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Ameliorating effect of *Cichorium Intybus L.* Against testicular dysfunction associated with type 2 diabetes and other diabetic complications in rats

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Abstract: The major sign of diabetes mellitus (DM), hyperglycemia, has a variety of harmful consequences on all bodily functions, including reproduction. Chronically elevated blood sugar is linked to an excess of reactive oxygen species (ROS), which weakens the body's antioxidant defense system and causes a variety of health issues. Male diabetics' testicular dysfunction is mostly caused by testicular oxidative stress, inflammation, apoptosis, and hormonal abnormalities. Diabetes is also linked to a wide range of metabolic problems, such as decreased protein levels and changes in certain blood enzymes and minerals.

The anti-diabetic, antioxidant, and anti-inflammatory qualities of many naturally occurring plants have played an important part in human health maintenance. Chicory (Cichorium intybus) is a vital medicinal herb. Anti-inflammatory, anti-diabetic, and antioxidant benefits are only some of the many examples of chicory extracts' wide spectrum of biological and pharmacological use. Its widespread use meant that it could be used to treat anything from lesions to diabetes.

This research demonstrated the preventative effects of chicory against diabetes-induced dysfunction of the testicles in male rats. Chicory seed extract (250 mg/kg) was given via stomach tube once daily to diabetic rats for 30 days after the establishment of diabetes. Chicory's ameliorative impact on testicular diseases was confirmed by an increase in the body and testis weights of treated diabetic rats compared to untreated rats. Both fasting blood sugar and serum insulin were dramatically reduced. A rise in testosterone hormone was observed in diabetic rats given chicory. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were also seen to be attuned. In addition, alkaline phosphatase (ALP) and acid phosphatase (ACP) activity in the serum of the diabetic group were reduced by chicory. Also, administration of chicory seed extract restored serum and testis total protein content due to its ameliorating effect on protein metabolism . Diabetes is associated with serum mineral disturbances. Treatment with chicory recorded an improvement of serum minerals like zinc (Zn) and copper (Cu). Moreover, histological examinations confirmed the protective effects of chicory testicular dysfunction. In conclusion, treatment with chicory seed extract can attenuate many unfavorable complications associated with diabetes mellitus.

keywords: chicory, hyperglycemia, testicular dysfunction

1.Introduction

Approximately 425 million persons were diagnosed with diabetes mellitus in 2017, with a projected 629 million by 2045 [(1)]. Chronic hyperglycemia is a hallmark of this metabolic condition, along with the disarray of carbohydrate, lipid, and protein metabolism. Because cells in people with type 2 diabetes mellitus (T2DM) are unable to properly respond to insulin, glucose levels rise, leading to an increased production of insulin and subsequent hyperinsulinemia [(2)] which causes more insulin secretion resulting in hyperinsulinemia. Persistent hyperglycemia can lead to complication in multiple body organs[(3)]. Studies in both animals and diabetic men have shown that long-term hyperglycemia increases the risk of infertility through altering testicular structure and function due to oxidative stress,

inflammation, and cell death.[4].

Researchers have found evidence that people with diabetes mellitus have an enzyme deficiency in their ROS scavenging system [5,6]. Increased oxidative stress (OS) has been linked to sperm cell loss, DNA damage in the testicles, and a slowed spermatogenesis in diabetic rats, according to previous research[7]. Seminiferous tubules, body and testis weights, serum testosterone as well as FSH and LH levels, and sperm parameters were all drastically changed by streptozotocin (STZ)-induced diabetes[8,9].

Diabetes is associated with the abnormal metabolism of blood lipid. High triglycerides, high cholesterol, low HDL-C and high LDL-C are also related to diabetic complications[10]. In addition, diabetes incidence reduces the rate of protein synthesis in diabetic rodents[11]. Otherwise, diabetes problems have been linked to mineral deficits, specifically zinc and chromium[12]. Elevated enzyme levels have also been linked to diabetes. Patients with diabetes mellitus have had an increased serum ALP levels for [13]. ACP also is significantly raised in epididymal fat tissue in streptozotocin-diabetic rats[14].

There are several medications for treating hyperglycemia, but for some patients the trade-offs of side effects and expense make them less than ideal[15]. Hence, the search for more safe and effective hypoglycemic agents has continued[16]. For thousands of years, people have turned to the healing properties of plants to alleviate a wide range of medical issues[17]. Some of the natural products have been used as alternative and complementary treatment for diabetes[18].

