Effects of Colocynth (Citrullus colocynthis) Pulp on Serum Levels of Testosterone and Changes in Reproductive Organs in Streptozotocin-Induced Diabetic Rats

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Original Article

Abstract

Introduction: Investigating the effects of colocynth pulp on levels of testosterone and changes in the reproductive system organs in streptozotocin-induced diabetic rats.

Methods: In this study, 32 Wistar rats were divided into four groups of normal (N), normal+colocynth pulp (N+C), diabetic control (D), and diabetic+colocynth pulp (D+C). Groups N and D received orally 2 mL normal saline for two weeks, and groups N+C and D+C received orally 10 mg/kg colocynth pulp for two weeks. Diabetes was induced in rats through intraperitoneal injection of 65 mg/kg streptozotocin (STZ).

Results: Group D showed a significant increase in glucose levels compared with Group N ($P<0.0001$), and testosterone levels, body weight, and reproductive organs weight ratio of group D decreased significantly compared with Group N ($P<0.0001$). In addition, a significant reduction in glucose level ($P<0.01$) and a significant increase in testosterone level and reproductive organs weight ratio ($P<0.01$) were observed in Group D+C in comparison to Group D.

Conclusion: This study showed that consumption of 10 mg/kg colocynth for two weeks relatively improved glucose and testosterone levels as well as reproductive system damage in streptozotocin-induced diabetes in rats.

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Introduction:

Diabetes mellitus is a heterogeneous metabolic disorder which is characterized by altered metabolism of carbohydrates, lipids, and proteins (5). Diabetes leads to hyperglycemia and increased production of free radicals, resulting in oxidative stress in different tissues (15). Oxidative stress induced by advanced glycation end products (AGEs) is one of the complications of diabetes. AGEs are intermediates of reactive species production and can damage different tissues, including reproductive system (26). Diabetes exerts harmful effects on the reproductive system in patients and diabetic animals (3,20,22). Diabetes mellitus is known to cause male infertility,

impotence, retro-ejaculation, hypogonadism, etc. (12). It also induces molecular changes resulting in alterations in the quality and performance of sperms. Impaired reproductive system in diabetics may result from changes in hypothalamic-pituitary-gonadal axis (11). These changes are mostly the result of decreased insulin secretion or resistance to insulin (13). Furthermore, testosterone replacement cannot modify reproductive behavior to normal state, while insulin replacement improves the effects of diabetes on reproductive system (24). The reason for low testosterone in diabetics is not fully understood. Caoand et al. showed that the antioxidant defense system in Leydig cells involves in development of oxidative stress and increased oxidative damage. The researchers also suggest that increased oxidative stress may contribute to age-related defects in testosterone secretion (8). Other researchers have shown that reactive species modulate the function of mature rats Leydig cells at psychological conditions through a variety of functions such as decreasing the activity of steroidogenic enzymes and increasing oxidative stress and apoptosis (10). The use of medicinal plants is increasing given the numerous side effects of synthetic anti-hyperglycemic drugs. As a member of Cucurbitaceae family, colocynth commonly known as bitter apple is one of these herbs. It is a tropical plant and is grown in many Arabic countries and widely in other parts of the world. In traditional medicine, this herb is used to treat constipation, diabetes, edema, fever, leukemia, jaundice, bacterial infections, etc. (18,25).

The present study has evaluated the effects of colocynth pulp on levels of glucose, testosterone, and weight changes in the reproductive system of streptozotocin-induced diabetic rats.

Methods:

In this experimental study, dried colocynth was purchased from grocery and its pulp was separated from seeds and skin, the isolated pulp was then ground, and the obtained powder was kept in a cool, dry place.

