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Tribulus terrestris Hydroalcoholic Extract Administration Effects on Reproductive Parameters and Serum Level of Glucose in Diabetic Male Rats

Efectos de la Administración del Extracto Hidroalcohólico *Tribulus terrestris* sobre los Parámetros Reproductivos y el Nivel Sérico de Glucosa en Ratas Macho Diabéticas

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SUMMARY: Usage of medicinal plants for the treatment of diseases is a remarkable strategy considered in both clinical and experimental studies. This study was conducted to evaluate the effect of *Tribulus terrestris* hydroalcoholic extract administration on the serum level of glucose and reproductive parameters in diabetic male rats. In this experimental study, 30 adult male Wistar rats (200–250 g) were divided into 5 groups (n= 6). Diabetes was induced by a single intraperitoneal injection of 50 mg/kg streptozotocin. Experimental groups received daily Intraperitoneal injection of the extract (100, 250 and 500 mg/kg) since one week after inducing diabetes for two weeks. At the end of the experiment, the serum levels of testosterone and glucose were examined by ELISA and Glucodr devices respectively. Sperm parameters including morphology, count and motility were evaluated from the cauda epididymis. The diameters of seminiferous tubules were assessed based on 5 μm paraffin section of H&E stained section of testes. The data were evaluated by One Way ANOVA followed by post-hoc test using SPSS software. In *T. terrestris* extract groups, there were significant reductions in the levels of blood glucose while increased sperm motility, sperm count and seminiferous tubules diameter, percentage of sperms with normal morphology, level of testosterone hormone and final body weight compared with diabetic group ($p < 0.001$). In conclusion, *T. terrestris* hydroalcoholic extract decreases serum level of glucose and diminishes the side effects of diabetes on male reproductive system showing by increasing in the serum level of testosterone, improving sperm parameters and returning the seminiferous tubules to normal diameter by a dose dependent manner.

KEY WORDS: Diabetes; Glucose; Rat; Streptozotocin; Testosterone; *Tribulus terrestris.*

INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent diseases in developing countries. It has been estimated that by the year 2030, the number of diabetic's patients in the world will reach to 336 million (Wild *et al*., 2004). Diabetes mellitus is characterized by hyperglycemia, changing in the metabolism of lipids, carbohydrates and proteins that are consequences of reducing cellular secretion of insulin or insulin resistance (Amaral *et al*., 2008).

Side effects of diabetes can be seen in all body systems. Physical disability, renal impairment, visual impairment, heart disease and sexual dysfunction are the main side effects of diabetes (Engelgau *et al*., 2004).

On male reproductive system, diabetes mellitus decreases libido and testosterone production, inhibits spermatogenesis, declines in the diameter of seminiferous tubules, and degenerate testicular germ cells (Guneli *et al*., 2008). There are several techniques to induce experimental diabetes in laboratory animals including chemical destroying beta cell mass and surgical excision of the pancreas. Also the various chemical factors like Streptozotocin can cause diabetics changes (Rasch *et al*., 1979). Streptozotocin insert its diabetic effects within two or three days by damaging the beta cell's membrane and induction of DNA strand breaks, that causes beta cells death and leads to type I of diabetes in experimental animals (Yamamoto *et al*., 1981).

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Currently, effective therapies for diabetes are using insulin and hypoglycemic drugs, but these compounds also have numerous adverse effects (Suji & Sivakami, 2003). Another strategy for diabetes treatment is using medicinal plants and their derivatives (Shapiro & Gong, 2002; Salimi *et al*., 2015). In this regard, World Health Organization (WHO) has a particular emphasis on the development of herbal antidiabetic drugs (Suji & Sivakami; Stanley *et al*., 2000).

