International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **3(8),** 89-97, August **(2014)** *Int. Res. J. Biological Sci.*

Ameliorative Effect of Rocket Leaves on Fertility in Streptozotocin-Induced Diabetic Rats

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Available online at: www.isca.in, www.isca.me
Received 17th March 2014, revised th 2014, accepted th 2014

Abstract

In the present study, Rocket leaves were evaluated for the acute toxicity and fertility of male diabetic rats. Leaves were subjected to extraction with water. Extract was administered orally to Swiss albino mice to explore their acute toxic effects (LD_{50}). For the fertility experiment, 24 rats were divided into 4 groups, containing 6 rats in each group; one group serve as normal control, while in others groups, animals were rendered diabetic by single injection of STZ (55 mg/kg, i.p.). One group serving as diabetic control, and the others two groups were treated with aqueous extract of rocket (AER) at doses of 250 and 500 mg/kg for 8 consecutive weeks. Animal were assessed for fertility protective activity after 24h of the last dose. The results showed significant ($p<0.01$) increase in the sexual organ weights (testes, epididymis, seminal vesicles and prostate) and serum quality in the AER treated groups when compared with the diabetic control group in dose dependent manner. AER was also found to increase serum testosterone, testicular GSH level and decreased testicular TBARS levels. The obtained results demonstrated that AER significantly improve diabetes complications in rat's testis. The present study suggested that rocket might have a promising effect against diabetes-induced impaired testicular damage in male rats.

Keywords: Rocket, testes, male fertility, histopathology, streptozotocin.

Introduction

Infertility may be defined as, if a couple sexually intercourse without protection for one year and unable to conceive[1]. Infertility affects almost 15% of all married partner, but ~50% of these abnormalities evident in the male. Because of low sperm count, the infertility rate in male is increasing worldwide. Over the last 50 years, in Western countries, sperm counts have been decreased by 1% each year[2].

Diabetes has been related with reproductive problems of both partners. Diabetic patients (~90%) have turbulence in sexual problem that includes decrease in libido, impotence, and infertility[3,4]. Sperm produced by diabetic patient, have more abnormalities[5]. These abnormal or damage sperm may lead to conceiving problems and if conception does occur, a higher chance of miscarriage. In patient with diabetes, or any insulin resistance condition, hormonal regulation becomes disturbed and even a single hormone is out of balance, it may affect other hormones such as estrogen, progesterone, testosterone etc., which may cause a number of adverse effects, for example, ovarian cysts, impotence, infertility etc..

The name "rocket" is widely used to indicate different species of Brassicaceae family (also known as salad rocket). Most commonly found in Mediterranean region, and are used as a new ingredient in salads for their pungent flavor[6]. Salad intake has been increased all around the world in last 10 years. *Eruca sativa* salad is a good source of natural antioxidants like vitamin C, carotenoids, and polyphenols[7]. The 4-(methylthio) butyl isothiocyanate (erucin or ER) has been previously reported as a main constituent present in rocket salad leaves[8,9], has ability to prevent selectively cancer cell growth[10,11]. Recently, rocket leaves have been also reported for chemo-prevention on human colon cancer cells[12]. Rocket seeds oil is tried for prevention and treatment of alloxan induced - diabetic rats[13]. Rocket is also known to have strong aphrodisiac effect since Roman times[14] and considered as a medical plant with many reported properties. In experimentally induced diabetic animal models, STZ results in impaired testicular function and degeneration[15]. Rocket can decrease STZ's harmful effects on sperm parameters and testis by reducing oxidative stress. Therefore, the present study was designed to determine the effects of rocket on spermatogenesis and testicular tissue disorders against STZ-induced diabetes in male rats.

Material and Methods

For the present research, fresh rocket leaves were purchased from Al-Kharj vegetable market, Saudi Arabia and authenticated by expert taxonomist. Leaves were shade dried; coarsely pulverized and percolated for about 3 days at room temperature with water. The collected percolate was concentrated under reduced pressure.

Male Wistar albino rats (180-200g) and Swiss albino mice weighing 25-30 g were procured from the Animal House, College of Pharmacy, Salman bin Abdulaziz University, Kingdom of Saudi Arabia. Rats and mice were maintained on a stable temperature (22±2°C), moisture (55±1%), light-dark

conditions (12:12 h light dark ratio) for acclimatization and Purina chow diet was provided with free access to drinking water.

Acute Toxicity Test: For acute toxicity test, leaves extract was administer to different groups of mice at different doses, ranging from (50–5000 mg/kg) by oral route. Another control group received the vehicle (3% v/v Tween 80 in distilled water) and kept under the same conditions. The animals were continuously observed for 48 h for clinical signs and toxicity symptoms[16].