To induce diabetes in rats, 65 mg/kg streptozotocin (STZ) purchased from Sigma Company was dissolved in cold normal saline and injected intraperitoneally. Those rats with fasting glucose levels of above 300 mg/dL after 72 hours were considered diabetic. In this study, 32 Wistar rats weighing 270-230g were randomly divided into four 8-member groups:

Group 1 or control group (N) received 2 mL normal saline per day through gavage; Group 2 (C+N) received 10 mg/kg/day colocynth pulp powder dissolved in 2 mL normal saline; Group 3 or diabetic control group (D) received 2 mL normal saline per day; and Group 4 or diabetic treatment group (C+D) received 10 mg/kg/day colocynth pulp powder dissolved in 2 mL normal saline.

After two weeks, the rats were anesthetized by CO_2 and beheaded by guillotine. Their blood was then collected and centrifuged after 20 minutes at laboratory air, and the serum was separated and kept at -20°C. Testosterone assay was performed using the kit of Darman Kav Company.

The obtained data were analyzed with SPSS and the mean data of different groups with ANOVA (One-way ANOVA) and Tukey test. Final results were reported as mean±SEM, and *p*-values of less than 0.05 were considered significant.

Results:

Glucose

The effect of colocynth pulp on serum glucose is depicted in Figure 1. In comparison with Groups N and N+C, Group D had a significant increase ($P<0.0001$) and Group D+C had a significant decrease ($P<0.01$). Group D+C showed a significant reduction compared with Group D ($P<0.01$).

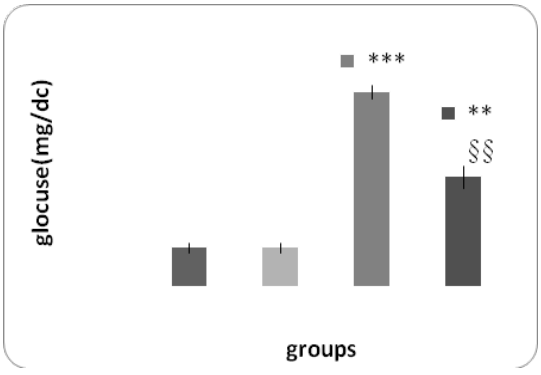

Figure 1. Effect of Oral Consumption of Colocynth Pulp on Serum Levels of Glucose

Each column represents the Mean ± ESM, n=8.
**Significant difference compared with Groups N+C and N (P<0.01). ***Significant difference compared with Groups N+C and N (P<0.0001). §§Significant difference compared with Groups D+C and D (P<0.01).

Testosterone

The effect of colocynth pulp on serum testosterone is depicted in Figure 2. In comparison with Group N, Group D had a significant decrease (P<0.0001) and Groups N+C and D+C had a significant decrease (P<0.05). Group D+C showed a significant increase compared with the diabetic group (P<0.05).

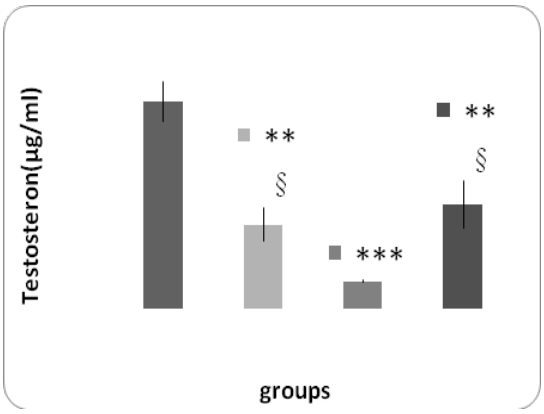

Figure 2. Effect of Oral Consumption of Colocynth Pulp on Serum Levels of Testosterone
Each column represents the Mean ± ESM, n=8.
**Significant difference between Groups N+C and D+C and Group N (P<0.01). ***Significant difference compared with Group N (P<0.0001). §Significant difference compared with Group D (P<0.01).

Body weight

The effect of colocynth pulp on body weight is depicted in Figure 3. In comparison with Group N, Groups D and D+C had a significant decrease (P<0.0001). There was also a significant decrease in Groups D and D+C compared with Group N (P<0.05).

