Coadministration of Ginger Roots Extract and Vitamin E Improves Male Fertility of Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Ginger is an important medicinal plant in folk medicine. Antioxidant, antidiabetic, and anti-inflammatory effects have been reported in ginger extracts. This study was performed to examine how ginger extract (GE) and vitamin E (Vit E) have affected the fertility in Streptozotocin-induced diabetic male rats. Sixty adult male rats were randomly assigned into six groups (n=10). Group 1 served as a negative control, while the other animals were intraperitoneally injected with Streptozotocin (50mg/kg) after fasting for 16 hours to induce diabetes. Group 2 was kept as a diabetic positive control. While groups 3 and 4 were orally given GE alone at 300 and 600mg/kg for 65 consecutive days to cover the whole spermatogenic cycle in rats, respectively. For the same period, groups 5 and 6 received GE (300 and 600mg/kg) plus Vit E (200mg/kg). Serum samples were obtained to estimate blood glucose, insulin, testosterone, FSH, and LH levels. Sex organs have been weighed, besides semen picture and histopathology of the testis were carried out. The results revealed that co-administration of GE and Vit. E increased the fertility index, weights of the testis and epididymis, serum testosterone level and improved semen quality in Streptozotocin-induced diabetic rats. Moreover, co-administration of GE and Vit. E caused hypoglycemia and hypoinsulinemia in administered rats compared to the diabetic positive control. Mild to moderate testicular degeneration and incomplete arrest of spermatogenesis were seen by histopathological examination of diabetic control group. Moreover, GE and Vit. E alleviated testicular lesions induced by diabetes, and improved spermatogenesis in treated groups. Conclusively, diabetic patients who are sexually impotent may benefit from a dietary supplement containing ginger roots and vitamin E.

Key words: Male fertility; Ginger; Testis; Testosterone; Sperms; Histopathology; Vitamin E; Glucose.

INTRODUCTION

Diabetes mellitus (DM) is a disease of inadequate control of blood glucose levels. DM, the most common endocrine disorder, is characterized by persistent hyperglycemia caused when the pancreas fails to secrete insulin (insulin deficiency) or resistance to insulin (insulin resistance), or both. Hyperglycemia occurs when cells are unable to utilize glucose and/or the liver and skeletal muscles are unable to store glycogen (Luis-Rodríguez et al. 2012). Hyperglycemia induces oxidative stress, by increasing the generation of reactive oxygen species (ROS) and reducing the activity of antioxidant enzymes (Lucchesi et al. 2013). Complications of diabetes, particularly diabetic nephropathy, are strongly influenced by oxidative stress (Wang et al. 2011). Inflammation and oxidative stress are usually linked

with diabetes mellitus which leads to multi-organ damages during the course of disease. It is well known that persistent hyperglycemia induces testicular lesions, which adversely affects the process of spermatogenesis and reduces male fertility in Streptozotocin-induced diabetic rat (Kanter et al. 2012).

Male factors account for 40–50% of reported infertility cases; consequently, it has become a severe public health issue (Isidori et al. 2006). Several factors can impair spermatogenesis and have a negative impact on sperm quality and quantity. Male infertility can be caused by hormonal imbalances, anatomical abnormalities, or exogenous medication exposure. In addition, there are several pathological conditions which have negative impacts on male fertility and sperm production, such as coronary heart disease, chronic liver disease and diabetes (Brezina et al. 2012).

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Ginger (*Zingiber officinale*) is one of the most common herbal plants which belong to the family *Zingiberaceae* and is used as a popular spice in various foods and beverages as well as for medicinal purposes (Mekuriya and Mekibib 2018). It was reported that ginger have anti-inflammatory (Mascolo et al. 1989), antidiabetic (Shanmugam et al. 2011) and antioxidant (Joshua and Catherine 2010; Abdel-Azeem et al. 2013), effects. These effects are attributed to gingerols and shogaols existing in ginger (Zahang et al. 2017; Bakr et al. 2020). Ginger roots ethanolic extract attenuated oxidative stress and gene expression (Sief et al. 2021) and improved sexual performance and fertility of quail birds (Al-Kashef 2021). Ginger is commonly used to treat nausea and vomiting, pain, many types of cancers (Romero et al. 2018; Majeed et al. 2021), osteoarthritis, diabetes mellitus and hyperlipidemia, in Streptozotocin – induced diabetes (Al-Amin et al. 2017), and stomach aches (Bodagh et al. 2019). However, Morakinyo et al. (2008) reported the protecting effect of ginger aqueous extract against infertility of male rats is due to its antioxidant and androgenic effects. Zahra and Abbas (2020) mentioned that ginger and Echinacea extracts improve the quality of frozen ram spermatozoa. However, Eid et al. (2017) found that ingredients of ginger roots could alleviate diabetic prostatic complications. Furthermore, Banihani (2018) attributed the possible mechanisms of improved male fertility by ginger extract due to its ability to increase testosterone production, reduce oxidative stress, enhance the activity of antioxidant enzymes, and normalize blood glucose levels. Ginger extract has potential to improve semen quality and quantity in lead acetate-treated male rats (Odo et al. 2020). The purpose of this study was to see how coadministration of GE and Vit. E could affect the male fertility in Streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Animals