Cichorium intybus L., often known as chicory, is a plant of Egyptian origin that falls under the taxonomic classification of the family Asteraceae. It can be categorized as an annual, biennial, or perennial plant. Additionally, it is recognized for its edible properties [19]. A diverse array of chemical substances can be found in all morphological components of chicory[20]. Chicory has been widely utilized for the management of many illnesses linked to diabetes[21]. This particular botanical specimen is a consumable flora species that may be encountered in a wide range of temperate regions across the globe[22]. The chicory root has been subjected to phytochemical research, revealing the presence of several compounds including inulin, alkaloids, flavonoids, coumarins, sesquiterpene lactones, vitamins, minerals, and volatile oils[23]. Chicory has been extensively utilized in the management of diabetes mellitus. Furthermore, empirical evidence has demonstrated that it has notable radical scavenging properties and confers substantial defense against protein oxidation and DNA damage, owing to the presence of phenolic compounds[24].

Chicory possesses a notable concentration of chicoric acid, a compound that has been found to have immunostimulatory properties and a modest capacity to mitigate inflammation[25,26]. The role of chicory in increasing testosterone hormone, fructose levels, and sperm motility and vitality has been reported[27,28]. The use of chicory extract has been found to possess the ability to counteract the presence of free radicals, thereby mitigating the detrimental impact of reactive oxygen species (ROS) on the process of spermatogenesis. This protective action serves to safeguard the integrity of sperm cells and enhance overall male reproductive performance[29]. Moreover, chicory has the capacity to enhance many metabolic problems, such as reduced protein content and mineral levels. The administration of a diet supplemented with chicory has been shown to effectively inhibit protein oxidation and enhance the functioning of organs involved in the synthesis of plasma proteins[30].

Therefore, the current study was performed to study the therapeutic effects of chicory extract on testicular dysfunction and other complications in type 2 diabetic albino rats.

2. Materials and methods

2.1. Experimental animals

24 healthy adult male rats weighing between 110 and 120 (g) were employed in the current study. The Egyptian Vaccine Company (VACERA, Cairo, Egypt) was where the rats were purchased. The temperature of the room where the animals were kept was kept between 22 and 25 degrees Celsius, with 12-hour cycles of light and darkness, in stainless steel cages. Throughout the duration of the trial, they have unrestricted access to food and water while being kept in a designated pathogen-free area in

Animal House Lab., Faculty of Science, Mansoura University, Dakahleia, Egypt. They spent ten days getting acclimated to lab conditions before the experiment. Euthanasia, therapy, and other experimental procedures were all carried out in conformity with National Institute of Health (NIH) guidelines for the care and use of laboratory animals and the ethical committee of animal care and use , College of science, Mansoura University, Dakahleia, Egypt. Code number: MU-ACUC (SCMS.22.10.5).

2.2. Induction of diabetes

A single intraperitoneal injection of freshly prepared STZ at a dose of 50 mg/kg was used to cause diabetes. Sigma Aldrich Company, USA was where STZ was purchased. It was dissolved in citrate buffer (0.1M, pH: 4.5)[20]. A glucometer is used to measure serum glucose 72 hours after STZ injection to ensure that diabetes was successfully induced. The rats classified as diabetic had fasting blood glucose levels higher than 200 mg/dl.

2.3. Animal groups

The rats were haphazardly divided into four groups, six rats were included in each group.

1- Control group**:** The rats were not given any medication or special diets.

2- Chicory group: Rats received a daily oral dosage of 250 mg/kg chicory seed extract for 30 days[20].

3- Diabetic group: STZ 50 mg/kg was administered intraperitoneally to rats as a single dose[20].

4- Diabetic + chicory group: Rats with diabetes were given an oral a daily dosage of 250 mg/kg chicory seed extract for 30 days[31].

2.4. Blood and tissue sampling

At the end of the experiment, all rat groups were fasted the night before being sacrificed while sedated with ketamine and xylazine. By immediately puncturing the hearts, blood samples were immediately drawn and placed in clean test tubes. Blood tubes were centrifuged for 15 minutes at a speed of 3000 rpm to obtain sera. Samples were labeled before being immediately frozen at -20°C for additional biochemical investigation. Once the blood had been collected, the animals were quickly dissected. For later histological staining, the right testes from each animal were carefully removed and preserved in neutral formalin (10%).

2.5. Determination of body weight and relative testes weight

To calculate the body weight gain for each group, the starting and ending weights (g) were taken at the beginning and end of the trial, respectively. The absolute testis weight was divided by the final body weight (×100) to calculate the relative testis weight.