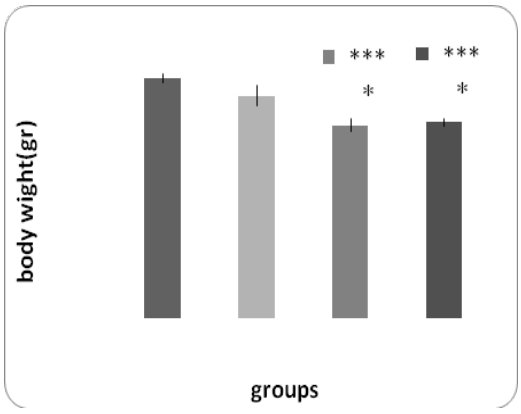

Figure 3. Effect of Oral Consumption of Colocynth Pulp on Body Weight
Each column represents the Mean ± ESM, n=8.
*Significant difference compared with Group N (P<0.05).
***Significant difference compared with Group N (P<0.0001).

Testicular weight ratio

The effect of colocynth pulp on changes in testicular weight to body weight is depicted in Figure 4. Groups D, N+C, and D+C had a significant decrease compared with Group N (P<0.05). There was also a significant decrease compared with Groups D and N+C (P<0.01).

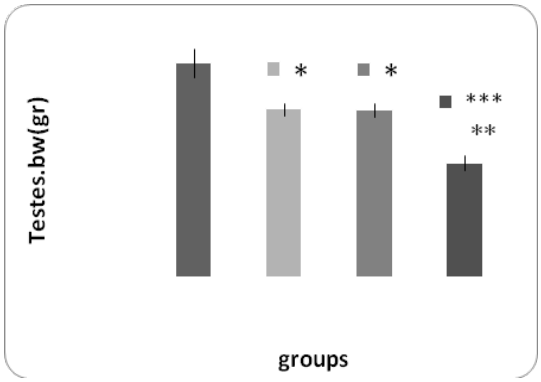

Figure 4. Effect of Oral Consumption of Colocynth Pulp on Testicular Weight
Each column represents the Mean ± ESM, n=8.
*Significant difference compared with Group N (P<0.05).
***Significant difference compared with Group N (P<0.0001).
§§Significant difference compared with Groups D and D+C (P<0.01)

Epididymis weight ratio

The effect of colocynth pulp on changes in epididymis weight to body weight in the studied groups is depicted in Figure 5. Group D had a significant decrease compared with Group N (P<0.0001). There was also a significant increase

in Group D+C ($P<0.01$) and Group N+C ($P<0.0001$) compared with Group D.

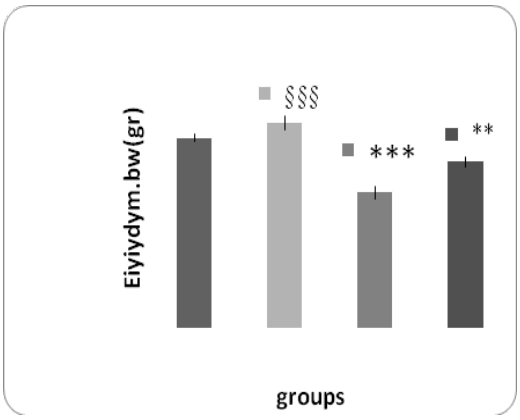

Figure 5. Effect of Oral Consumption of Colocynth Pulp on Epididymis Weight

Each column represents the Mean ± ESM, n=8.
***Significant difference compared with Group N ($P<0.0001$).
**Significant difference compared with Group D ($P<0.01$).
§§§Significant difference compared with Group D ($P<0.0001$).

Vas deferens weight ratio

The effect of colocynth pulp on changes in vas deferens weight to body weight in the studied groups is depicted in Figure 6. Group D ($P<0.0001$) and Group D+C ($P<0.01$) had a significant decrease compared with Groups N and N+C. There was also a significant decrease in Group D+C compared with Group D ($P<0.0001$).

