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Research Article

Effect of Teucriumpolium on oxidative damages and sperm parameters in diabetic rat induced with streptozotocin

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ABSTRACT

Summary: Diabetes is one of the most prevalent metabolic diseases. It raises oxidative stress and thereby causes testicular damage. Utilization of antioxidants culminates in a reduction in oxidative stress and so brings about the testicular damage. In this study, we assessed the anti-oxidative potential of *Teucrium polium* extract on oxidative damages in the testes and sperm parameters of diabetic rats.In this experimental study, 32 male Wistar rats were segregated in 4 groups: Control, Diabetic and Diabetic treated with two doses of *Teucrium polium*extract. An instillation of distilled water was performed in the control and diabetic groups. Also, treatment groups received *T.polium*extract (50 and 100 mg /kg body weight) for 6 weeks. After treatment period all of animals were anesthetized, their blood sample were taken from Harte and measured the serum level of insulin and glucose thenthe testicles and epididymis wereremovedthenthe sperm parameters and oxidative stress markers were assessed. Treatment of diabetic rats with *T. polium* extract significantly reducedthe glucose plasma levels ($p=0.001$) and increased insulinlevels ($p=0.001$).Moreover,during diabetesan upsurge in the level of MDA and a decreasein the levels of SOD and CAT enzymes activity were observed in the testes. Administration of*T.polium* extract (100 mg /kg BW) significantly rectified these parameters ($p<0.05$), but no significant differences were observed between 50 mg /kg BW of *T.polium* and diabetic group. In addition, the sperm parameters decreased in diabetic group, while the use of *T.polium*significantly improved the above disorders in treatment groups ($p<0.05$). The results of this study confirm the antioxidant role of hydro-alcoholic extract of *T.polium* in amelioration the testicular oxidative damage caused by diabetes.

Keywords: Oxidative Stress, Diabetes, *Teucriumpolium*, Testis, sperm parameters

INTRODUCTION

One of the foremost common metabolic disorders is Diabetes which is related to high levels of glucose (Shrilatha 2007). The defects ensued from the mentioned one are related to

insulin production (insulin dependent diabetes) or owing to hormone resistance in peripheral tissues (Non-insulin dependent diabetes) with a decrease in the secretion of hormone from β-cells of the pancreatic islets. The number of people with diabetes is increasing globally and it is expected that by 2025the number would even rise to approximately 300 million people worldwide (Agbaje, Rogers et al. 2007).One of the serious complications of diabetes in men is sexual dysfunction and at the same time decreased testicular weight, semen quality parameters, the level of testosterone, increased abnormal sperm and infertility (Agbaje, Rogers et al. 2007, Vignera, Condorelli et al. 2012).

During diabetes, hyperglycemia causes an increased advanced glycated end products (AGEs), changes in the activity of protein kinase C, a disruption in the balance of prostanoids and increased production of mitochondrial superoxide; these effects themselves causean increased oxidative stress due to excess free radicals (Pop-Busui, Marinescu et al. 2002, Jakuš and Rietbrock 2004, Kaneto, Katakami et al. 2007, Salimnejad, Jalali et al. 2014). Some studies have shown that reinforcingantioxydant system can reduce the complications of diabetes. Herbal remedies prescribed since the ancient times have hardly been eficaciasfor treating diabetes(El-Demerdash, Yousef et al. 2005).

Teucrium polium is one of the wild-growing flowering species belong to *Teucrium (Laminaceae)* genus that has been used for medical purposes in Iran (Mousavi, Niazmand et al. 2015). Severalstudies have reported that *T.polium* has hypoglycemic, hypotensive, antibacterial, and antipyretic characteristics (Mirghazanfari, Keshavarz et al. 2010, Bahramikia and Yazdanparast 2012, Tatar, Qujeq et al. 2012). Various compounds such as flavonoids, iridoids and cirsiliols have been detected in *T.polium* by phytochemical analyses (Mirghazanfari, Keshavarz et al. 2010, Mousavi, Niazmand et al. 2015). Four major flavonoids are obtained from fractionation of *T.polium* methanol extract. Flavonoids are secondary plant phenolics with remarkable antioxidant and chelating effects that are able to scavenge the free radicals produced during lipid peroxidation through donating hydrogen atoms (Sharififar, Dehghn-Nudeh et al. 2009, Fiorentino, D'Abrosca et al. 2010). Some studies have shown that *T.polium* prevents diabetes-induced oxidative stress (KARIMI, ABBASI et al. 2002, Yazdanparas, Esmaeili et al. 2005, Esmaeili, Zohari et al. 2009).In 2010, Wasnaa studied the effect of *T.Polium* aqueous extract on testicular and renal function of white male mice and also the histological changes induced by the mentioned extract. They showed that *T.Polium* aqueous extract significantly increased testicular weight and testosterone levels and the number of Leydig's cells, spermatogonia, and spermatozoa in the treatment group (M. 2010). In this study the impact of hydro-alcoholic extract of *T.polium* on oxidative damages in the testes and sperm parameters of diabetic rats has been investigated.