Tribulus terrestris is a yearling plant widely distributed in the Mediterranean and the warm parts of Europe, Asia, USA, Africa and Australia (Chen *et al*., 2013). This plant is used in traditional medicine in China, India, Iraq, Iran, etc. Studies show that *T. terrestris* contains steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins, potassium, nitrate, aspartic acid and glutamic acid (Yan *et al*., 1996). This plant has many medical effects including; antimicrobial, antibacterial, anti-oxidant and anti-toxic. The plant is also used for the treatment of cardiovascular diseases, cancers, respiratory diseases and joint pain (Kadry *et al*., 2010). The use of *T. terrestris* extract increases body's ability to build muscle mass and strength and also improves circulation and oxygen transfer (Arsyad, 1996). Long term use of *T. terrestris* causes dilatation of the vessels and improves coronary arteries without side effects (Wang *et al*., 1990). Trybstan is one of the *T. terrestris* compounds that increases libido and counteract of cold-natured infertility and menopausal disorders. Researchers have shown that *T. terrestris* extract increases sexual potency in men by increasing level of free testosterone and regulating the amount of estrogen, progesterone and pregnenolone (Ghosian Moghaddam *et al*., 2013). Further, the use of the plant in traditional medicine in India and China is to treat sexual dysfunction and increase libido by increasing the level of testosterone and LH (Martino-Andrade *et al*., 2010; Park *et al*., 2006). However, a study has shown that *T. terrestris* had no effect on the organs such as the prostate, seminal vesicles, uterus and vagina that are sensitive to endocrine system (Martino-Andrade *et al*.). On the other hand, using *T. terrestris* with other herbs including ginseng, soy and berries improves erectile and sexual behavior in rats (Park *et al*.). Finally, Saponin derived from *T. terrestris* had a significant effect in reducing serum glucose levels that could introduce the plant as a potent medicinal plant for the treatment of diabetes (Li *et al*., 2002).

According to the literature, there was no report about the effect of *T. terrestris* extract on reproductive parameters in diabetic subjects. In this regards, present study was conducted to determine the effect the extracts of on glucose and testosterone serum levels, testis tissue, and sperm parameters of STZ induced diabetic rats.

MATERIAL AND METHOD

Animals. The present study worked on adult male Wistar rats and the mean age of 70 days weighting 200-250 g that were obtained from Pasteur Institute. They were kept in the departmental animal house at temperature 26±2 ºC and relative humidity 44-56 %, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 18-24 h before the experiment though water was allowed ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Animal Care Committee of Kermanshah Medical Sciences University, Iran.

Preparation of extract. The seeds of *T. terrestris* were prepared from a traditional medicine center and were identified and approved by a botanist. Extracting was performed by percolation method as described earlier (Raoofi *et al*., 2015).

Experimental induction of diabetes in rats. Diabetes was induced by using streptozotocin as diabetogenic agent.streptozotocin (STZ) (50 mg/kg) produced by the USA Sigma company was dissolved in citrate buffer (pH 4.5) immediately before use. The solution was injected intraperitoneally in a dose of 50 mg/kg. One week after STZ administration, Blood was taken from the tail of the rats. Then by using Glucodr devices, Super Sensor model, making South Korea, blood glucose samples were measured. Glucose level over 200 mg/dL was considered diabetic (Lenzen, 2008).

Experimental design. Rats were divided into five groups of six rats in each group.

Group I: Normal control group; the rats were treated with distilled water.

Group II: Diabetic control group; the rats were treated with distilled water.

Group III: Diabetic A group; the rats were treated with 100 mg /kg *T. terrestris* extract.

Group IV: Diabetic B group; the rats were treated with 250 mg /kg *T. terrestris* extract.

Group V: Diabetic C group; the rats were treated with 500 mg /kg *T. terrestris* extract.

Antidiabetic activity of *T. terrestris* hydroalcoholic extract was evaluated by Glucodr device to estimate blood glucose levels and also by body weight measurement. The extract was dissolved in distilled water and administered (I.P) to rats daily with specified doses for two weeks. At the end of the experiment (day 22), the rats were weighed (secondary weight) and anesthetized 24 h after the last injection. Blood samples were taken from the heart and its serum was separated by centrifuge.

Analysis of testis weight and testosterone hormone. The animals were anesthetized 24 h after the last injection. Blood was taken from the heart and preserved at a temperature of 37 ºC for 30 min and the collected blood was centrifuged at 25 ºC for 10 min with 4000 rpm to obtain the serum. The serum samples were then kept in deep freezer (-18 ºC) until measurement of testosterone hormone by ELISA method. Also, the testes were separated and weighed separately and then preserved in neutral buffered formalin 10 % (Keshtmand *et al*., 2015; Moradi *et al*., 2015).

