) were determined according to the methods described by Richmond (1973) and Fossati and Prencipe (1982), respectively. High density lipoprotein–cholesterol (HDL–c) was determined according to the methods of Lopez et al. (1977). Serum LDL–cholesterol (LDL–c)
level was calculated according to Friedewald (1972) formula: LDL-c = total cholesterol - (HDL-c + triglycerides)/5. Very low density lipoprotein cholesterol (VLDL-c) levels were calculated by using the following formula of Prakasam et al. (2003): VLDL-c = triglyceride/5.

Preparation of homogenate tissue

The excised testicular tissue was washed with deionized water for the removal of blood, and later the fatty parts were removed. Homogenization was performed in a phosphate buffer solution with a pH value adjusted to 7.4, and the supernatant was separated by means of centrifugation at 20,000 rpm for 1 h. The supernatant and hemolysate obtained were used for the analyses of all oxidative and antioxidant parameters.

Lipid peroxidation (LPO) level: Lipid peroxidation was determined in supernatant of homogenate testicular tissue by the thiobarbituric acid (TBA) method which estimates the malondialdehyde formation (MDA) according to Esterbauer and Cheeseman (1990). The concentration of MDA was calculated by the absorbance coefficient of MDA–TBA complex (1.56 X 10^5 M^-1 cm^-1). LPO was expressed as nano moles MDA/g tissue.

Antioxidant enzymes: The specific activity of testicular superoxide dismutate (SOD, EC.1.15.1.1) was determined according to the method described by Misra and Fridovich (1972). Activity of SOD was expressed as units/mg protein. The testicular catalase (CAT) activity (CAT; EC 1.11.1.6) was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme (Aebi, 1984). Activity of catalase (CAT) was expressed as units/mg protein. Glutathione peroxidase (GPx) activity was determined as described by Hafeman et al. (1974). The peroxide substrate (ROOH), glutathione reductase (GRx) and NADPH are included in the reaction mixture. The formation of oxidized form of glutathione (GSSG) catalyzed by GPx is coupled to the recycling of GSSG back to reduce form of glutathione (GSH) using GRx. NADPH is oxidized to NADP^+. The change in A 340 due to NADPH oxidation is monitored and is indicative of GPx activity. Glutathione-S-transferase (GST; EC 2.5.1.18) activity of testicular was measured spectrophotometrically by the method of Alin (1985) using S-2,4-dinitrophenyl glutathione (CDNB) as a substrate. The activity of GST was expressed in terms of μmol/min/mg protein.

Reduced glutathione content: Reduced glutathione content (GSH) of supernatant was performed by the method of Beutler (1963) using commercial glutathione reduced kits (Bio diagnostic for diagnostic reagents: Dokki, Giza, Egypt). Determination of GSH is based on the reaction of DTNB (50,5- dithiobis-(2-nitrobenzoic acid)) with GSH and yield of a yellow colored chromophore; 5-thio-nitrobenzoic acid with a maximum absorbance at 412 nm. The amount of GSH present in the testicular tissue was calculated as nano mole/g tissue.

Histopathological evaluation

Histological examination of the tissue was conducted after removal of testis from rats. The tissues were gently rinsed with a physiological saline solution (0.9% NaCl) to remove blood and adhering debris. Testes were taken and fixed in a 10% neutral-buffered formalin solution for 24 h. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4–6 μm thickness and stained with Hematoxylin and Eosin (H&E) then examined microscopically according to Luna (1968).

Statistical analysis

Data are expressed as mean values ± SE (n = 6). Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. For each significant effect of treatment, the post hoc Tukey’s test was used for comparisons. The criterion for statistical significance was set at P < 0.05. All statistical analyses were performed using SPSS statistical version 20 software package (SPSS Inc., USA).

RESULTS

Phytochemical screening

Qualitative phytochemical screening of aqueous extracts of TT showed the presence of alkaloids, tannins, saponins and cardiac glycosides.