Induction of diabetes: In overnight-fasted Wistar rats, diabetes was induced by a single dose of streptozotocin (55 mg/kg, i.p.) in citrate buffer (0.1 M, pH 4.5)[17]. Rats were confirmed diabetic by measuring the fasting blood glucose level, 3 days after injection of STZ. Animals were included in the experiment with a blood glucose level above 300 mg/dl.

Fertility experiment: For studying the effect of AER on fertility, 24 male rats were randomly divided into four groups (n=6). The 1st group was kept as normal control. Among the other three diabetic groups, one left as diabetic control, and other two groups were given AER at doses of 250 and 500 mg/Kg respectively orally, for 8 consecutive weeks to cover the spermatogenic cycle of the rats[18]. After 24h of last dose, retro-orbital plexus was punctured to collect the blood samples and then serum was separated for glucose and testosterone estimation[19].

Body weight and organ weight: Body weights of each rat were recorded for observation at weekly interval and the final body weights were recorded at the end of experiment. Animals of each group were sacrifice under light ether anesthesia and organ (testis, epididymis, seminal vesicles and ventral prostate) were dissect out, cleared by washing in ice-cold saline and weighed. Gonadosomatic index (GSI) was calculated by using the formula (organ weight/body weight) × 100.

Epididymal sperm characters: After cutting the epididymis tail of treated rats, semen samples were obtained from cuda epididymis, gently place on clean microscopical slide and observed for the sperm motility and sperm cell count under the microscope[20]. Epididymal sperm abnormalities were also determined by microscopical examination of seminal smears[18].

Determination of TBARS and GSH: Testicular tissue after washing with ice cold water was homogenized in phosphate buffer (1:9). Testicular homogenate was used for TBARS estimation[21], and then PMS (Post Mitochondrial supernatant) was prepared by centrifuging remaining homogenate at 4000 rpm at 4ºC for 15 min for GSH[22].

Histological evaluation: Testes were fixed in freshly prepared formalin solution (10%) and embedded in paraffin sections. Sections (5µm) were cut with a rotary microtome and stained with haematoxylin and eosin for histological evaluation.

Statistical analysis: All data were reported as mean ± SEM and analyzed by using one way ANOVA followed by post hoc Tukey's test for the comparison between different groups, using SPSS program (version 8) software package (SPSS_ Inc., USA).

Results and Discussion

Acute toxicity test: Toxicity symptoms were not observed. The LD_{50} value could not be calculated because lethality was not observed even up to 5 g/kg dose of the AER in mice by oral route.

Glucose and Testosterone estimation: The blood glucose levels at the time of sacrifice were significantly increased in the diabetic group compared to the normal control (546.6±17.47 Vs 115.67±12.35 mg/dl respectively). The AER treatment found to decrease blood glucose level significantly ($p<0.01$) at dose dependent manner (figure 1). The results depicted that STZ significantly decreased the serum Testosterone level as compared to normal control group and AER treatment for 8 consecutive weeks to male rats causes significant increased in serum Testosterone level as compared to diabetic control group (figure 2).

Testicular TBARS and GSH: The results showed that STZ significantly increased the TBARS and decreased the GSH level in testes. Rats treated with oral administration of AER at a dose of 250 and 500 mg/Kg for consecutive 8 weeks significantly decreased the TBARS level and increased the GSH level in testes, as compared to diabetic control group (figure 3 and 4).

Body weight, Organ Weight and GSI: The final body weight was found to be decrease in diabetic control group, as compared to normal control group while body weight increase in the AER treated groups (figure 1). The weights and GSI of the testes, epididymis, seminal vesicles and ventral prostate were significantly decreased in diabetic control group as compared to normal group while the rats treated with AER found to have increased organ weight and GSI significantly as compared with diabetic control group (figure 5 and 6).

Epididymal sperm characters: STZ caused a drastic reduction in sperm count and motility, but increased the percentage of sperm cell abnormality in diabetic rats as shown in figure 7. The most common abnormalities seen in the examined seminal smears of diabetic rats were detached head and coiled tail.

Histological Evaluation: Photomicrograph of control group showed, normal seminiferous tubules and the thickness of the basement membrane was also seems to be normal (figure 8a). Testicular slides of diabetic rat showed numerous seminiferous tubules, thickened basement membrane and marked decrease in spermatogonia cells. Only few tubules still contain spermatogonia but sperms were absent in the tubules. The interstitial cells are increased in between the tubules (figure 8b). AER treatments at doses of 250 and 500 mg/kg/body wt showed

most of the seminiferous tubules have few spermatogonia and sertoli cells. More regeneration and focal proliferation of spermatogonia cells observed in seminiferous tubules. The interstitial cells are decreases in some areas. There is evidence of starting spermatogenesis in very few tubules at a dose of 500 mg/Kg (figure 8c and 8d).