2.6. Determination of fasting blood glucose and serum insulin

Kits from Bio-diagnostic Egypt (Catalogue #: GL 13 20) and Biovendor R&D, Czech Republic (Catalogue #: RTC018R) were used to assess blood glucose and serum insulin levels, respectively.

2.7. Estimation of fertility hormones

The serum levels of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were determined by My-BioSource (San Diego, USA), ELISA kits provided by My-BioSource (San Diego, USA), (Catalogue #: MBS282195, MBS2502190 and MBS764675) respectively.

2.8. Estimation of serum and testis total protein

Utilizing kits from Bio-diagnostic Egypt (Catalogue #: TP 20 20), we were able to calculate the total protein content (TP) of the serum and testis.

2.9. Estimation of serum enzymes and minerals

The serum alkaline phosphatase (ALP) activity was measured using kits provided by Biodiagnostic Egypt (Catalogue #: AP 10 20), while the acid phosphatase (ACP) activity was assessed using ELIZA kits from Assaygenie (London, UK; Catalogue # RTFI01238). With the aid of kits from Bio-diagnostic Egypt (Catalogue #: Zn 21 20), the serum zinc (Zn) levels was determined. Meanwhile, serum copper (Cu) was measured using ELIZA kits from Assaygenie (London, UK; Catalogue # BA0035).

2.10. Histopathological analysis

The testes were removed, cut into slices that

were 4 m thick, embedded in paraffin, and fixed for an overnight period at room temperature in 4% paraformaldehyde before being deparaffinized and stained with hematoxylin and eosin. (H&E). The morphology of the testes as well as the number of spermatogonia and spermatocytes were evaluated under a light microscope using the testicular grading system created by Johnsen. Using Johnsen's standards, thirty cross-sectioned tubules of each group were evaluated[32].

2.11. Statistical analysis

The data from this study were statistically analysed using GraphPad prism 9.0 software (GraphPad prism software Inc., San Diego, California, USA). Results are presented as mean ± SEM of n = 6. The statistical comparison was examined using one-way analysis of variance (ANOVA), then the Neuman-Keuls post-hoc test. Percent of changes in different groups were evaluated, comparing to control and diabetic groups respectively. When the P value was less than 0.05, the difference was declared significant, and any higher significance level was reported. The following significance level symbols were used to display statistical differences: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ and $****P <$ 0.0001versus control animals. $^{@}P < 0.05$, $^{@@}P < 0.01$, $^{@@@}P < 0.001$ and $^{@@@@}P <$ 0.0001versus diabetic animals.

3. Results

3.1. Body weight change and relative testes weight

There was no observed variation in the beginning body weight among all the groups. In contrast, the diabetic rats exhibited a substantial reduction in body weight (-25.2%, p<0.0001) compared to the control group at the conclusion of the trial period. In the interim, the administration of chicory seed extract for a duration of 30 days resulted in a noteworthy increase in body weight (+27%, p<0.0001) as compared to the diabetic group that did not receive therapy (Fig. 1.i).

The ingestion of chicory extract to rats with normal physiological conditions resulted in a modest rise in the relative weight of the testes, as compared to the control group. Nevertheless, a notable decline in the relative weight of the testes was observed in rats with diabetes when compared to the control group, with a fall of 51.9% (p<0.0001). The administration of chicory seed extract resulted in a significant increase in the relative weight of the testes when compared to diabetic rats who did not receive treatment (91.1% increase, p<0.001), (Fig.1.ii).