MATERIALS AND METHODS

Preparation of extract:

Teucrium polium was collected from Ferdos, south Khorasan, Iran, and then controlled by botanists of Ferdowsi University of Mashhad, Iran. After the deposition of a voucher specimen, the plants were dried at room temperature. To prepare the hydroalcoholic extract, 50g of the dried aerial parts of the plant were chopped and then soaked in ethanol (50%) for 72h. After filtering through a paper filter, the extract was dried with rotary vacuum evaporator.

Study design and experimental groups:

In this experimental study 32 male Wistar rats (10 weeks old and weighing 250 ± 20 g) were kept at 22 ± 2∘C and 12h light/dark cycle at 7:00 a.m. They were randomly divided into four groups and treated according to the experimental protocol: (1) Control, (2) Diabetic, (3) Diabetic-Extract 50 mg/kg BW (Dia- Ext 50), (4) Diabetic-Extract 100 mg/kg BW (Dia-Ext 100).The animals of groups 3 and 4 were administered orally once daily with 50 and 100 mg/kg BW of the extract for the period of 6 weeks.

Diabetes was induced in groups 2–4 by a single intraperitoneal (i.p.) injection of streptozotocin (60mg/kg).Only the animals with serum glucose higher than 250mg/dL at 3 days afterstreptozotocin injection were included in

the study. Animal handling and all related procedures were confirmed by Mashhad University of Medical Sciences, Ethical Committee. After 6 weeks the rats were anesthetized. Their right testes were removed,and then kept at a temperature of -80 °C for biochemical (malondialdehyde, Catalase and superoxide dismutase) measurements.The doses of*Teucriumpolium* were selected on the basis of previous studies that demonstrated significant changes in glucose plasma level and testicular histology (Mousavi, Niazmand et al. 2015), (M. 2010).

Biochemical assays:

In order to measure the changes in the glucose plasma level, blood samples were obtained from the rats' ophthalmic veins in 3 phases including before the injection, the third, and the sixth week after the beginning of the study. All the blood sampling phases were performed12 hours after the fast. Immediately after sampling, blood samples were centrifuged and the serum was stored at -80 °C until analysis. The concentration of glucose level was measured by Iran Pars Azmoon kit usingglucose oxidase method via the auto-analysis device. Plasma levels of insulin were measured by the ELISA methodusing the commercial kit of Rat Insulin (mercodia). The intra assay variation was 4.9%. The level of insulin in serum was expressed in mIU/ml.

Measurement of lipid peroxidation:

The level of lipid peroxidation was indicated by the amount of malondialdehyde (MDA) in the testis tissue. In order to provide a solution of TBA-TCA-HCL, 375 mg of thiobarbituric acid (TBA) were dissolved in 2 ml of hydrochloric acid (HCL) and then was added to 100 ml of 15%trichloroaceticacid (TCA). For complete dissolution of sediment, the 50°C water bath was used. The tissue was weighed and homogenized immediately with a solution of potassium chloride 5.1 percent to obtain a 10% homogenized mixture. Then 1ml of homogenized tissue mixture was mixed with 2 ml of TBA-TCA-HCL solution and then it was heated in boiling water for 45 minutes (pink-orange solution). After cooling, it was centrifuged at 1000 rpm for 10 minutes. The absorption (A) at 535 nm was read using a spectrophotometer. Finally, it was calculated using the following formula ((Mousavi, Niazmand et al. 2015)):