Sperm characteristics and relative organs weight

Figure 1 shows the effect of treatment of TT-extract on sperm characteristics in diabetic rats. Our findings indicate that in diabetic rats, sperm count was 77.66% lower than normal, non-diabetic rats. In diabetic rats, total sperm count was approximately 33.0 ± 15.21 million/mL which was lesser than the normal rats. 60-days treatment with the MET or TT-extract caused a significantly higher count (2.33- and 2.73-fold, respectively) as compared to non-treated diabetic rats. No significant difference in the count was noted between treatment with MET and TT-extract. The sperm viability was 28.0% in diabetic rats than normal, non-diabetic rats, while the viability was 66.0% for control animals (Figure 1). 60-days treatment with MET resulted in a significant increase in the viability to 49%. Meanwhile, treatment with TT-extract caused increase in sperm viability of diabetic rats, significantly to normal value of control (60.0%).

Figure 1 shows percentages of abnormal sperm in control and treatment groups. Sperm morphology assessment showed that the sperm abnormalities (head and tail) were more frequent in diabetic male rats (60%) than those of the normal control (17%). On the other hand, the sperm abnormalities reduced (P < 0.05) in normal group treated with the TT-extracts for 60 days to 10%. When the diabetic groups received MET or TT-extract, the abnormality decreased by 33 and 29%, respectively as compared to those of the diabetic animals.

The tissue weights expressed as relative organ weights are shown in Table 1. Changes in the relative weights of the testes were recorded among all the treated-group. Significantly lower relative weights of the prostate glands vs. controls were found in the diabetic-group. In groups treated with MET and TT extract, a significant (P<0.01) decrease in the relative mass of the prostate was also found as compared to diabetic animals. No difference in
Figure 1. Sperm evaluation in control and diabetic male albino rats treated with MET and TT extract. Values as mean ± SEM. n=6, One Way ANOVA followed by Duncan multiple comparison tests. *p<0.05 when compared with normal control group, **p<0.05 when compared with diabetic group.

Table 1. Effect of MET and TT extract on relative organ weight in normal and streptozotocin-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups and Treatment</th>
<th>Mean ± SE of sexual organs relative weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testes</td>
</tr>
<tr>
<td>Control</td>
<td>0.65±0.051</td>
</tr>
<tr>
<td>MET</td>
<td>0.77±0.085</td>
</tr>
<tr>
<td>TT extract</td>
<td>0.76±0.025</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.28±0.023a</td>
</tr>
<tr>
<td>Diabetic and MET</td>
<td>0.51±0.024b</td>
</tr>
<tr>
<td>Diabetic and TT extract</td>
<td>0.62±0.121b</td>
</tr>
</tbody>
</table>

Values as mean ± SEM. n=6, One way ANOVA followed by Duncan multiple comparison tests. *p<0.05 when compared with normal control group, **p<0.05 when compared with diabetic group.

the relative weights of the epididymis tail between the control and the diabetic-rats and also between the diabetic animals and other treatment was seen.

Biochemical parameters

Table 2 shows that after eight weeks of treatment, apart from the treated control rats (MET and TT-extract groups) where the blood glucose continued to increase, those groups remained statistically unchanged as compared to their respective baseline values (initial blood glucose level) but remained high as compared to the normal control rats. Diabetic rats displayed characteristic high blood glucose and low plasma insulin levels. The glucose levels decreased significantly by 55.19 and 34.91% after treating the diabetic rat with MET and TT-extract, respectively. The levels of insulin were improved significantly in the groups treated with MET and TT-extract by 2.55- and 3.0-fold, respectively.