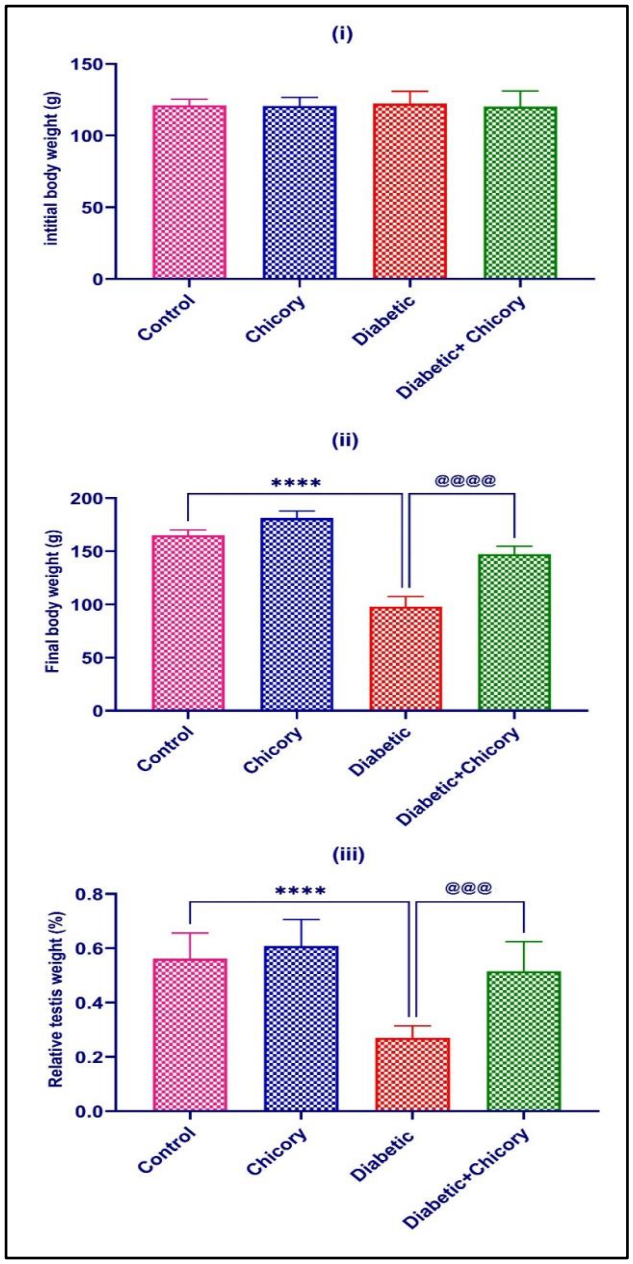

Figure 1. Effect of diabetes and chicory on: (i) Initial body weight (g); (ii) Final body weight (g), (iii) Relative testis weight (%) in the different experimental groups. Values are expressed as means ± SEM; (n = 5). (**** indicate statistical significance at P < 0.0001, compared to the control group. $^{@@@}$ and $^{@@@@}$ indicate statistical significance at P < 0.001 and P < 0.0001, respectively, compared to the diabetic group).

3.2. Fasting blood glucose and serum insulin

The data presented in Figure 2 indicates that there was no statistically significant alteration observed in the levels of fasting blood glucose (FBG) or insulin in normal rats following treatment with chicory seed extracts. The respective mean values for FBG and insulin were 98.2 mg/dl and 2.53 ng/ml. In contrast, the diabetic rats exhibited a notable elevation in fasting blood glucose and serum insulin levels when compared to the normal rats, with an increase of 492.7% and 250% respectively ($p<0.0001$). The administration of chicory seed extract to diabetic rats resulted in a considerable reduction in fasting blood glucose (FBG) levels and serum insulin levels, compared to diabetic rats that did not receive treatment. The reduction observed in FBG levels was 69.7%, while the reduction in serum insulin levels was 55.5% ($p<0.0001$).