TBARS concentration: C (m) =Absorbance/1.65×10^5

Determination of superoxide dismutase activity :

Superoxide dismutase (SOD) activity assays were based on Madesh method (Jadhav, Sarkar et al. 2006). In brief, the reaction mixture contained 0.65 ml of phosphate buffered saline (PBS) (pH 7.4), 30 µl of MTT (1.25; mM), 75 µl of pyrogallol (100 µmol), and 10µl of tissue homogeneity. The mixture was kept in a laboratory incubator at room temperature for 5 minutes. The reaction was stopped by adding 0.75 ml of dimethyl sulfoxide. Absorbency of the solution was read using an ELISA plate reader at 570 nm. The enzyme activity was achieved by matching the inhibition percentage with the standard curve and was reported, based on unit/mg of total protein.

Catalase activity assays:

The catalase activity assays were based on Aebi method (Aebi. 1984). Briefly, the activity was determined by measuring the decrease in absorbance of a reaction mixture consisting of 30 mM H2O2, in sodium phosphate buffer (pH=7), and requisite volume of tissue homogenized at 240 nm. The specific activity was calculated and was expressed as units per mg of total protein.

Evaluation of sperm parameters:

The epididymis from both testes was removed and minced in 5 ml phosphate buffered saline (PBS, pH 7.2).Then were put in the incubator of 37 ° C CO2 for 30 minutesand removed the100 µl of the solution and dissolved in 900 µl from PBSand again this was repeated for new solutions. One drop of solution was mixed thoroughly added into Neubauer's chamber. The sperm count was conducted according to the standard Protocol in 8 squares of 0.1 cm2 each except the central erythrocyte area. The total count was then multiplied by correction factor, 5 x 10^6 m.

Morphometry of sperm:

For accessing the Morphometry of sperm after preparing the Smears of sperm, the slides was

Dried in Exposed to the air and fixed with alcohol 96%. Then the slides were stained with H & E. For each of samples, 100 sperm were counted in each slide and then the percentage of normal and abnormal sperm was determined.

STATISTICAL ANALYSIS:

All statistical analyses were carried out by using the SPSS software (SPSS version 11.5). All data were expressed as mean ± (SE). The data were analyzed using the one way analysis of variance (ANOVA) followed by Tukey's range test. P values ≤0.05 were considered statistically significant.

RESULTS

The MDA levels in the testis tissue:

A highly significant increase (p=0.001) in the MDA levels was recorded in the testes of diabetic group compared to control group. Treating diabetic rats with 50 mg/kg of T.polium did not significantly reduce increasing of testicular MDA level caused by diabetes (p=0.54). However, treating diabetic rats with 100 mg/kg of T.polium did prevent the diabetes-induced increase in the testis MDA level and significantly decreased its level compared to untreated diabetic rats(p=0.001) (Figure 1).

The activity of the catalase enzyme in testis tissue:

These results demonstrated that diabetes caused a significant decrease in the activity of the catalase enzyme in contrast to the control group (p=0.001). Treating diabetic rats with 50 mg/kg of T.polium has not made any significant differences in comparison with untreated diabetic group (p=0.16). However, treating diabetic rats with 100 mg/kg of T.polium showed significant increase (p=0.001) in catalase enzyme activity in comparison with diabetic group (Figure 2).

Superoxide dismutase enzyme activity in testis tissue:

The results showed that SOD activity has more significant decrease in diabetic group (p=0.001) than control group. Comparison between treatment group (50 mg/kg) and diabetic group showed no significant increase in SOD enzyme activity (p=0.72) but treatment of diabetic rats with 100 mg/kg of T.polium showed significant enhance in the enzyme activity in comparison with the diabetic group (p=0.001)(Figure3).

The Sperm count, morphology and motility:

The number of sperm was significantly decreased in diabetic group as compared with control group (p=0.001). In both therapeutic group, the number of sperm was significantly increased when compared with diabetic group (p=0.001). Diabetes led to increase the number of abnormal sperm when compared with control group (p=0.001). Also treatment with T.poliumwas significantly decreased the number of abnormal sperm as compared with diabetic group (p=0.001). On the other hand the, the motility of sperm was significantly decreased in diabetic group as compared with the control group (p=0.001). Treatment of diabetic rats with T.polium significantly improved the sperm motility in both therapeutic groups.