Sixty adults (200-210g bwt, 3 months old) male Sprague-Dawley rats were used. Rats were obtained from Laboratory Animal Colony, Agricultural Research Center, Giza, Egypt. Animals were housed under hygienic condition at 24°C, 50% relative humidity and 12hr light/12hr dark cycles. Rats were fed freely on locally manufactured rat pellets, and water was available at all the times. The experiments were carried out according to rules of International Animal Care and Use Committee at Pharmacology department, Faculty of Veterinary Medicine, Cairo University (Vet. CU. IACUC, dated 16 March 2021).

Drugs and chemicals

Streptozotocin (STZ) (Sigma-Aldrich Chemical Company, St. Louis, MO, USA) is a monofunctional methyl nitrosourea that can induce diabetes mellitus type 1 through its toxic effects on pancreatic beta cells. The administered dose of STZ used in this study was 50mg/kg injected by intraperitoneal route daily for 7 days after fasting rats for 16 hours as previously mentioned by Lenzen (2008). STZ was supplied and before the use of STZ, it was freshly prepared by dissolving it in 0.01M sodium citrate buffer solution with a pH of 4.5. Vitamin E (Pharco Company for Pharmaceuticals, Alexandria Egypt)

has been provided in form of soft capsules containing 1000mg of Vit. E. Glucose and insulin radio-immunoassay kits (Gamma Trade Company, Egypt) have been provided for estimating blood glucose and insulin level, respectively.

Induction of diabetes mellitus

Male rats had been fasted for 16 hours then injected intraperitoneally by Streptozotocin in a dose of 50mg/kg (equal to a volume of 1mL/rat). To prevent STZ-induced hypoglycemia, the rats were given a 5% sucrose solution overnight. The blood glucose level was determined using a Bayer Contour meter (Japan). Blood glucose level of more than 250mg/dL is considered an indicator of diabetes mellitus incidence. Rats with blood glucose level of 250mg/dL or more were chosen for the study (Francis and Sudha 2016).

Preparation of ginger roots extract

Dried ginger roots were purchased in Cairo, Egypt, from a local market of Agricultural Herbs, Spices, and Medicinal Plants. Ginger roots were authenticated at Cairo University Botany Department, Faculty of Science Herbarium. The air-dried ginger roots were ground into a fine powder with a blender before being subjected to aqueous extraction. Five hundred grams (500g) of dried ginger roots were added to two liters (2000mL) of distilled hot but not boiling water and shaken intermittently for three days. After that, the content was filtered to obtain a 25% aqueous ginger extract.

Evaluation of the male fertility index

The effect of GE on male fertility index was evaluated using a serial mating procedure. One treated male rat was mated with three normal females with a regular estrous cycle. Each male's fertility index was determined by dividing the number of females which become pregnant on the number of mated females with treated male rats as previously mentioned by Amann (1982).