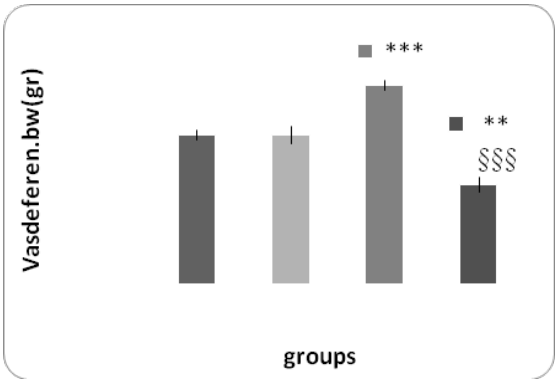

Figure 6. Effect of Oral Consumption of Colocynth Pulp on Vas Deferens Weight

Each column represents the Mean ± ESM, n=8.
**Significant difference compared with Groups N and N+C ($P<0.01$). ***Significant difference compared with Groups N and N+C ($P<0.0001$). §§§Significant difference compared with Group D ($P<0.0001$).

Seminal vesicle weight ratio

The effect of colocynth pulp on changes in seminal vesicle weight to body weight in the studied groups is depicted in Figure 7. Group D had a significant decrease ($P<0.0001$) and Group N+C had a significant increase ($P<0.01$) compared with Group N. There was also a significant decrease in Group D+C compared with Group N+C ($P<0.05$).

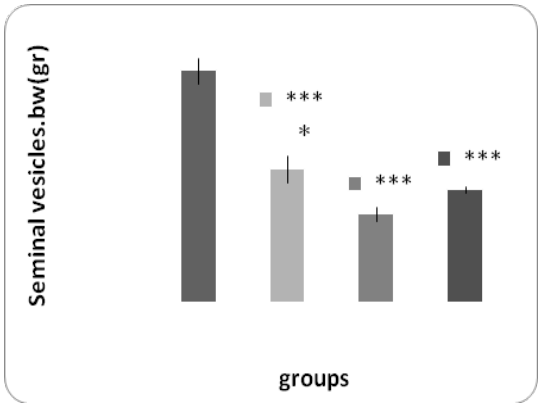

Figure 7. Effect of Oral Consumption of Colocynth Pulp on Seminal Vesicle Weight

Each column represents the Mean ± ESM, n=8.
*Significant difference compared with Group D ($P<0.05$).
***Significant difference compared with Group N ($P<0.0001$).

Prostate weight ratio

The effect of colocynth pulp on changes in prostate weight to body weight in the studied groups is depicted in Figure 8. Group D ($P<0.0001$) and Group N+C ($P<0.05$) had a significant decrease compared with Group N. There was also a significant increase in Group N+C compared with Group D ($P<0.0001$). The weight ratio was higher in Group D+C than Group D, but this increase was insignificant.

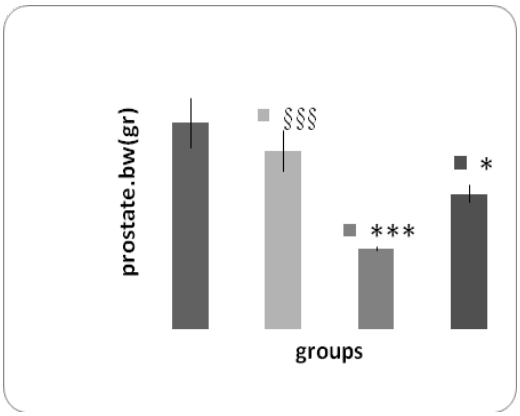

Figure 8: Effect of Oral Consumption of Colocynth Pulp on Prostate Weight

Each column represents the Mean ± ESM, n=8.
*Significant difference compared with Group N (P<0.05).
***Significant difference compared with Group N (P<0.0001).
§§§Significant difference compared with Group D (P<0.0001).