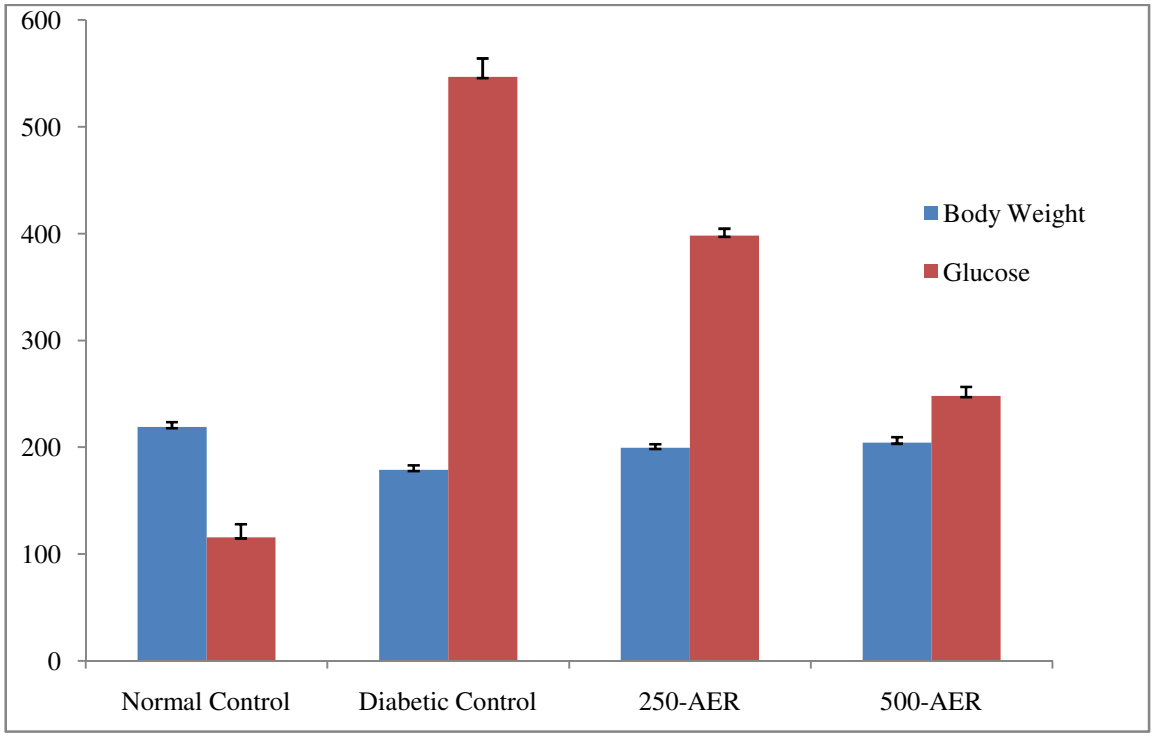

Figure-1
Effect of AER on Body weight (g) and glucose (mg/dl) level in STZ induced Diabetic male rats