Evaluation of sperm characteristics. Both cauda epididymis from each rat were placed in 2 mL of normal saline, pre-warmed at 37 ºC. Cauda epididymis was separated into small segments and placed in the medium DMEM/F12 containing FBS 5 % which had been previously balanced in the incubator with the temperature of 37 °C and CO_2 5 %. The prepared suspension was used for the analysis of sperm parameters including: motility, count and morphology. Sperm motility was divided into four levels: (0): without motility, (I): minor in situ motility. (II): circumferential motility and (III): progressive motility (Jequier, 2000).

Morphologic analysis of sperm count and motility. To count the sperms, after putting the sperm suspension on Neubauer's chamber, the sperms on the four corners of the central square were counted. To examine the sperm cells morphology, smear was prepared from the samples and was stained and investigated by Papanicolaou method (Lotfi *et al*., 2013).

To determine the motility, one drop of the sperm cells suspension was placed on the chamber and the motile and immotile sperm cells were analyzed by microscope with magnification 400X (Keshtmand *et al*., 2015).

Histological analysis. The testes were preserved in neutral buffered formalin 10 %. The paraffin blocks were prepared and were cut in 5 μm thick sections, which were stained with hematoxylin and eosin and examined by light microscope at X400 magnifications. Seminiferous tubules diameter was measured by Motic camera and software (AE3; Motic S.L.U., Barcelona, Catalonia, Spain). Briefly, more than 20 sections were prepared from each block. The sections numbered 5, 10, 15, and 20 were selected and photographed separately from two random scopes. The mean of seminiferous tubules diameter (mm) was determined for each testis.

Statistical analysis. The results were presented as Mean±SEM (Standard Error of Mean) and data were analyzed by One way ANOVA followed by post-hoc test to determine the statistical significance between different groups using SPSS software (version 20) and $p < 0.05$ was considered as statistically significant.

RESULTS

Weight of Body and Testis: Streptozotocin induced diabetic rats showed significant reduction in body weight compared to normal control group ($p < 0.001$). The mean of final weight in diabetic extract groups (100, 250 mg/kg) revealed a significant decrease compared to normal control group ($p < 0.001$) (Table I). Further, the rats receiving 250 and 500 mg/kg doses of *T. terrestris* extract indicated significant increase in body weight compared to diabetic control group ($p < 0.001$) (Table I). Significant weight testis decrease were seen after STZ administration in diabetic control groups compared to normal control one ($p < 0.05$) (Table I).

Table I. The effect of different doses of *T. terrestris* extract on body weight, testis weight and seminiferous tubule diameter in sham and experimental groups. The data are shown as Mean± Standard Error of Mean.

Groups	Body weight (g)	Testis weight (g)	S. T. D (_m)
Normal control	237.8±16.1	1.45±0.1	270±30.2
Diabetic	156.3±12.2*	1.2±0.07*	173±22.5**
Diabetic+ *T. terrestris* (100 mg)	177.5±14.3$^{**, \#}$	1.33±0.3	201±22.1$^{**, \#}$
Diabetic+ *T. terrestris* (250 mg)	184.2±14.8$^{**, \#\#}$	1.36±0.06	204±20.6$^{**, \#}$
Diabetic+ *T. terrestris* (500 mg)	217.8±15.1$^{\#}$	1.38±0.05	216±30.1$^{*, \#\#}$

S.T.D= Seminiferous tubule diameter; **= $p<0.001$, the mean difference is significant compared to Normal control group. ##= $p<0.001$, the mean difference is significant compared to Diabetic group. #= $p<0.001$, the mean difference is significant compared to Diabetic group. *= $p<0.05$, the mean difference is significant compared to normal control group.

Blood sugar: The mean of blood glucose in experimental groups was increased significantly compared to normal control group ($p < 0.001$), while blood glucose in diabetic rats receiving the extract (100, 250 and 500 mg/kg) reduced significantly compared to diabetic group ($p < 0.001$) (Table III).

Sperm parameters: The mean of sperm count in diabetic and experimental groups was decreased significantly in comparison with normal control group ($p < 0.001$) (Table II). Analysis of sperm motility indicated that percentage of progressive motile sperms (fast) in diabetic and diabetic+ extract groups decreased significantly compared to normal control group ($p < 0.001$) (Table II). The percentage of sperms with normal morphology in diabetic and diabetic+ extract groups revealed a significant decrease in comparison with sham group ($p < 0.001$) (Table II). Sperm abnormal morphology included twisted cauda sperm, round cauda sperm, and headless sperm.