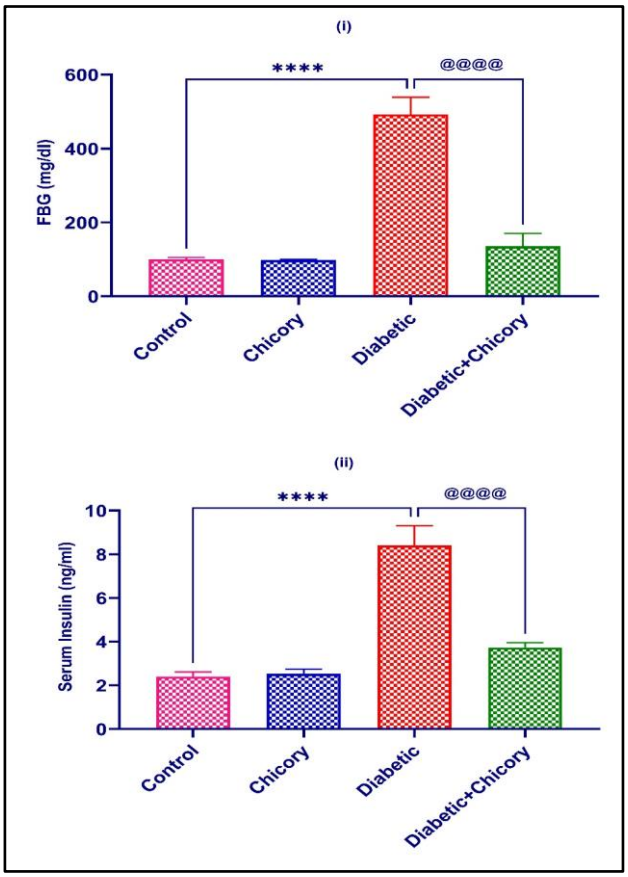

Figure 2. Effect of diabetes and chicory on: (i) FBG (mg/dl); (ii) serum insulin (ng/ml) in the different experimental groups. Values are expressed as means ± SEM; (n = 6). (**** indicate statistical significance at $P < 0.0001$, compared to the control group and @@@@ indicate statistical significance at $P < 0.0001$, compared to the diabetic group).

3.3. Hormonal assay

According to the findings presented in Figure 3, the daily administration of chicory did not result in a statistically significant alteration in the blood testosterone level, as compared to the control group of rats (10.56 ng/ml). The data demonstrated a substantial reduction in serum testosterone levels when comparing the experimental group of rats with the control group, showing a decrease of 95.9% ($p<0.0001$). In contrast, it was shown that diabetic rats treated with chicory seed extract exhibited a noteworthy elevation in serum testosterone levels (+1157.3%, $p<0.01$) compared to diabetic rats who did not receive any treatment.

The data obtained from the experiment revealed that there was no statistically significant alteration in the levels of follicle-stimulating hormone (FSH) or luteinizing hormone (LH) in rats that received daily doses of chicory seed extract, when compared to the control group of rats. The blood FSH levels in the experimental group were measured to be 5.28 mIu/ml, while the LH levels were found to be 5.04 mIu/ml. In contrast, a notable elevation in serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels was observed in diabetic rats when compared to the control group, with a substantial rise of 92.8% and 83.5% respectively ($p<0.0001$). Treatment of diabetic rats with chicory seed extract,caused a significant decrease in serum FSH level when comparing with diabetic rat group, (-10.1, $p<0.05$), while this treatment recorded non-significant decrease in serum LH level (-6.3) when compared with diabetic rats group (fg.3).

3.4. Serum and testis total protein content

Observed data in (fig.4) displayed no significant change in the level of serum or testis TP level by daily administration of chicory seed extracts comparing to normal rats, (7.46 g/dl & 7.19 mg/g) respectively. Otherwise, there was a decrease in serum and testis TP levels in diabetic rats if compared with control rats group, (-17.89 % &-28.1 %, $p<0.0001$) respectively. An improvement in serum and testis TP level was recorded when diabetic rats treated with chicory seed extract, (+27.1 %, $p<0.0001$) & (16.4 %, $p<0.01$) respectively.

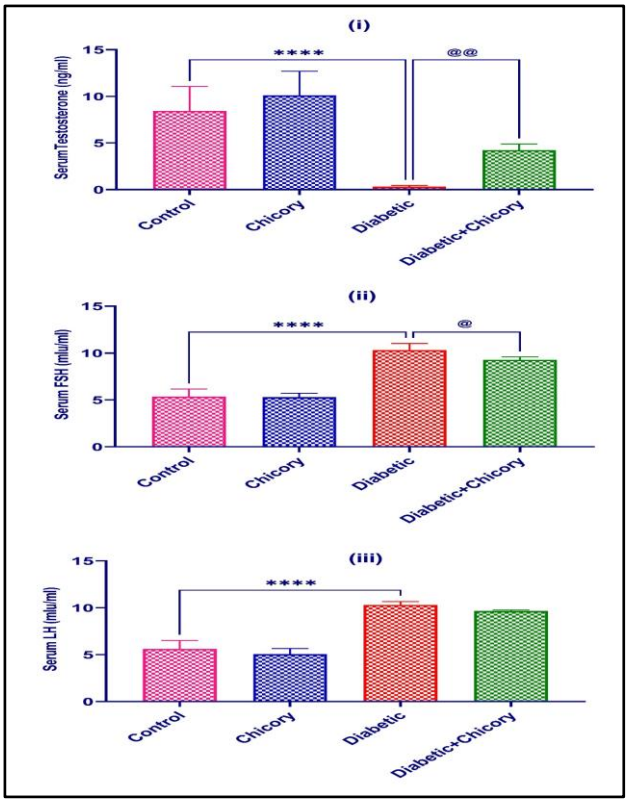

Figure 3. Effect of diabetes and chicory on: (i) serum Testosterone (ng/ml); (ii) Serum FSH (mIu/ml); (iii) serum LH (mIu/ml) in the different groups. Values are expressed as means ± SEM; (n = 6). (**** indicate statistical significance at P < 0.0001, respectively, compared to the control group. @, @@, indicate statistical significance at p<0.05, P < 0.01, respectively, compared to the diabetic group).