Experiment design and sampling

Sixty mature (200-210g bwt and 3 months old) male Sprague-Dawley rats were randomly allocated into six groups of ten rats each. Group 1 served as a negative control, while the other groups were diabetic for 7 days after receiving a daily intraperitoneal injection of Streptozotocin at a dose of 50mg/kg. Group 2 was kept diabetic positive control, while groups 3 and 4 were orally given GE at 300 and 600mg/kg for 65 consecutive days, respectively. Groups 5 and 6 were given the same doses of GE (300 and 600mg/kg) plus Vit. E @200mg/kg for the same period of time and by the same route. The dose of Vit. E used in this study for male rats was 200mg/kg as mentioned by ELaraby et al. (2019). To cover the spermatogenic cycle of male rats, oral coadministration of GE and Vit. E was continued for 65 days (Amann 1982). At the end of the experiment, rats were with an anesthetized with an intraperitoneal anesthetic dose of 50mg/kg pentobarbital sodium (Nesdonal® 10g vials) and blood samples were withdrawn by puncture of the orbital plexus's veins of the eye. Serum samples were obtained by centrifugation for 15min at 2200-2500rpm within one

hour of blood collection and the kept frozen at -20°C . Testis, epididymis, and seminal vesicles were removed and weighed in relation to the animal's body weight.

Examination of semen picture

The cuda epididymis was cut and seminal contents of the epididymis were squeezed in a clean dry glass watch containing 2% sodium citrate solution. The seminal contents were microscopically examined for progressive motility, sperm cell count, viability (alive/dead) ratio and epididymal sperm cells abnormalities (Bearden and Fluquary 1980).

Biochemical analysis

Serum samples were used for estimation of blood glucose, insulin and male sex hormones (Testosterone, FSH, and LH) levels. BioMeriueux kit (Catalog No. 61269; BioMeriueux, Marcy l'Etoile, France) was obtained for determination of blood of glucose level according to Beach and Turner (1958) and insulin was measured as adopted by Wu et al. (2014). Testosterone concentrations were estimated using radioimmunoassay (RIA) method (Chen et al. 1994). While the concentrations of LH and FSH were estimated using ELISA technique according to Ballester et al. (2004).

Histopathological examination

Testicular specimens were collected and fixed in 10% neutral buffered formalin before being dehydrated in increasing concentrations of ethyl alcohol (70-100%). Specimens were prepared using standard procedures of staining with H&E (Luna 1968).

Statistical analysis

The data were presented as $\text{mean} \pm \text{SD}$. The one-way analysis of variance (ANOVA) test was used for the statistical analysis, followed by the Tukey test for multiple comparisons. The significance of differences between experimental groups was tested at a probability level of $P < 0.05$.

RESULTS

Diabetes mellitus induced by Streptozotocin in male rats decreased the fertility index to 50% versus 90% in negative normal control rats. Oral coadministration of GE at 300 and 600mg/kg alone and combined with Vit. E at 200mg/kg for 65 consecutive days increased the fertilizing capacity of male diabetic rats to 60, 70, 80 and 80%, respectively. Male diabetic rats administered GE at doses of 300 and 600mg/kg combined with Vit. E (200mg/kg) for 65 consecutive days significantly ($P < 0.05$) increased weights of the testis and epididymis as depicted in Table 1. In addition, coadministration of GE at doses of 300 and 600mg/kg and Vit. E to male rats for 65 consecutive days significantly ($P < 0.05$) increased sperm cells count, progressive motility and viability (alive/dead) percent (Table 2). Sperm cells abnormalities in semen of male control positive diabetic rats were 12.5% versus to 3.9% of normal control rats. The sperm cell abnormalities of diabetic rats included detached head (8.6%), double head (1.0%), coiled head (1.2%) and bent tail (1.7%) as demonstrated in Fig. 1. Concurrent administration of rats

with GE at 300 and 600mg/kg and Vit. E decreased sperm cell abnormalities to 8.9 and 12.5% as compared to 12.5% of control positive diabetic rats.

Table 3 showed that rats which were given GE at 300 and 600mg/kg along with Vit E for 65 days in a row, had lower blood glucose and insulin levels (hypoinsulinemia) than positive control diabetic rats. Serum testosterone, FSH, and LH levels were significantly increased in male rats given GE and Vit. E orally alone and concurrently for consecutive 65 days.

Histopathological examination of the testis of control negative rats revealed normal histological architecture of the seminiferous tubules, as shown in Fig. 2a. While testis of diabetic positive control rats showed mild to moderate spermatogenic cell degeneration, diffuse edema of interstitial cells, and complete spermatogenesis arrest in the majority of seminiferous tubules (Fig. 2b). However,

Fig. 1: Showing sperm cell abnormalities of positive control diabetic rats: (a) Detached head, (b) Double head, (c) Coiled head and (d) Bent tail. (Eosin-Nigrosine stain, X 200).