Histological study: The microscopic pictures of testes are shown in Figure 1. The ordered seminiferous tubules and low inter tubular spaces were seen in normal control and somehow in *T. terrestris* extract treated groups (Fig. 1A, C, D and E). In diabetic group, the shrinkage of seminiferous tubules as well as high intertubular spaces was seen (Fig

Table II. The effect of different doses of *T. terrestris* extract on sperm motility, sperm count and sperm normal morphology in sham and experimental groups. The data are shown as Mean± Standard Error of Mean.

Groups	Count (10^6)	Sperm motility (%)	Normal morphology (%)
Normal control	79.8±3.2	73.6±2.2	85.6±5.8
Diabetic	$15\pm0.5^{**}$	$26.8\pm1.5^{*}$	$55.6\pm3.5^{**}$
Diabetic+ *T. terrestris* (100 mg)	$38.4\pm1.1^{**, \#\#}$	$29.5\pm0.9^{*}$	$58\pm2.5^{**}$
Diabetic+ *T. terrestris* (250 mg)	$38.9\pm1.1^{**, \#\#}$	$36.2\pm0.9^{*, \#}$	$63.2\pm2.6^{**, \#}$
Diabetic+ *T. terrestris* (500 mg)	$43.6\pm2.1^{*, \#\#, @}$	$40.6\pm0.9^{**, \#\#, @}$	$65.3\pm2.5^{**, \#, @}$

**= $p<0.001$, the mean difference is significant compared to Normal control group. ##= $p<0.001$, the mean difference is significant compared to Diabetic control group. #= $p<0.05$, the mean difference is significant compared to Diabetic Control group. @= $p<0.05$, the mean difference is significant compared to Diabetic+100 mg/kg *T. terrestris* extract group.

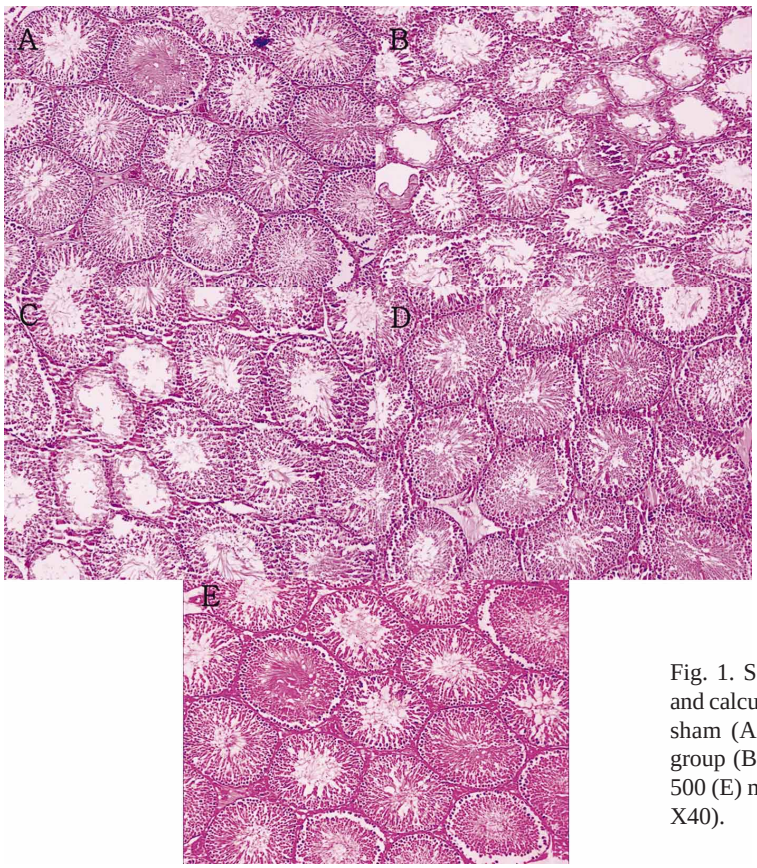

Fig. 1. Seminiferous tubules and calculation of diameter in sham (A), Diabetic Control group (B), 100 (C), 250 (D), 500 (E) mg/kg extract (H&E, X40).

Table III. The effect of different doses of *T. terrestris* extract on blood glucose and Testosterone Hormone in different groups. The data are shown as Mean± Standard Error of Mean.