As shown in Figure 2, TT extract treatment increased significantly, the serum testosterone levels in diabetic animals as compared to the diabetic rats by 2.79-fold. The treatment of diabetic rat with MET increased the level of testosterone by 2.33-fold as compared to diabetic-group. FSH increased significantly in diabetic
Table 2. Effect of MET and TT extract on blood glucose and insulin levels in normal and streptozotocin-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dL)</th>
<th>Insulin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93.40 ± 2.04</td>
<td>1.20 ± 0.08</td>
</tr>
<tr>
<td>MET</td>
<td>104.20 ± 7.12</td>
<td>0.92 ± 0.03</td>
</tr>
<tr>
<td>TT extract</td>
<td>110.20 ± 2.69</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>Diabetic</td>
<td>212.00 ± 11.58a</td>
<td>0.20 ± 0.01a</td>
</tr>
<tr>
<td>Diabetic and MET</td>
<td>95.60 ± 3.64b</td>
<td>0.71 ± 0.01b</td>
</tr>
<tr>
<td>Diabetic and TT extract</td>
<td>138.20 ± 15.84b</td>
<td>0.80 ± 0.11b</td>
</tr>
</tbody>
</table>

Values as mean ± SEM, n=6. One way ANOVA followed by Duncan multiple comparison tests. a p<0.05 when compared with normal control group, b p<0.05 when compared with diabetic group.

Figure 2. Testosterone and FSH levels in control and diabetic male albino rats treated with MET and TT extract. Values as mean ± SE (n=6), One way ANOVA followed by Duncan multiple comparison tests. a p<0.05 when compared with normal control group, b p<0.05 when compared with diabetic group.

Rats by 3.94-fold as compared to the control animals (Figure 2). FSH was reduced by 49.27 and 46.83% in diabetic rats treated with MET and TT-extract, respectively as compared to diabetic animals.

All the parameters of lipid profile (TC, TG, LDL-c and VLDL-c) were increased except the HDL-c which was decreased in diabetic rats (Table 3). The effect of TT extract on the lipid profile of the diabetic rats was significantly different from the MET-treated group. Treatment of the TT extract to diabetic group significantly decreased TC, TG, LDL-c and VLDL-c as compared to MET-group.

Our results revealed an increase of LPO in the testis of the diabetic-treated group as evidenced by the enhanced malondialdehyde levels in the testis homogenates as compared to negative the controls (Table 4). The administration of MET and TT extract alleviated LPO induced by STZ treatment and significantly modulated the malondialdehyde levels in the testis of rats by 62.19 and 65.74%, respectively.

The result clearly indicated that treatment with MET resulted in a significant increase in the activity of testes GST as compared to diabetic animals by 1.66-fold (Table 4). However, diabetic rats treated with TT-extract showed significant increase in GST by 1.33-fold as compared to diabetic rats.

The result of testicular reduced glutathione (GSH) level is presented in Table 4. These results indicated that
respectively). The treatment of MET and TT-extract (3.81- and 4.45-fold, respectively) has been noticed in the GSH levels that there is an increase by diabetic animals, it was capable of recovering the GSH level to approximately the normal values (Table 4). It has been noticed in the GSH levels that there is an increase by the treatment of MET and TT-extract (3.81- and 4.45-fold, respectively).

Histological study

Figure 3 illustrates the histological examination of testicular tissues of different treatment groups. Testicular histology of control group revealed normal spermatogenesis, depicting all the germ cells types, viz. spermatogonial, primary spermatocytes (non-pachytene and pachytene) and spermatids (round and elongated), sperms with normal morphology and concentration in the seminiferous tubules. The Sertoli and interstitial Leydig cells are also showed normal morphology (Figure 3A). Moreover, all layers of germ cells had normal basement membrane and interstitial tissue. Figure 3B and C shows normal testis of control rats treated separately with MET and TT-extract, respectively. Figure 3D demonstrates that diabetes caused degenerative changes such as loss of germ cells, abnormality of germinative epithelium, interruption in meiosis, sperm with abnormal shape and concentration.

These changes were markedly reduced with oral administration MET or TT-extract, revealing a marked repairing of testicular abnormalities, as shown in Figure 3E and F, demonstrating maximum antioxidant and healing effects against STZ induced diabetes testicular damage, showing sperm with normal morphology and concentration close to the control group. Histopathological findings are in accordance with the results of the above studied parameters for testicular toxicity.