Fig. 2: Histopathological examination of the testis of normal control rats showed normal histological architecture of the seminiferous tubules (S) as illustrated in a) the testis of diabetic positive control rats showed mild to moderate degeneration of spermatogenic cells, diffuse edema (E) of interstitial cells and complete arrest of spermatogenesis in most of seminiferous tubules (b). The testis of diabetic rats given orally GE at 300mg/kg concomitantly with Vit. E (200mg/kg) revealed partial arrest of process of spermatogenesis in some of seminiferous tubules and the other seminiferous tubules were normal (c). The diabetic rats co-administered orally GE at 600mg/kg plus Vit. E (200mg/kg) showed that most of seminiferous tubules were normal and full of sperms and only few of them had partial arrest of spermatogenesis process (d). H&E x200.

Table 1: Effect of oral administration of ginger extract (GE) alone and combined with Vit. E (200mg/kg) on weights of sexual organs of male diabetic rats (n=10)

Parameters Groups	Testes (g)	Epididymis (g)	Seminal Vesicles (g)
Negative control	0.82±0.016a	0.46±0.002a	0.57±0.01a
Diabetic control	0.56±0.014b	0.30±0.028c	0.26±0.03c
GE (300mg/kg)	0.66±0.015b	0.33±0.018b	0.45±0.07b
GE (600mg/kg)	0.68±0.001b	0.35±0.018b	0.46±0.04b
GE (300mg/kg)+Vit. E	0.72±0.025b	0.39±0.019b	0.48±0.04b
GE (600mg/kg)+Vit. E	0.79±0.016a	0.41±0.002a	0.49±0.01a

Mean±SD is a column bearing different letters differ significantly (P<0.05).

Table 2: Effect of oral administration ginger extract (GE) alone and combined with Vit. E on sperm cell characters of male diabetic rats (n=10)

Parameters Groups	Sperm cell count (10 ⁶ /mL)	Progressive Motility (%)	Viability (%)
Negative control	75.0±1.3a	92.6±1.6a	18.2±1.2a
Diabetic control	45.0±1.4d	57.0±3.9d	6.6±1.4d
GE (300mg/kg)	55.0±0.5c	65.0±2.0c	8.0±1.1c
GE (600mg/kg)	57.0±0.6c	68.0±2.1c	10.8±1.3c
GE (300mg/kg)+Vit. E	64.0±1.1b	72.0±0.8b	12.4±0.4b
GE (600mg/kg)+Vit. E	66.0±1.2b	75.6±1.9b	13.2±1.2b

Mean±SD with different letters in the same column differ significantly (P<0.05).

Table 3: Effect of oral administration ginger extract (GE) alone and combined with Vit. E (200mg/kg) on blood glucose (BG), insulin and sexual hormone levels of male diabetic rats (n=10)

Groups	BG (mg/dL)	Insulin (μU/mL)	Testosterone (ng/mL)	FSH (ng/mL)	LH (ng/mL)
Negative control	190±8.0d	10.95±0.15a	2.95±0.04a	2.80±0.03a	0.57±0.01a
Diabetic control	385±9.0a	12.89±0.13c	1.55±0.07d	1.72±0.05d	0.26±0.03d
GE (300mg/kg)	300±6.0b	3.62±0.24b	2.94±0.07c	1.80±0.04c	0.45±0.07b
GE (600mg/kg)	295±7.0b	3.63±0.12b	3.10±0.04c	1.86±0.05c	0.46±0.04b
GE (300mg/kg)+Vit. E	227±5.0c	2.75±0.14b	3.20±0.06b	2.10±0.03b	0.48±0.04b
GE (600mg/kg)+Vit. E	225±4.0c	2.70±0.11b	3.40±0.06b	2.50±0.02b	0.49±0.01b

Mean±SD with different letters in the same column differ significantly (P<0.05).

the testis of diabetic rats given orally GE at 300mg/kg concomitantly with Vit. E revealed partial arrest of spermatogenesis in some of seminiferous tubules while, the other seminiferous tubules were normal (Fig. 2c). The diabetic rats administered orally GE at 600mg/kg plus Vit. E showed that most of seminiferous tubules were normal and full of sperms and only few of them had partial arrest of spermatogenesis process (Fig. 2d).

