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Effect of *Zingiber officinale* Roots and *Cinnamon zeylanicum* Bark on Fertility of Male Diabetic Rats

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Abstract: The effects of *Zingiber officinale* (ZO) roots and *Cinnamon zeylanicum* (CZ) bark extracts on fertility of male diabetic rats and blood glucose, insulin and testosterone levels were studied. The experiment was carried out on sixty male rats allocated into 6 equal groups of 10 animals each. One group was kept as a normal control, while rats of the other groups were given alloxan (120 $mg.kg^{-1}$ / day for 3 days) by intraperitoneal injection for induction of diabetes. Group (2) was left as a diabetic control, while rats of groups (3) and (4) were given ZO extract at 250 and 500 $mg.kg^{-1}$ b.wt., respectively, for 65 consecutive days to cover the period of spermatogenesis in the rat. Rats of groups (5) and (6) were given orally CZ extract at the same doses and for the same period. Results showed that oral administration of ZO and CZ extracts to diabetic rats for 65 days increased the weight of testes and seminal vesicles; improved semen quantity and quality; decreased blood glucose level, but increased serum insulin and testosterone levels. The large dose of both extracts ameliorated the degenerative lesions which seen in the testes of diabetic rats, This study recommend that intake of ZO roots and CZ bark as a drink may be beneficial for diabetic patients who suffer from sexual impotency as it improve fertility and lower the high blood glucose level in male diabetic rats.

Key words: *Zingiber officinale* · *Cinnamon zeylanicum* · Male fertility · Diabetes · Rat

INTRODUCTION

Infertility is one of the major health problems in life and approximately 30% of this problem is due to male factors [1]. Several factors can interfere with the process of spermatogenesis and reduce sperm quality and quantity. Some diseases such as coronary heart diseases; diabetes mellitus and chronic liver diseases have been reported to cause deleterious effects on spermatogenesis [2]. On the other hand, intake of antioxidants and vitamins A, B, C and E can increase stability of testicular blood barrier and protect sperm DNA from oxidative stress that caused by active free radicals [3].

Diabetes mellitus is a chronic metabolic disease which affects millions of people all over the world. It is characterized by hyperglycemia due to insulin deficiency or insulin resistance. Hyperglycemia occurs when the liver and skeletal muscles can not store glycogen and/or the cells become unable to utilize glucose. The prevalent treatment of diabetes mellitus besides controlling food intake; treating obesity; proper exercise and changing life style includes administration of oral hypoglycemic drugs and injection of insulin [4].

Several medicinal plants were examined for their hypoglycemic and antidiabetic activities and some of them have been selected to develop new drug sources to be safety used for treating diabetes mellitus [5]. Some of these plants decreased blood glucose level in experimental animals such as *Zizyphus spina christi* [6], Fenugreek [7], *Urtica dioica* [8] and *Balanites aegyptiaca* [9]. Other plants were reported to increase the level of insulin in man, rats and mice such as *Urtica dioica* [8], *Rhazya stricta* [10] and *Viscum album* [11].

Zingiber officinale (Family *Zingiberaceae*) roots are commonly used as a culinary spice and medicinally used for their antioxidant [12] androgenic [13] and hypoglycemic [14] activities which were reported in animal models. The active ingredients of *Zingiber officinale* roots such as zingerone, zingiber officinalediol, zingibrene and shogaols produced antioxidant activity [15]. Previous study showed that natural antioxidants can protect DNA and other molecules from cell damage which induced by oxidation and can improve sperm quality and increase reproductive efficiency of men [16]. *Zingiber officinale* was also found to possess a protective effect against DNA damage induced by H_2O_2 and enhanced sperm quality in rats [17].

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Cinnamon zeylanicum (Family *Lauraceae*) bark is commonly used in Arabian countries as a spice for most foods. In Eastern and Western folk medicine, it used for treating abdominal and chest pains, chronic diarrhea, hypertension, kidney disorders and rheumatism. Intake of 3g or 6g of *Cinnamon zeylanicum* bark reduced serum glucose level in people with type 2 diabetes [18]. *Cinnamon zeylanicum* extract was found to induce hepatoprotective and antioxidant effects in CCl4 - intoxicated rats [19].