DISCUSSION

The aim of the present study was to evaluate the aphrodisiac effects of the aqueous extract of TT in streptozotocin-induced type 1 diabetic rats. Streptozotocin-induced type 1 diabetes in rats provides a relevant model to study the reproductive dysfunction under diabetic conditions, as they exhibit a number of reproductive deficits that resemble those seen in human diabetes (Soudamani et al., 2005). It is well known that diabetes is positively associated with lowered male fertility and sexual dysfunction (Shalaby and Mouneir, 2010).

To the best of our knowledge, this study reported for...
Figure 3. Effect of MET (350 mg/kg) and TT extract (20 mg/kg) on testicular histology of normal and diabetic rats. A: Section from testis of control rat showing intact seminiferous tubules, permatocytes, non-pachytene (NP), pachytene (P), round spermatids (R), and elongated spermatids (E). B and C: Testis of treated-rats with Met and TT extract, respectively; showing the normal architecture of the seminiferous tubules. D: Diabetic group showing degeneration of seminiferous tubules and decreased amount of mature spermatozoa in tubular lumen (L) as well as seminiferous epithelium exhibiting cytoplasmic vacuolization (V). E: Diabetic rats treated with MET showing normal histological structure of most seminiferous tubules. F: Diabetic rats treated with TT extract showing normal histological structure of most seminiferous tubules with mild changes in the cellular components of germinal epithelium (arrow). Scale bars: 50 µm.

The first time comparison of the effect of TT-extract and MET on sperm characteristics and oxidative stress in testis of diabetic rats. Higher sperm count, percentages of sperm forward motility, viability and lower percentage of abnormal sperm were observed following TT-extract treatment to STZ-induced diabetic rats. Moreover, it increased the weight of testes and seminal vesicles and decreased blood glucose level, but increased serum insulin and testosterone as well as FSH levels and ameliorated the degenerative lesions seen in the testes of diabetic rats. Our findings have further shown that TT extract was able to lower the level of testis oxidative stress as evident from lower amount of LPO and the higher activities of endogenous antioxidant enzymes (SOD and CAT) as well as the non-enzymatic antioxidant (GSH) in testis of diabetic rats.

An evaluation of sperm characteristics is useful when investigating the underlying cause of male infertility (WHO, 2010). In the present study, TT extract administration to diabetic rats prevented or reduced impairment in sperm characteristics, abnormal sperm percentages and abnormal appearances of sperm. The effect of diabetes on these sperm endpoint parameters was consistent with other reports in rats and humans (Bal et al., 2011; Rabbani et al., 2010). Oligozoospermia could predispose diabetic males to subfertility or infertility (Noguchi et al., 1990). The observed decrease in sperm count was supported by diminished sperm intensity in the epididymal lumen. Treatment with TT extract has resulted in higher sperm count and epididymal sperm density which suggests that this herb protect the sperm against diabetes-induced damage.

Diabetes induces oxidative-stress has been reported to cause peroxidation of sperm membrane lipid which might interfere with membrane fluidity and transport processes (Sanocka and Kurpisz, 2004). In view of this, appearance of various abnormal sperm shapes could be due to abnormal membrane or cellular and nuclear changes induced by diabetes (Suresh et al., 2013). More studies could be needed to elucidate mechanisms underlying abnormal sperm appearances in diabetes. Treatment with TT extract prevents the increase in the amount of testis LPO in diabetic rats. In both diabetic rats (Nelli et al., 2013) and humans (Karimi et al., 2011), LPO was the major cause of sperm damage. Administration of TT extract to diabetic rats alleviates oxidative stress via
several mechanisms which include reduced amount of free radicals such as superoxide and preservation of total antioxidant capacity via main tainting near normal activity level of endogenous enzymatic/non-enzymatic antioxidant. The later effects may be attributed to higher amount of total phenolic content in the TT extract as revealed by phytochemical analysis. Meanwhile, ability of TT extract to lower lipid profile levels in diabetic rats could also help to reduce the risk of acquiring abnormal sperm morphology and characteristics and sperm oxidative stress (Kanter et al., 2012). Scarano et al. (2006) reported that sperm counts in diabetic rats diminished following short-term exposure to hyperglycemia, while Amaral et al. (2006) reported that prolonged hyperglycemia in rats adversely affect sperm concentration and motility due to oxidative stress.