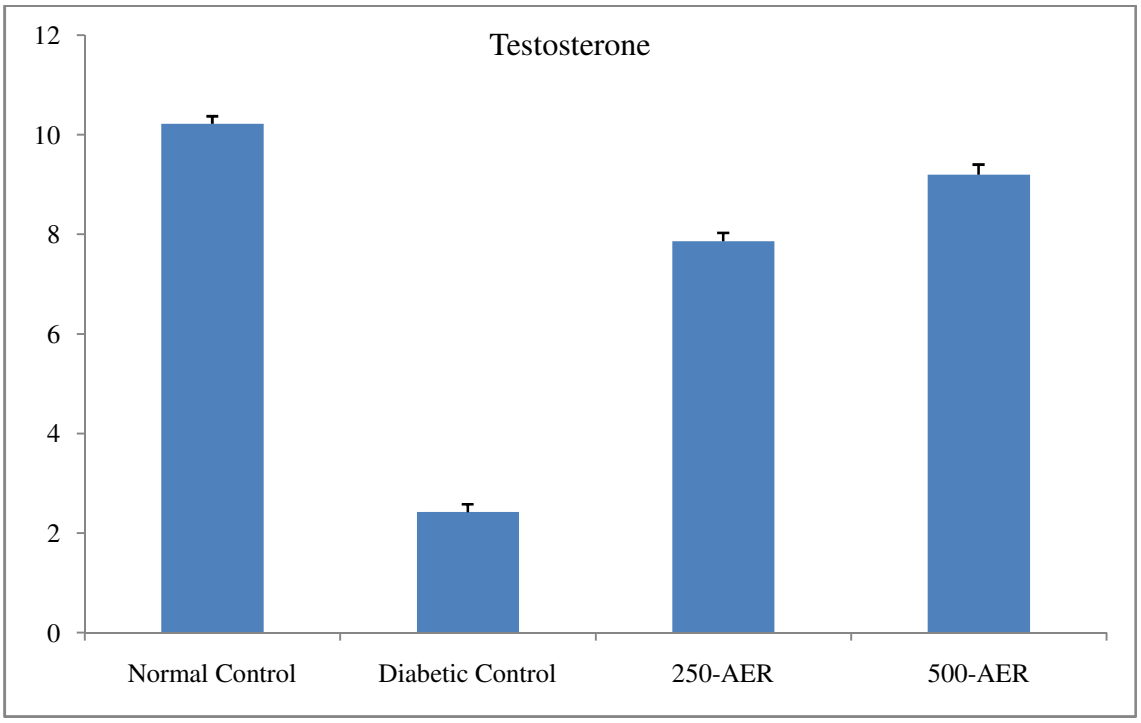

Figure-2
Effect of AER on Testosterone (ng/ml) level in STZ induced Diabetic male rats

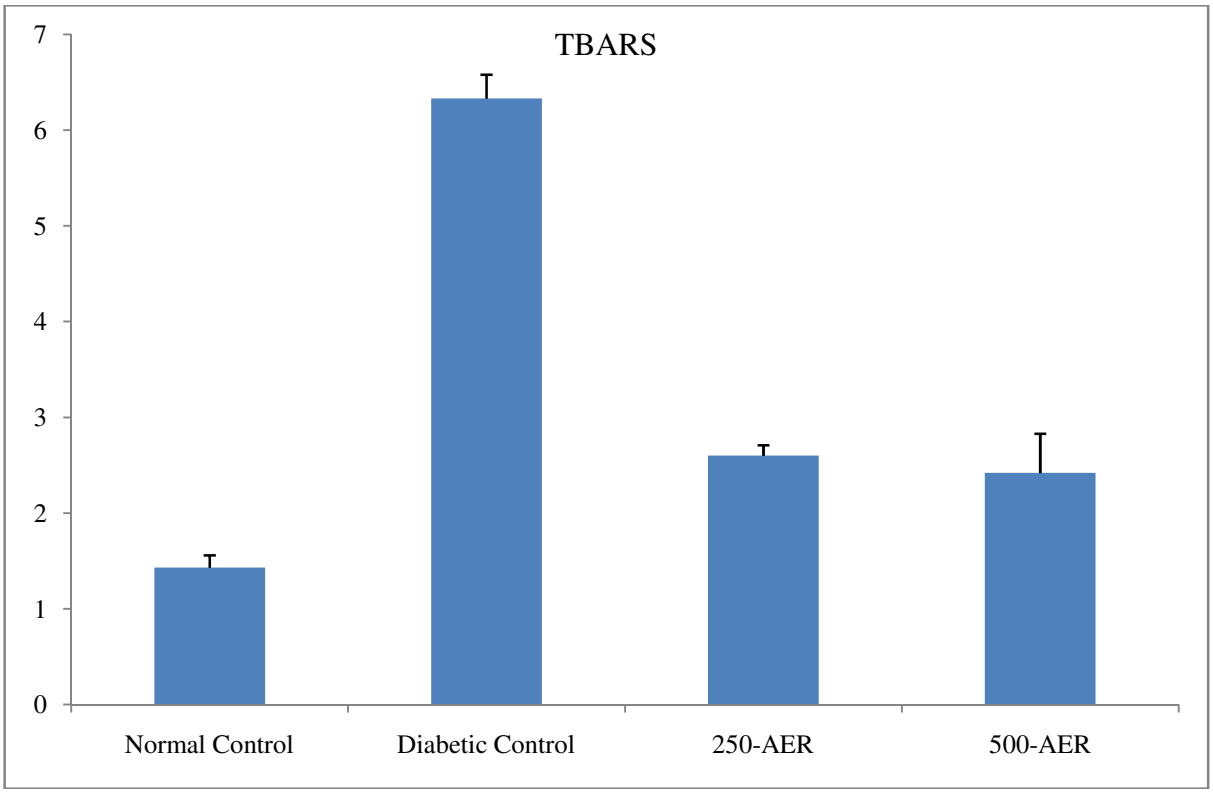

Figure-3
Effect of AER on testicular TBARS (nmol of MDA formed/min/g Tissue) level in STZ induced Diabetic male rats