DISCUSSION

This study was conducted to figure out the impact of coadministration of ginger extract (GE) and vitamin E (Vit. E) on the male fertility in Streptozotocin-induced diabetic rats. Streptozotocin was used in this study to induce diabetes mellitus in male rats as reported by Francis and Sudha (2016). Streptozotocin (STZ) is currently the most used diabetogenic agent in testing insulin and new antidiabetic drugs in animals (Quinna and Badwan 2015). The dose of STZ used in this study 50mg/kg was similar to that previously used by Lenzen (2008). According to the later author, STZ causes pancreatic cell damage, resulting in and hyperglycemia and hypoinsulinemia. The mechanisms by which STZ induced testicular toxicity may be due to induction of oxidative stress and reduction of the activity of antioxidants enzymes (Zha et al. 2018). Also, Streptozotocin-induced testicular damage may be attributed to oxidative damage, inflammation, and apoptosis (Zahang et al. 2017 and Nna 2019).

Vitamin E (alpha-tocopherol) is a potent antioxidant that regulates the body's oxidation processes. According

to Kurutas (2016), utilization of Vit. E can help to mitigate the harmful effects of oxidative stress caused by oxygen free radicals (ROS). It was reported that diabetes mellitus can cause deleterious effects on the testis and process of spermatogenesis in rats (Maresch et al. 2018). Furthermore, Khorramabadi et al. (2019) referred to the ability of Vitamins E and C to prevent oxidative damage in sperm DNA in the rat testis. ELaraby et al. (2019) reported that oral administration of Vit. E supplement at 500,1000 and 2000mg/kg in rats for 90 days was safe for the testis

The current study found that coadministration of GE and Vit. E to male diabetic rats increased the fertility index, testis and epididymis weights, serum testosterone levels, and semen quality and quantity. There was a decrease in blood glucose levels and an increase in insulin levels. These results agreed with those obtained by Morakino et al. (2008) and Sief et al. (2021) who concluded that GE improves fertility in diabetic male rats due to its powerful antioxidant and androgenic properties. According to Khaki et al. (2009) and Zahra and Abbas (2020), GE treatment dramatically enhanced sperm count, viability, motility, and serum total testosterone levels. These data suggested that ginger may help maintain healthy sperm parameters (Al-Kashef 2021). Recently, ginger extract had potential to improve semen quality and quantity in lead acetate-treated male rats (Banihani 2018; Odo et al. 2020). Moreover, Morakinyo et al. (2008) found that GE was significantly increase the relative weight of the rat testis as well as serum testosterone levels. The previous authors concluded that GE have an androgenic activity in rats. However, the improvement of

semen quality and parameters of biochemical analysis agreed with alleviations of histopathological lesions seen in the testis of rats co-administered with GE and Vit. E (Al-Kashef 2021).

According to the previous findings, it could be assumed that mechanism (s) by which GE alleviated testicular lesions in diabetic rats may be attributed to its antioxidant, androgenic, and hypoglycemic effects. Also, co-administration of GE and Vit. E assisted to decline the hyperglycemic effect existed by STZ-induced diabetes. The hypoglycemic effect of ginger roots that reported herein agreed which that was reported by Nammi et al. (2009) who concluded that in rats fed a high-fat diet for 6 weeks, the blood glucose, insulin, total cholesterol, LDL cholesterol, triglycerides levels were significantly reduced by *Zingiber officinale* treatment rats. The authors concluded that GE has hypoglycemic, hypoinsulinemic, and regulates diabetes and dyslipidemia. Moreover, ginger have a protective effect against abnormalities in diabetic rats due to its antioxidant properties (Al-Qulaly 2021).

Conclusion

Coadministration of GE and Vit. E increased the fertility index, weights of the testis and epididymis, serum testosterone levels and improved the process of spermatogenesis in rats. It decreased blood glucose and insulin levels and alleviated testicular lesions seen in diabetic rat testis. GE and Vit. E improve male fertility in diabetic rats. According to findings of the current study, utilization of ginger roots as a boiled drink and intake of Vit. E as a dietary supplement may be beneficial to improve the fertility in male diabetic patients who suffering from sexual impotency.

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Author's Contribution

MAS suggested the idea of the manuscript, planned the study and wrote the references. SRI performed the experimental part of this work. RAG examined the sperm cell characters and counted the sperms. All authors shared writing, drafting and approval of the manuscript.

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