Serum glucose level:

A significant increase has been observed in serum glucose level during the third and sixth weeks of the study in the diabetic group compared with the control group (p=0.001). Additionally, a significant decrease was observed in serum glucose levels in the third and sixth weeks in diabetic group that were under treatment with 50 and 100 mg/kg of hydro-alcoholic extract of T.polium in contrast to untreated diabetic group(p=0.001)(Table 1).

Serum level of insulin:

Serum insulin level between the subject groups showed that diabetes causes a significant decrease in the level of serum insulin in contrast to control group (p=0.001). Treatment of diabetic rats with 50 and 100 mg/kg of T.Polium extracts improved the decrease of serum insulin level when compared with the diabetic group (p=0.03)(Figure 4).

DISCUSSION:

The present study evaluated the ameliorative effect of *T.polium* extract against damages induced by diabetes in the male reproductive system.Diabetescauses testicular dysfunctions in

male reproductive system and *T.polium* extract treatment improves these functional deficits by antioxidant and anti-diabetic roles. The results of this study showed that hydro-alcoholic extract of T.polium changed blood glucose levels and also the level of oxidative stress enzymes in the testes of diabetic rats. T.polium extracts reduced blood glucose levels in treated diabetic rats. The exact mechanism of lowering blood sugar by T.polium is not still known, however, the hypoglycemic activity of T.polium is due to the increased insulin secretion (Tatar, Qujeq et al. 2012). T.poliuminduces this process by increasing endogenous insulin production,by insulin releasing and by increasing target tissue sensitivity to insulin(Mirghazanfari, Keshavarz et al. 2010, Tatar, Qujeq et al. 2012).

During diabetes, in addition to increased amount of glucose, the balance between production and elimination of free radicals is also disrupted; As a result, free radicals increase and cause oxidative stress (Maritim, Sanders et al. 2003, Vincent, Russell et al. 2004)Oxidative stress causes cell damage through mechanisms such as lipid peroxidation and DNA and protein oxidative damage (Tremellen 2008). The results of this study showed that diabetes significantly increases the levels of MDA (as Lipid peroxidation marker) in the testis of diabetic rats compared with the control group.This result corresponds to the finding of several previousstudies onoxidative stress in the testis of diabetic rats (Anwar and Meki 2003, Aitken and Roman 2008). Therefore, MDA levels increasing in the testis of diabetic group emphasize the enhancement of lipid peroxidation. In the present study, treatment of diabetic rats with *T.polium* extract showed significant reduction of MDA level in testis tissue.Previous studies have suggested that flavonoids in *T.polium* scavenge the free radicals produced during lipid peroxidation (Sharififar, Dehghn-Nudeh et al. 2009). Thereby testis MDA level reduction in the treated groups with the *T.polium* extract may be related to antioxidant effects of compounds present in *T.polium*, including flavonoids.

Our study indicated that SOD activity was significantly decreased in diabetic rats compared with control group. These findings correspond to previous studies (Armagan, Uz et al. 2006). SOD is one of the most important enzymes of the antioxidant system. Main action of SOD is decomposition of superoxide anion radicals to H_2O_2, and through this process toxicity of superoxide fades and no free radicals from superoxide are produced(Khan, Telang et al. 2013). On the other hand, in this study, SOD activity had a significant increase in testes of diabetic rats being under treatment with extract of *T.polium* in contrast to diabetic group.

CAT is another antioxidant enzyme that has detoxification effects against free radicals (Aitken and Roman 2008, Tremellen 2008). In the present study, the activity of CAT enzyme in diabetic group had more significant decline than control group, where as in the group treated with *T.polium* extract it showed more significant increase than diabetic group. A decrease in CAT activityin our study can be resulted from the increase in H_2O_2production because of glucose autoxidation and non-enzymatic protein gyration that cause the generation of oxygen-free radicals (Sivajothi, Dey et al. 2010, Saravanan and Ponmurugan 2013). It is known that the administration of antioxidants causes an increase in CAT activity, as confirmed in the present study too.