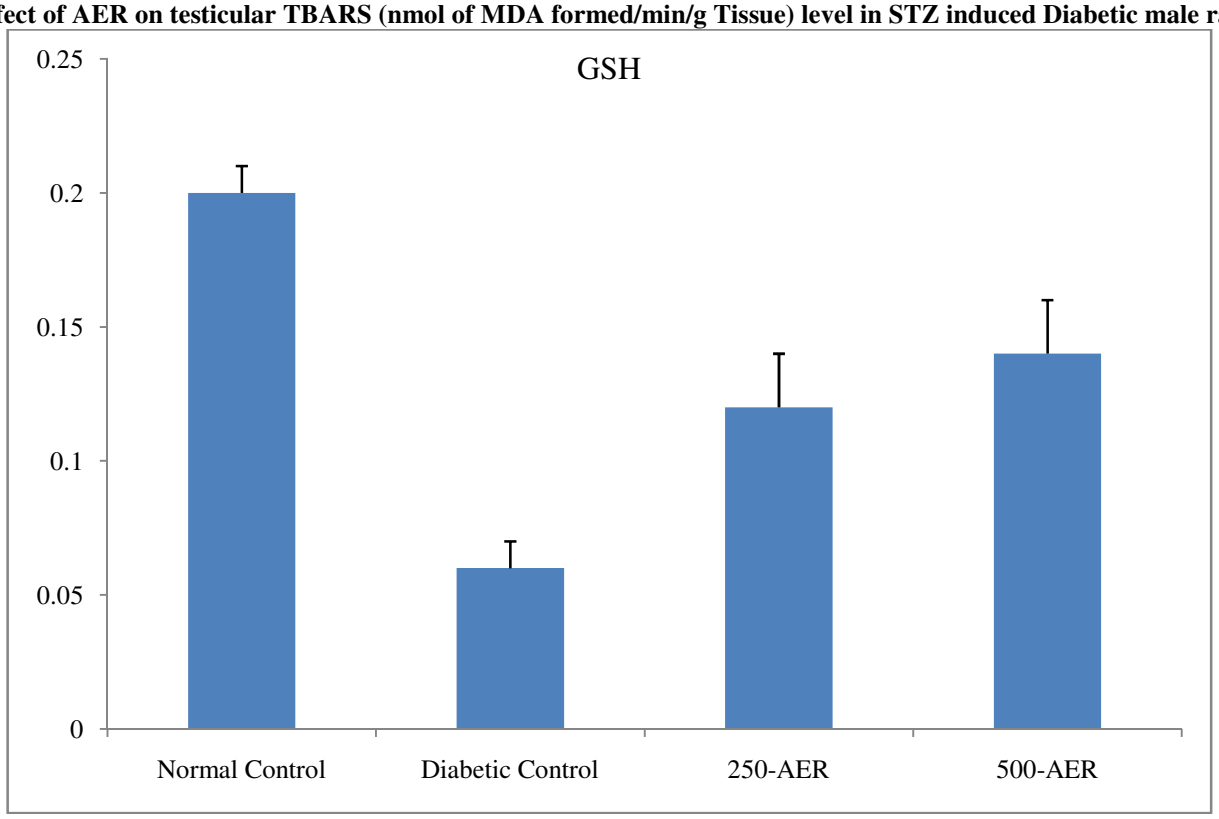

Figure-4
Effect of AER on testicular GSH (µmoles / g Tissue) level in STZ induced Diabetic male rats