Groups	Blood glucose (ng/dL)	Testosterone hormone (ng/mL)
Normal control	93.5±19.3	3.48±0.6
Diabetic	300±30.4**	2.58±0.4**
Diabetic+ *T. terrestris* (100 mg)	190±29.4$^{*, \#\#}$	2.85±0.3**
Diabetic+ *T. terrestris* (250 mg)	175.3±26.2$^{**, \#\#}$	3.11±0.4$^{\#}$
Diabetic+ *T. terrestris* (500 mg)	173.3±26.8$^{**, \#\#}$	3.16±0.6$^{\#\#}$

**= p<0.001, the mean difference is significant compared to Normal control group. ##= p<0.001, the mean difference is significant compared to Diabetic Control group. #= p<0.05, the mean difference is significant compared to Diabetic Control group.

1B). Quantitative analysis of microscopic pictures of testes showed that seminiferous tubules diameter in diabetic rats was significantly reduced in comparison with both normal control and diabetic + extract groups (p <0.001). When compared to normal control group, a significant boost in seminiferous tubules' diameter was observed in diabetic+ *T. terrestris* extract (500 mg/kg) (p <0.001) and diabetic + *T. terrestris* extract (250 and 100 mg/kg) (p <0.05) respectively (Table I).

Serum testosterone level: The mean of testosterone level in diabetic control and diabetic + 100 mg/kg *T. terrestris* extract groups was decreased significantly in comparison with normal control group (p <0.001). Increasing the dose of *T. terrestris* extract showed increased level of testosterone hormone in comparison with diabetic control group (p <0.001) (Table III).

DISCUSSION

In the present study, we demonstrated the protective effect of *T. terrestris* extract on the complication of diabetes such as body weight, blood sugar, testes weight, serum level of testosterone and sperm parameters in the rats.

STZ was used to induce Type1 diabetes and the increased blood glucose was the same as in other studies and that could be contributed to cell death induction of the drug in beta cells of pancreas gland, leading to decrease serum level of insulin (Shenoy & Ramesh, 2002).

The decreased level of blood sugar in diabetics rats treated with *T. terrestris* extract was similar to results of El-Tantawy & Hassanin (2007) and could be contributed to antioxidant effect of the extract.

Our study also showed a significant decrease in the weight of diabetic rats compared with normal control group. These data were similar to other studies (Shenoy & Ramesh; Suthagar *et al*., 2009; Kianifard *et al*., 2011). The weight gain in diabetic rats that were treated with *T. terrestris* confirmed Keshtmand *et al*. (2015). The study showed that weight loss caused by diabetes can be compensated by *T. terrestris* extract first time. We can conclude that *T. terrestris* extract can be helpful for weight loss in chronic diseases such as diabetes. A significant decrease was observed in testicular weight in diabetic group. These data were in parallel with Cameron *et al*. (1985), that showed that diabetes had caused tubule atrophy and reduced spermatogenic cells, which could be considered signs of morphological abnormalities in spermatogenesis. We demonstrated that testicular weight was increased in the diabetic + *T. terrestris* treated rats. Thus, it can be concluded that *T. terrestris* extract has had a positive effect on testis weight. The reduction of seminiferous tubule diameter in diabetic rats confirms the hazardous effect of diabetes in testis tissue. Further, the recovery of *T. terrestris* extract in this issue showed in beneficial effect of the extract both in histological and gross levels. The beneficial effect of *T. terrestris* on testis tissue is in parallel with previous studies (Suthagar *et al*.; Kianifard *et al*.).

Moreover, studies suggested that diabetes causes reducing testicular weight by launching of the mechanism of cell death. Atrophy of tubules will be followed by reducing the diameter of the tubules and loss of spermatogenic cell (Guneli *et al*.). Thus, in the diabetic rats that were treated by *T. terrestris* extract, it can be said that increase in testicular weight may be due to inhibition of apoptosis in spermatogenic cells and this cellular process affects sperm production by increasing sperm parameters.

The present study confirmed other studies and offered that diabetes decreases secretion of androgens. This side effect of diabetes can be due to interference with performance of interstitial endocrine cells (Leydig cells) (Ferlin *et al*., 2008; Feng *et al*., 1999). Maneesh *et al*. (2006) and Stanciu *et al*. (2010), showed that diabetes affected

Hypothalamus-Pituitary-gonadal axis in males and decreased testosterone significantly.