Saponins, one of the active components of TT, have been proposed to regulate lipid metabolism (Yang et al., 1999) and hyperglycemia (Li et al., 2002). This protective reaction observed after TT treatment might also be related to the action of the saponin component of TT extract affecting lipid profile parameters. It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition. Hence, STZ induced diabetic rats have altered lipid profile. In this study, diabetic control rats exhibited significantly elevated cholesterol and triglyceride, LDL-c and VLDL-c levels as compared to normal control rats. Chronic administration of TT-extract and MET significantly reduced all the previous parameters. Therefore, normalization of lipids in diabetic rats treated with TT extract may be partly due to its stimulatory effect on insulin secretion from pancreatic β-cells (confirmed by serum insulin levels).

Histopathological changes revealed marked degeneration of most seminiferous tubules including atrophied seminiferous tubules with absence of spermatogenic series and sperms in tubular lumen (Figure 3D) and decrease in both diameter of seminiferous tubules and height of germinal epithelium of testes and epididymis as compared to those in the normal controls. These changes may be due to DM which induces subtle molecular changes that are important for sperm quality and function and alters conventional sperm parameters. Various mechanisms may explain the sperm damage observed in patients with DM. These include endocrine disorders, neuropathy, and increased oxidative stress (La Vignera et al., 2012). These effects may due to DM which decreases serum testosterone levels (Shalaby and Mouneir, 2010; Maiorino et al., 2014) which are associated with a stereidogenetic defect in Leydig cells. Furthermore, DM is associated with an increased oxidative stress, which damages sperm nuclear and mitochondrial DNA. Finally, spermatogenesis derangement and germ cell apoptosis in type 1 DM may relate to a local autoimmune damage, whereas insulin resistance, obesity, and other related comorbidities may impair sperm parameters and decrease testosterone serum levels in patients with Type 2 DM (La Vignera et al., 2012).

Oral administration of the plants extracts to the diabetic rats for 60-days caused enhancement of the histological changes of both testes and epididymis besides enhancing the diameter of seminiferous tubules, diameter of epididymal tubules and height of epithelium of testes and epididymis. These results indicate that extract of plant used in this study act to attenuate the degenerative changes in testes and epididymis because it contains many compounds that act separately or synergistically to enhance testes function and retard normal value. This may due to presence of some phytochemical compounds such as saponin, flavones, tannins and terpenes and its action can be related to insulin-like action and had ability to induce DNA repair systems due to antioxidant activities which reduce or prevent generation of free radicals.

The results of this study were confirmed by Gauthaman et al. (2003) using non-castrated rats at various TT concentrations (2.5, 5 and 10 mg/kg). They study also confirmed an increase in pressure, which is a widely accepted index of penile erection. Further studies showed increases in testosterone and dihydrotestosterone, indicating a T. terrestris-induced increase in male sex hormones (Gauthaman and Ganesan, 2008).

Conclusion

The results indicate improved testicular and epididymal and reduced sperm abnormalities by oral chronic administration of TT extract used in this study, suggesting its protective potential against spermatotoxic and testicular toxicity in diabetic male rats. Based on the reduced sperm abnormalities, antihyperglycemic, antioxidant, antihyperlipidemic and histological changes exhibited by TT-extract, it can be suggested that the extract could be useful in reducing the male reproductive defects associated with DM. Although the MET decreased the glucose level in blood more than TT extract, but TT extract administration improved most of lipid profile and oxidative parameters more than MET. The present investigation has opened an excellent opportunity in the development of herbal formulation from TT extract to control diabetes.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES


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