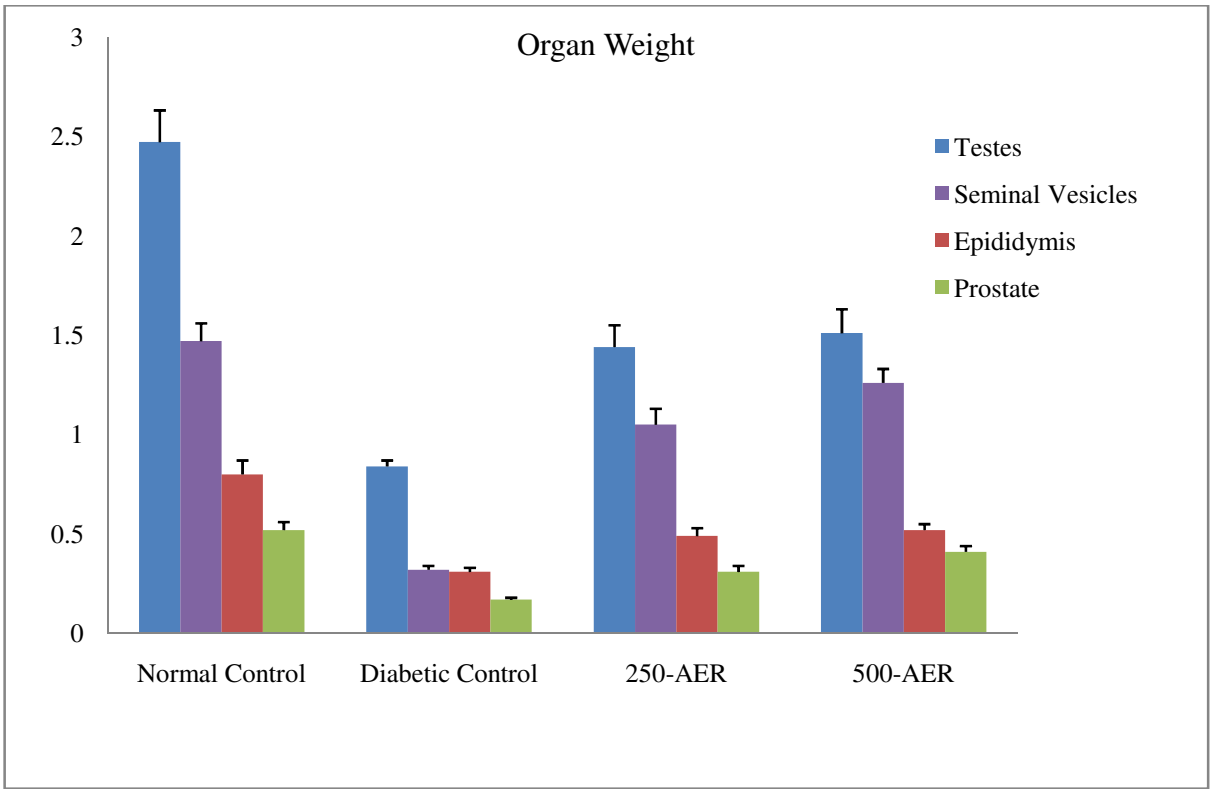

Figure-5
Effect of AER on Organ weight (g) in STZ induced Diabetic male rats

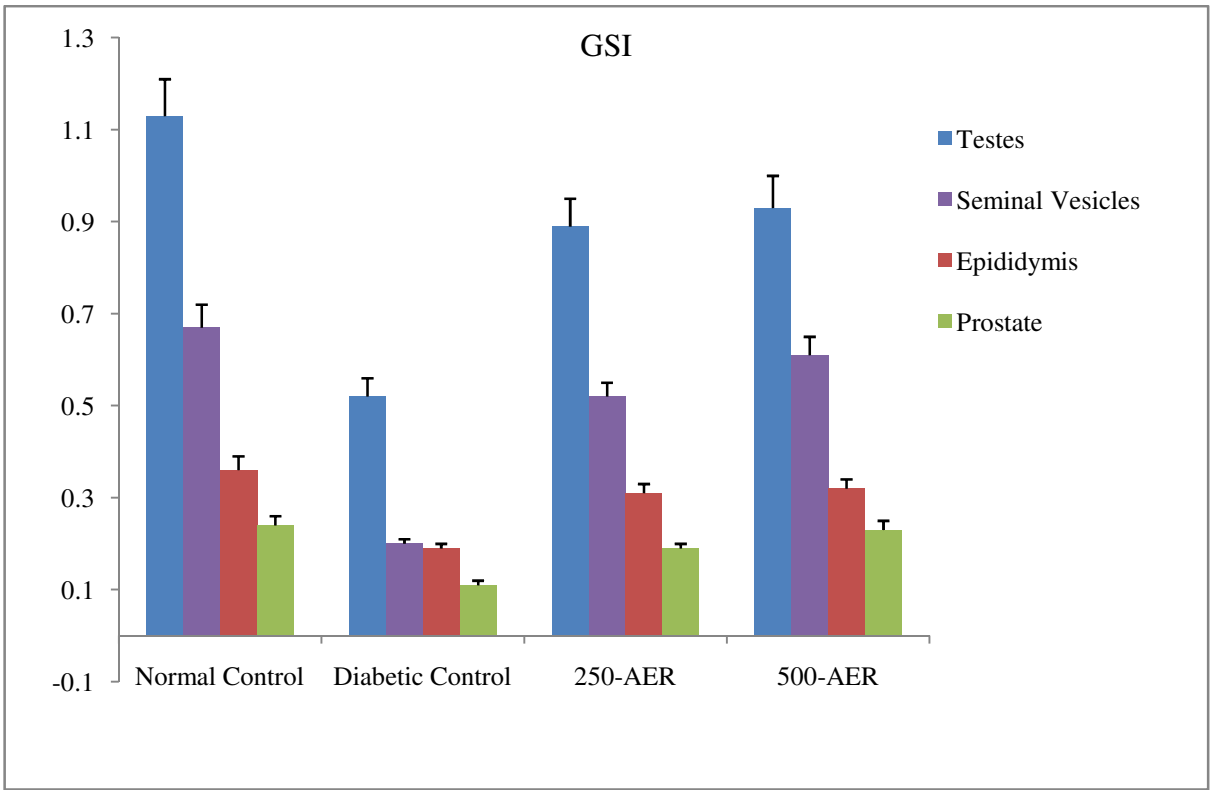

Figure-6
Effect of AER on GSI (organ weight/body weight x 100) in STZ induced Diabetic male rats