Conclusion:

Increased blood glucose levels causes several structural and functional changes in different organs of diabetic patients (8). In this study, the mean blood glucose in STZ-induced diabetic rats was significantly increased above normal levels and this increase remained constant in untreated diabetic rats during the study. In line with increase in blood glucose, serum testosterone levels were significantly reduced in diabetic rats; this decrease is consistent with other studies (4). It is suggested that this decline in testosterone in diabetic rats is the result of the disease impact on changes in proliferation, differentiation, and function of Leydig cells as well as hyperplasia of Leydig cells in diabetic rats (21,23). Some researchers have suggested that neuro-endocrine damage in diabetes occurs at the level of hypothalamus (7,17). Therefore, it is likely that reproductive damage specified in diabetic men is a result of impact of the disease on hypothalamic-pituitary-gonadal (HPG) axis at several levels (7). In addition, insulin affects testosterone through stimulation of the HPG axis (11). It is thought that decreased secretion of testosterone in two weeks is the result of slight obstruction of LH secretion in diabetic rats (16). Hence, it is suggested that poor function of Leydig cells and impairment of the HPG axis resulted from increased glucose and decreased insulin due to diabetes lead to decreased testosterone production in Leydig cells of diabetic rats. Declined weight of body and sexual organs including testes, epididymis, vas deferens, seminal vesicles, and prostate in diabetic rats compared to normal ones was also evident in this study. Body weight loss results from body protein breakdown and lack of hormones (growth, thyroxine, etc.) and their effects on anabolic activity of the body (1,19). In addition, diabetes results in reproductive glands weight loss, and it has also been shown that diabetes reduces the number of androgen receptors on reproductive glands (6), because differentiation, development, and function of the reproductive organs and glands is androgen-dependent. Along with androgen, other hormones such as insulin, glucocorticoids, and estrogen affect the prostate gland (9,18). Therefore, it is suggested that following decreases in levels of insulin and androgens during diabetes, the regulatory effect of these hormones on the body and reproductive organs decreases the weight of body and the weight ratio of reproductive organs. Treatment of diabetic rats in this study with 10 mg/kg colocynth pulp showed that this dosage significantly reduced glucose compared with the diabetic group. This decreased sugar in treated rats arises from the hypoglycemic effect of colocynth. Anti-hyperglycemic effects of colocynth pulp are attributed to saponosids (saponins) which are components or the main compounds of colocynth and these types of plants and may react with several metabolic pathways or insulin metabolism and directly or indirectly affect glucose homeostasis (17). Other researchers have shown that this plant contains glycosides, steroids, and terpenoides. Charantin is a steroidal saponin isolated from Cucurbitaceae family which has an insulin-like activity *via* enhancing the release of insulin and reducing glucogenesis (14).

The study also found that levels of testosterone and weight of sexual organs was increased compared to the diabetic group. Increase in testosterone levels and the weight of testis, epididymis, and vas deferens was significant, while increase in the weight of gonads was not significant compared to the diabetic group. Therefore, according to the results of this study and previous studies, it is suggested that colocynth exerts hypoglycemic effects with its glycosidic compounds, steroids, saponosids, and terpenoides

through increasing glucose consumption and secretion of insulin *via* restoring the damaged beta cells of pancreas.

Increased levels of insulin prevent oxidative stress caused by increased levels of glucose, and hence increase testosterone levels and reduce the developed reproductive damage resulted from increased glucose in diabetics. Since insulin directly affects the hypothalamic-pituitary-gonadal (HPG) axis (2), it is suggested that increase in insulin in the treated diabetic rats and elimination of insulin effect on the HPG result in increased testosterone levels and reduced effects of insulin and testosterone deficiency on sexual organs.

As a general conclusion, it can be stated that administration of colocynth pulp for two weeks has favorable effects on blood glucose and reproductive complications from diabetes, and these effects arise from specific compounds of this plant. It is advised to prolong the period of treatment to achieve better results.

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