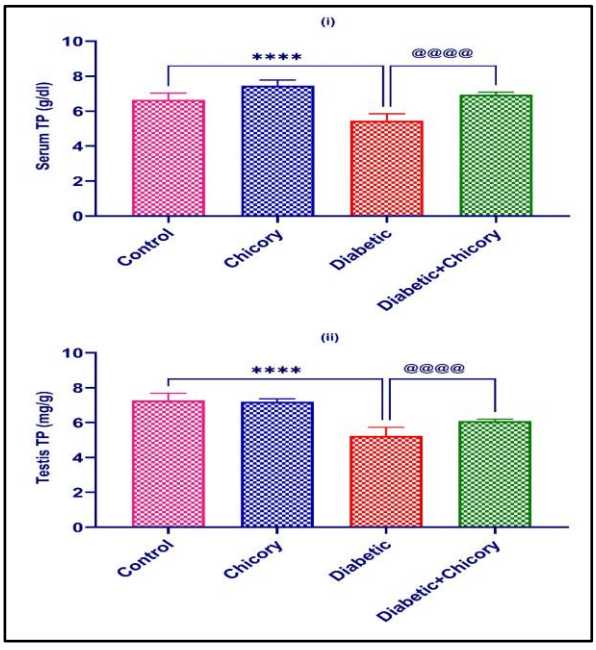

Figure 4. Effect of diabetes and chicory on: (i) serum TP (g/dl); (ii) testis TP (mg/g) in the different experimental groups. Values are expressed as means ± SEM; (n = 6). (**** indicate statistical significance at P < 0.0001, compared to the control group and @@@@ indicate statistical significance at P < 0.0001, compared to the diabetic group).

3.5. Serum enzymes and minerals

Daily administration of chicory didn't show any significant change in the activity of serum ALP or ACP when compared with control group, (262.50 & 1.49 mg/dl) respectively. Meanwhile, a significant increase in serum ALP & ACP activities were observed in diabetic rats comparing to control animals, (+47.3, +122.6% p<0.0001) respectively. Otherwise, when diabetic rats treated with chicory seeds there was a significant decrease in serum ALP and ACP activities if compared to untreated diabetic rats. (-26.4 & -31.1 %, p<0.0001) respectively (fig5).

As shown in (fig.5), chicory treated normal rats didn't show any significant change in the level of serum Zn or Cu when compared with control rats group, (107.33 & 101.16 mg/dl) respectively.

STZ treatment showed a significant decrease in serum Zn and Cu (-24.5%, -15.7%, p<0.01) respectively, if compared with control rats group. Moreover, treatment of diabetic rats with chicory recorded a significant increase in serum Zn (+24.3, p<0.05) & serum Cu (+22.6%, p<0.01) when comparing with diabetic rats group.

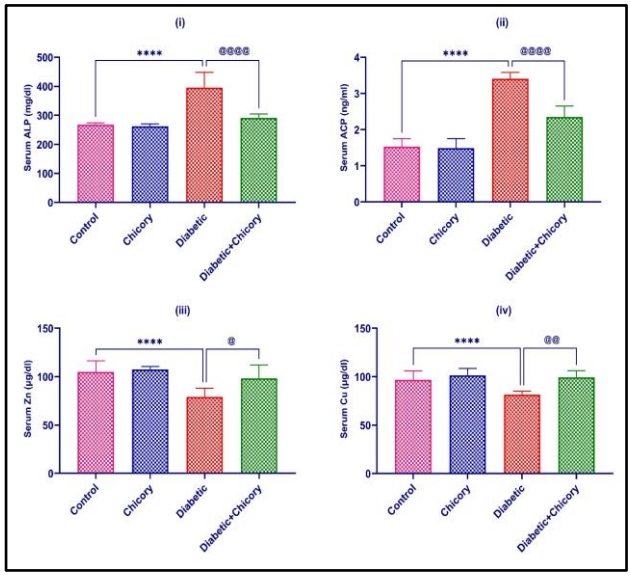

Figure 5. Effect of diabetes and chicory on: (i) serum ALP (mg/dl); (ii) serum ACP (mg/dl), (iii) serum Zn (µg/dl) (iii) serum Cu (µg/dl) in the different experimental groups. Values are

expressed as means ± SEM; (n = 6). (** and **** indicate statistical significance at P < 0.01 and P < 0.0001, respectively, compared to the control group. @, @@ and @@@@ indicate statistical significance at P < 0.05, P < 0.01 and P < 0.0001, respectively, compared to the diabetic group).