The results of present research indicated that the diabetes decreased the sperm parameters (count, motility and morphology) and treatment with T.polium extract can increase the number of sperm and improve the motility and morphology of sperm in diabetic rat. Its maybe due toT.polium extractcontain flavonoids as an antioxidant that can counteract free radicals.In confirmed with our results, previous study indicated that plants with flavonoids compounds can improve the sperm parameters and testosterone level in diabetic rats(Sudnikovich, Maksimchik et al. 2007, Guneli, Tugyan et al. 2008).

Possible mechanisms involved in amelioration of testicular oxidative stress in diabetic rats by *T.polium* extract can be expressed as follows: *T.polium* has anti-oxidant properties; it decreases blood glucose levels, and increases insulin secretion (KARIMI, ABBASI et al. 2002, Yazdanparas, Esmaeili et al. 2005, Sharififar, Dehghn-Nudeh et al. 2009,

Fiorentino, D'Abrosca et al. 2010). During diabetes, free radicals cause lipid peroxidation and consequently, damages to the testes. The antioxidant properties of *T.polium* enhance the antioxidant system and can reduce testicular damages. It has also been demonstrated that the administration of a hydroalcoholic extract of *T.polium* increases serum insulin levels in rats by increasing insulin secretion (Tatar, Qujeq et al. 2012). These can be stated that *T.polium* increases insulin secretion and reduces testicular damage.

CONCLUSION:

The present findings reveal that,diabetes has a negative effect on testis and sperm parameters through oxidative stress. T.polium has potent antioxidant effect in reducing the oxidative stress induced by diabetes. However, many researches are necessary to clarify these results.

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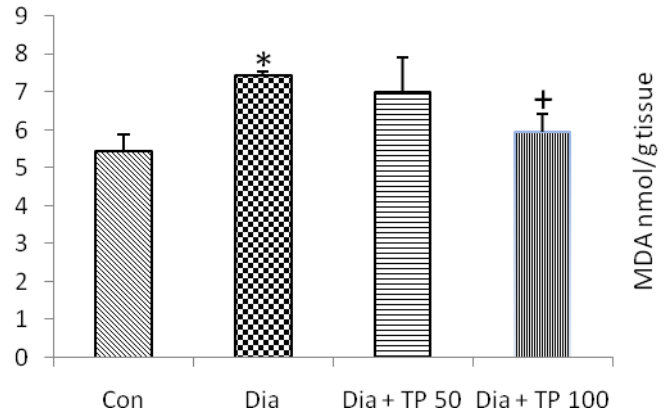

Fig 1. The MDA concentration in rat's testes tissues of 4 groups. Data are shown as Mean ± (SE). * in Comparison with control group (p=0.001) and + in Comparison with diabetic group (p=0.001).

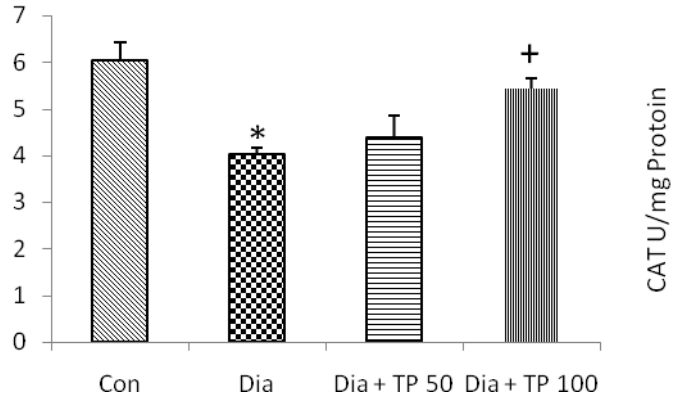

Fig 2. Comparison of catalase (CAT) enzyme activity in testes tissue.Data are shown as Mean ± (SE). *in Comparison with control group (p=0.001) and + in Comparison with diabetic group (p=0.001).