On the other hand, improving sperm parameters in *T. terrestris* treated rats could be due to increasing testosterone. Xu *et al*. (2001), have shown that *T. terrestris* was able to improve sexual function, including an increase in sperm count, improving erectile function and increasing libido, and it could be the effect of saponin compound of *T. terrestris* on testosterone production. Brown *et al*. (2001) and his colleagues have indicated that Frostanole, one of *T. terrestris* saponins, stimulated spermatogenesis by increasing production of pituitary gonadotropins that triggered testosterone and improved sperm count. Various steroids of *T. terrestris* stimulate spermatogenesis and increase sperm production by affecting sustentocytes (Sertoli cells) (Adimoelja & Adaikan, 1997). Natural glycosides of *T. terrestris* may have an intermediary and facilitating role in androgen production from estradiol (Ebisch *et al*., 2007).

Diabetes has general effects on whole body by reducing body weight. This disease also causes degradation of spermatogenesis. Diabetes affects spermatogenesis by reducing the number and function of interstitial endocrine cell and sustertocytes that could be seen by decreasing the seminiferous tubules' diameter, decreasing in the amount of testosterone, and diminishing in the sperm parameters. *T. terrestris* extract as a public body booster, compensates weight loss caused by diabetes. Considering the involvement of oxidative stress both in damaging of the tissues and diabetes, and antioxidant activity of the extract, it could be said that scavenging oxidative compounds is a cause for the beneficial effect of *T. terrestris* extract on complications of diabetes on male reproductive system as well as whole body systems and decreasing the amount of blood glucose of diabetic rats. A specified therapeutic pathway of *T. terrestris* extract administration on the male reproductive system could be explained by the presence of steroids compounds of the extract that increases the serum level of testosterone.

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GHANBARI, A.; MORADI, M.; RAOOFI, A.; FALAHI, M. & SEYDI, S. Efectos de la administración del extracto hidroalcohólico de *Tribulus terrestris* sobre los parametros reproductivos y el nivel sérico de glucosa en ratas macho diabéticas. *Int. J. Morphol., 34(2)*:796-803, 2016.

RESUMEN: El uso de plantas medicinales para el tratamiento de enfermedades es una importante estrategia considerada en estudios clínicos y experimentales. El objetivo fue evaluar el efecto de la administración de extracto de *Tribulus terrestris* hidroalcohólico en el nivel sérico de glucosa y los parámetros reproductivos en ratas macho diabéticas. En este estudio experimental, se dividieron 30 ratas Wistar (200–250 g) macho adultas, en 5 grupos (n= 6). Se indujo diabetes por una sola inyección intraperitoneal (i.p.) de 50 mg/kg de estreptozotocina. Los grupos experimentales recibieron inyección i.p diaria del extracto (100, 250 y 500 mg/kg) una semana después de la inducción de la diabetes durante dos semanas. Al final del experimento, los niveles séricos de testosterona y de glucosa se examinaron por ELISA y el dispositivos Glucodr, respectivamente. Además, fueron evaluados los parámetros de morfología, conteo y motilidad espermática. Los diámetros de los túbulos seminíferos de los testículos se evaluaron sobre la base de secciones de parafina de 5 μm teñidos con H&E. Los datos fueron evaluados por ANOVA de una vía seguido de prueba post-hoc utilizando el programa SPSS (versión 20). En los grupos tratados con extracto de *T. terrestris* hubo una reducción significativa en los niveles de glucosa en la sangre, mientras que se observó un aumento en el recuento y motilidad espermática, el diámetro de los túbulos seminíferos, el porcentaje de espermatozoides con morfología normal, el nivel de testosterona y el peso corporal final de las ratas en comparación con el grupo de ratas diabéticas ($p < 0,001$). En conclusión, el extracto de *T. terrestris* hidroalcohólico disminuye los niveles séricos de glucosa y los efectos secundarios de la diabetes en el sistema reproductivo mediante el aumento de los niveles séricos de testosterona, mejorando los parámetros de los espermatozoides y la restitución a diámetro normal de los túbulos seminíferos dependiendo de la dosis.

PALABRAS CLAVE: Diabetes; Glucosa; Rata; Estreptozotocina; Testosterona; *Tribulus terrestris* .

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