3.6. Histopathological examination

In both control and chicory group, seminiferous tubules examination revealed regular histoarchitecture, with intact spermatogenic epithelium. It was arranged neatly with abundant spermatozoa in the tubular lumen (Fig 6.a,b). In contrast, diabetic seminiferous tubules depicted degenerated germinal epithelium with irregular distribution of spermatogenesis stages. Inflamated tubules with occluded lumen could be noticed here and there in the diabetic testis (Fig 6.c). Otherwise, chicory administration to diabetic animals markedly improved the testicular architecture, concurrently the area and diameter of spermatogenic epithelium were increased, which led to the recovery of spermatogenesis to some extent (Fig.6.d). Testicular Johnson score revealed a remarkable decrease in the diabetic group (3.2, p<0.0001) with respect to normal control (8.8), while chicory treatment showed a significant improvement (8.2, p<0.0001) with respect to diabetic animals (Fig.6.e).

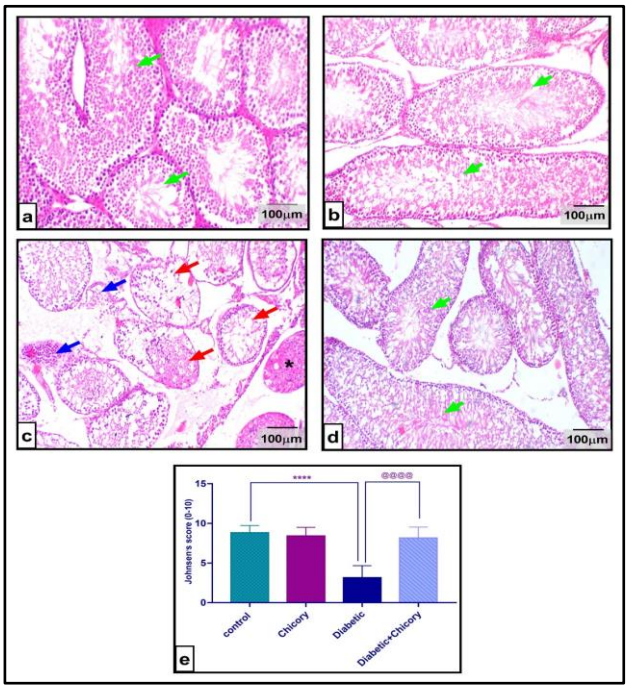

Figure 6. representative testicular sections of: **(a).** Control group; **(b)**. Chicory group showing normal seminiferous tubules with intact germinal epithelium (green arrow); **(c)**. diabetic group showing degenerated seminiferous tubules with abnormal germinal epithelium (red arrow), destructed cells in-between the tubules (blue arrow), and inflammatory tubules with occluded lumen (asterisk); **(d)**. diabetic animals treated with chicory revealing almost normal architecture and recovered spermatogenesis (green arrow); **(e)**. Johnsen's score. Values are expressed as means ± SEM; (n = 5). **** indicate statistical significance at P < 0.0001 compared to the control group. @@@@ indicate statistical significance at P < 0.0001 compared to the diabetic group.

3.7. Discussion

Diabetes mellitus is a chronic condition characterized by elevated blood glucose levels, which can lead to malfunction in several organs and systems, including the reproductive system[33]. The occurrence of hyperglycemia-induced apoptosis has been attributed to the significant involvement of oxidative stress and disruption of microcirculation[34]. Diabetes is characterized by disturbances in carbohydrate, protein and lipid metabolism[35]. The injection of STZ resulted in the rapid deterioration of pancreatic β-cells in rats, resulting in elevated blood glucose levels. Consequently, this event stimulated the secretion of insulin and the emergence of insulin resistance, which are notable features associated with type II diabetes[36]. Sustained hyperglycemia negatively affects sperm concentration and motility in rats due to oxidative stress[37].