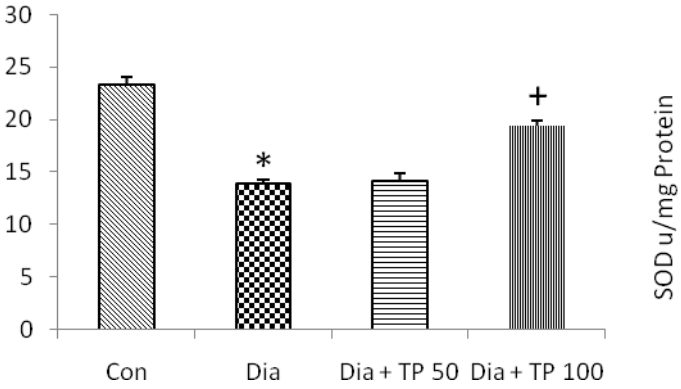

Fig 3. Comparison of SOD enzyme activity in testes tissues. Data are shown as Mean ± (SE). *in Comparison with control group (p=0.001) and + in Comparison with diabetic group (p=0.001).

Table1. The comparison of serum glucose level before the test, third week and sixth

Glucose (mg/dl) / Groups	Baseline Mean (±) SE	Third week Mean (±) SE	Sixth week Mean (±) SE
Control	99.78 + 4.13	121.91 + 3.57	124.29 + 4.38
Diabetic	449.00 + 6.54	462.55 + 7.50 *	513.45 + 7.33 *
Dia + TP 50	444.37 + 9.02	407.31+ 6.07 +	298.22+ 8.31 +
Dia + TP 100	445.97 + 6.87	343.38 + 4.59 +	194.17 + 4.59 +

* shows significant difference between control group and diabetic group and + shows significant difference between diabetic group and diabetic treatment with *T.polium* ($p<0.05$).

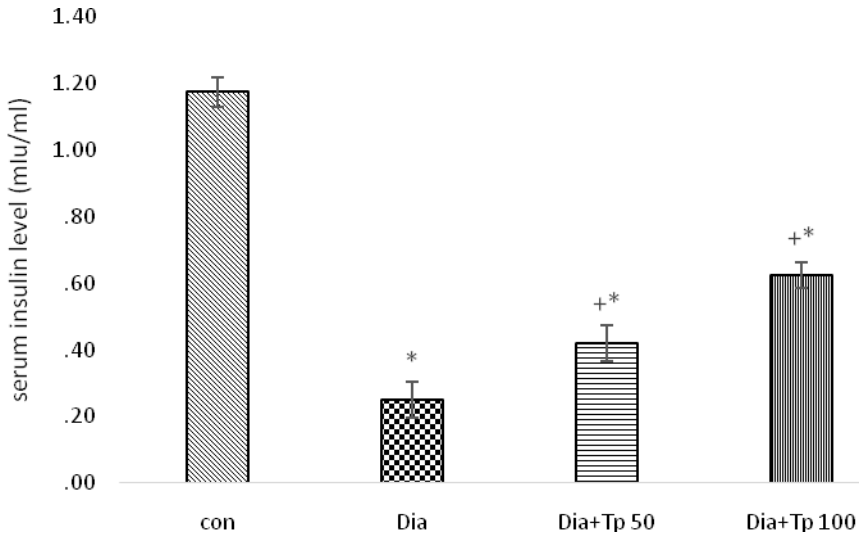

Fig 4: Comparison percentage changes of serum level of insulin between groups before test in week 3 and 6. *show significant difference with control group (p=0.001) and +show significant difference with diabetic group (p=0.03).

Table2. The comparison of sperm parameters after treatment period.

Sperm parameters / Groups	Sperm Count Mean (±) SE	MorphologyMean (±) SE		Sperm motility Mean (±) SE	
		Normal	Abnormal	motile	immotile
Control	115.5 x 106±5.48	86.2%±1.07	14.8%±1.07	75.2%±1.03	24.8±1.03
Diabetic	32.45 x 106±3.98*	36.5%±.50*	63.5%±1.17*	34.45%±1.15*	65.55±1.15*
Dia + TP 50	75.5 x 106±6.34+*	57.8%±.70+*	42.2%±.70+*	54.35±.84*+	46.65±.84*+
Dia + TP 100	91.35 x 106±4.52+*	68.2%±1.3+*	32.8%±1.3+*	65.05±1.04*+	34.95±1.04*+

*shows significant difference between control group and diabetic group and $^{+}$ shows significant difference between diabetic group and diabetic treatment with *T.polium* ($p<0.05$).