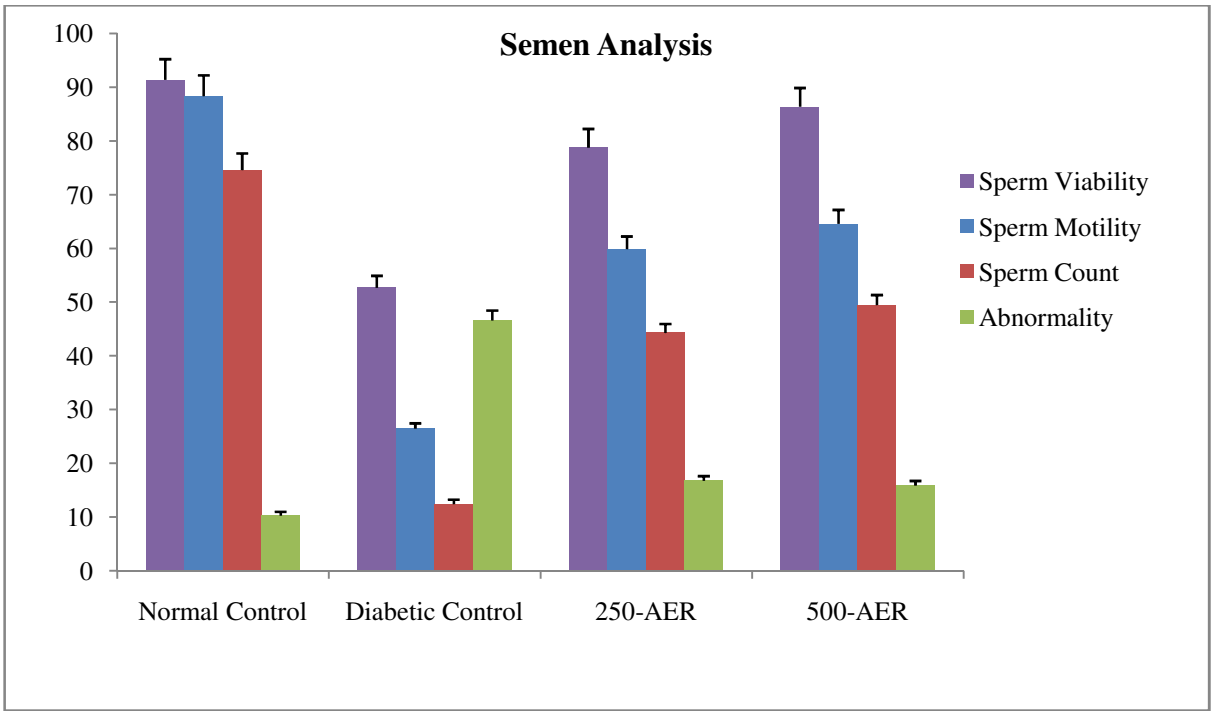

Figure-7
Effect of AER on Semen Analysis [Sperm viability (%), Sperm Motility (%), Sperm Count (10^6 /Epididymis), and Abnormality (%)] in STZ induced Diabetic male rats