Natural hypoglycemic medicines may serve as a viable alternative to synthetic pharmaceuticals currently in use, thereby mitigating the potential adverse effects associated with the latter. These substances are employed in the management of diabetes mellitus [38]. There is scientific evidence supporting the use of several bioactive flavonoids, including certain dietary compounds, as crucial components in the development of molecular treatments aimed at preventing diabetes[39]. The utilization of therapeutic botanicals has been employed for the management of diabetes and its associated problems. Chicory is considered to be a highly significant therapeutic herb due to its notable antidiabetic and antioxidant properties[40].

The observed decrease in body and testis weight in the present study can be attributed to the potential inhibitory effects of STZ on testosterone and growth hormone release, leading to disruptions in anabolic activities[41]. The induction of diabetes through the administration of Streptozotocin resulted in notable changes in various aspects, including the structure of seminiferous tubules, the weights of the body and reproductive organs, the concentration of testosterone in the bloodstream, and the characteristics of sperm[8]. However, treatment of diabetic rats with chicory seed extract revealed a significant increase in body and testis weight.

The present testicular dysfunction in the current investigation is due to increased production of reactive oxygen species and decreased antioxidant scavenging activities which are associated with diabetes leading to increased lipid peroxidation and DNA oxidation[42]. The current study proved that chicory has an ameliorating effect on lipid peroxidation as well as protection from DNA damage due to presence of natural antioxidants that enhance antioxidant defense mechanism and free radical scavenging activity. The antioxidant properties of chicory are ascribed to its high concentration of phenolic chemicals and flavonoids[43]. The antioxidant activity of the phenolic content is attributed to the presence of hydroxyl groups, which enable free radical scavenging action[44].

Production of sperms and hormone secretion particularly testosterone are the main functions of testis[45]. Testicular dysfunction caused by diabetes encompasses a range of adverse effects, such as diminished spermatogenesis resulting from an elevated rate of germ cell death, compromised sperm parameters, and reduced testosterone release, ultimately leading to infertility[46]. The diabetes group exhibited significantly elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) compared to the control group. This observation can be attributed to the well-established role of insulin in promoting the release of gonadotropin-releasing hormone (GnRH) by hypothalamic neurons[47]. In contrast, a significant reduction in serum testosterone levels was seen in rats with diabetes[48]. The present study revealed that, diabetes is associated with low testosterone level and high FSH and LH hormones. All these hormonal disturbances are restored and improved by administration of chicory.

The obtained decreased protein content is due to persistent hyperglycemia and excessive oxidative stress. A previous study revealed significant reduced protein content in diabetic patients[49]. In addition, an elevated level of alkaline phosphatase (ALP) was mostly correlated with the presence of insulin resistance. Elevated levels of alkaline phosphatase (ALP) have been found to potentially amplify the likelihood of developing diabetes, while also being linked to oxidative stress. [50]. The current study revealed that, diabetic group recorded a significant increase in ALP and ACP enzyme activities as well as significant decrease in serum and testis total protein content.

The aforementioned studies have demonstrated that the administration of chicory plant has resulted in improvements in testicular dysfunctions. These improvements were observed through an increase in total protein content and testosterone hormone levels, as well as a decrease in ALP and ACP activities. It can be inferred that the ameliorative effects of chicory on various biological processes in normal cells contribute to these observed outcomes[40,51]. Administration of chicory-supplemented diet resulted in an improvement of protein pattern by preventing protein oxidation in different body organs which synthesized plasma protein[30].

Furthermore, chronic illnesses such as chronic hyperglycemia have been found to be linked to trace-element shortages, leading to notable changes in the levels of certain micronutrients[52]. Two of the most crucial trace metals in redox reactions as antioxidants are copper and zinc. Copper plays an important role in the elimination of free radicals in cells as a component of copper/zinc superoxide dismutase (Cu/Zn SOD)[53]. The current study recorded a significant decrease in serum Zn and Cu was noticed in diabetic rats was partially restored by administration of chicory seed extract for 30 days. In addition to the chicoric acid, the main component of the chicory extract chicory contains inulin, phenolic compounds,

vitamins, and minerals like Cu, Zn, and others[54], which help in restoring Zn &Cu deficiency associated with diabetes.

The present findings suggested that chicory seed extract can ameliorate various complication associated with type 2 diabetes mellitus as testicular dysfunction, hormonal disturbances, metabolic disorders, increased enzyme activities and decreased minerals as well as total protein content, due to its antioxidant, antidiabetic, antiinflammatory and antiapoptotic effect.

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