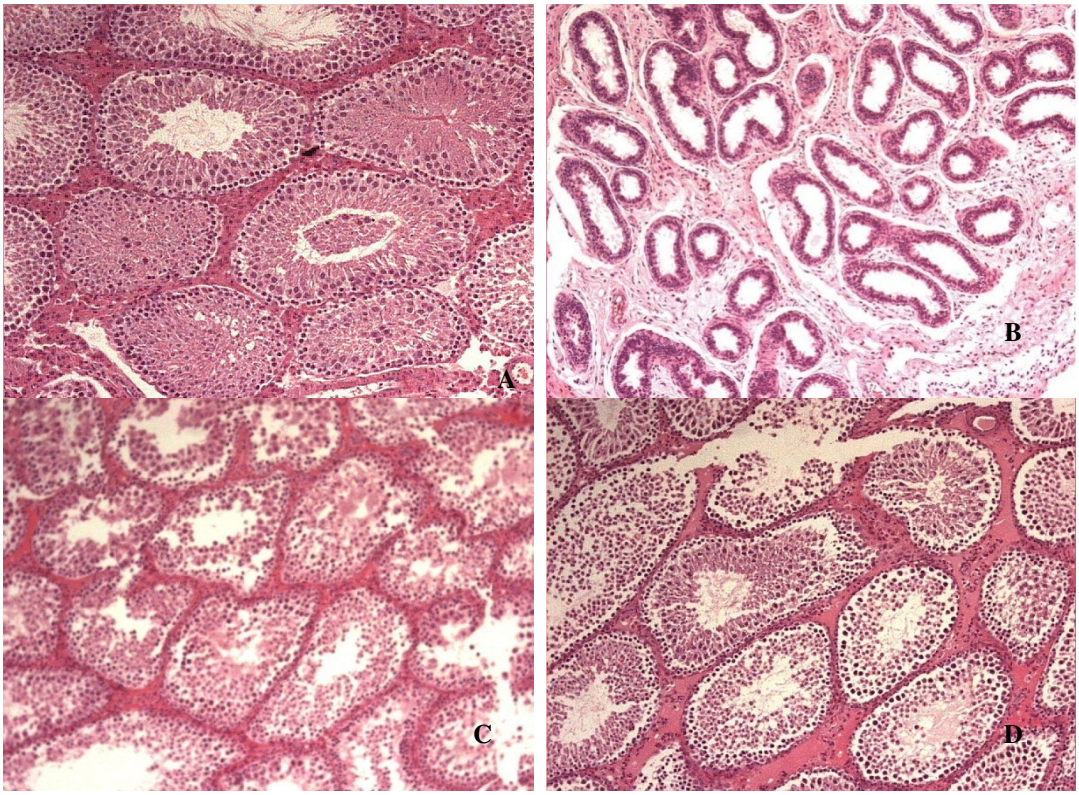

Figure-8
Rats testes after hematoxylin-eosin staining. Photomicrographs display representative images from control group (a), diabetic control group (b), *AER*-250 (c) *and AER*-500 (d).

Discussion: Diabetes mellitus markedly affect the sexual behavior and reproductive tract functions which can lead to reduced fertility[23]. The present study results showed that single intraperitoneal administration of STZ (55 mg/Kg) to normal healthy male rats, decreased the weight of testes, epididymis, and seminal vesicles, induced significant testicular degeneration, lowered semen quality and quantity, increased serum glucose and testicular TBARS levels, decreased serum testosterone and testicular GSH levels and created extensive histological changes in testes. These changes are indicative of morphologic disorders in spermatogenesis[24-25]. It is well known that diabetes causes the reduction of spermatogenic cells and decreases the tubules diameter by cell apoptosis and seminiferous tubules atrophy[26].

In this present research, oral administration of AER at doses of 250 and 500 mg/Kg for 8 weeks to male diabetic rats increased the weight of testes, epididymis and seminal vesicle, decreased serum glucose and testicular TBARS levels, increased serum testosterone and testicular GSH levels associated with the improved semen quality and quantity, and alleviate created extensive histological changes that seen in the testes of diabetic rats. These findings corroborate the research of Salem and Moustafa[27] and Hussein[28] who conclude that *Eruca sativa* may be capable in improving healthy sperm parameters and fertility. Diabetes increases the thickness of basal membrane in somniferous tubules which accompanies reduction of sperm production and total size of somniferous tubules[29]. That is compatible with last researches about *Eruca sativa* effects which showed increasing of spermiogenesis tubule's diameter[28].

On the other hand, in diabetes, reducing sugars level has been increased and easily react with lipids and proteins, thus the reactive oxygen species (ROS) production increases that gradually leads to the development of diabetic complications[30]. In the present research AER found to decrease serum glucose levels significantly.

In STZ-induced diabetes, ROS impaired Leydig cell function decreases testosterone level which results in altered seminiferous epithelium of diabetic animals[31]. Another probable mechanism in dysfunctioning of Leydig cells can be increased free radicals and oxidative stress that can prevent androgens production by Leydig cells[32]. Reduced level of testosterone hormone in diabetic rats and changes in structure and function of Sertoli cells can affect normal function of Leydig cells[33-34]. Testosterone secretion by Leydig cells relates to germinal cells health and their ability for mitotic divisions in seminiferous tubules. Disorder in testosterone biosynthesis in Leydig cells has harmful effects on male fertility. AER treatment significantly increases the testosterone level as compared with the diabetic group.

In testes of diabetic rats, significant increased TBARS levels and decreased GSH activity was observed. There are several ways through which ROS are increased in diabetes, i.e. glycation and decreased activity of enzymic and nonenzymic antioxidants[35]. Glutathione, a potent endogenous antioxidant is a first line of defense against free radicals. In diabetic rat's testes, GSH levels were found to be significantly lowered. These observations corroborate with the previous findings showing reduced GSH levels in diabetes[36-37]. In the testes, significantly decreased lipid peroxidation and re-generated intracellular GSH content were observed after AER treatment; may be because of antioxidant activity of AER.

Conclusion

In conclusion, oral administration of AER for 8 weeks, by reducing blood glucose level and oxidative stress has positive effects on morphology and hormone alterations in STZ-induced diabetic rats. Therefore, present study suggest that rocket leaves consumption as a salad may be beneficial for diabetic patients who have fertility problem, as their extracts produce anti-diabetic activity and exhibit fertility enhancing properties in male diabetic rats.

Acknowledgments

We are appreciative to Deanship of Scientific Research (DSR), Salman bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia for providing the funds to carry out this study under research grants No. 45/ص /33.

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