The present study was designed to investigate the effects of alcoholic extract of *Zingiber officinale* roots and *Cinnamon zeylanicum* bark on male fertility as well as on blood glucose, insulin and testosterone levels in alloxan- diabetic rats.

MATERIALS AND METHODS

Materials

Plants: *Zingiber officinale* (Family *Zingiberaceae*) dried roots and *Cinnamon zeylanicum* (Family *Lauraceae*) dried bark were obtained from a local company of Agricultural seeds, Spices and Medicinal plants, Cairo, Egypt. Authentication of both plants was carried out by staff members of Botany Department, Faculty of Science, Cairo University, Egypt.

Animals: Sixty mature male rats of Sprague Dawley strain weighing 180 -190 g body weight and 14-16 weeks old were used in this study. Rats were obtained from the Laboratory Animal Colony, Helwan, Egypt. Animals were kept under hygienic conditions in plastic cages, fed on basal diet and water was provided *ad libitum*.

Alloxan: It was purchased from El-Gomhoryia Company for Chemicals; Cairo, Egypt in the form of white powder packed in tightly closed bottles each containing 25g alloxan monohydrate.

Biochemical Kits: Glucose enzymatic kits were purchased from BioMeriuex (France)for determination of serum glucose level. Radioimmunoassay kits for estimating of the levels of serum insulin and testosterone were obtained from Biodiagnostica (USA).

Preparation of Basal Diet: Basal diet was prepared [20] and consisted of 20% protein (casein), 10% sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5% salt mixture and 5% fibers (cellulose). The remainder was corn starch.

Preparation of Plant Extract: The method of plant extraction was described [21]. In this method, 500 grams from the dried powder of *Zingiber officinale* or *Cinnamon zeylanicum* were soaked in 1 liter of 90% ethyl alcohol overnight and extracted by percolation several times till complete exhaustion. The solvent was evaporated using Rotavapour apparatus (made in Russia) connected to a vacuum pump. The bath temperature was adjusted to 50C till semisolid extract was obtained. Known grams of the extract were dissolved in few drops of Tween 80 (suspending agent) and distilled water was added to prepare a 10% solution.

Experimental and Grouping of Rats: The rats were divided into 6 groups of 10 animals each. The 1 group was fed on the basal diet and served as a normal control, while the other five groups were given alloxan by intraperitoneal injection of a single daily dose of 120 $mg.kg^{-1}$ b.wt.for 3 days to induce moderate stable diabetes [22]. The 2nd group of rats was left as a diabetic control, while diabetic rats of the 3rd and 4th groups were given *Zingiber officinale* extract at 250 and 500 $mg.kg^{-1}$ b.wt., respectively, for 65 days to cover the period of spermatogenesis in the rat. The 5th and 6th groups of diabetic rats were given *Cinnamon zeylanicum* extract at the same doses and for the same period. At the end of experiment period (65 days), blood samples were taken for separation of serum which was used for estimating blood glucose and insulin and testosterone hormones levels. Rats were anaesthetized and epididymal contents were taken from the tail of epididymis for semen analysis. The rats were then sacrificed and the testes, seminal vesicle and prostate gland were dissected out and weighed. The testes were preserved in 10% neutral formalin solution till processed for histopathological examination.

Blood sampling and Biochemical Analysis: Blood samples were collected from the orbital plexus of veins by capillary microtubes and left to clot for separating the serum after centrifugation at 3000 rpm for 15 minutes. Serum samples were directly frozen at –10 C till biochemical analyses. Estimation of blood glucose was carried out using enzymatic glucose kits [23]. Serum insulin level was determined using radioimmunoassay method [24] and testosterone concentration was determined using gamma counter apparatus [25]. RIA method to measure either insulin or testosterone level in the serum of rats was formulized, tested and validated.

The procedure measured both hormones equally well, whether or not estimation of recovery and chromatographic preceded the RIA.The results were equivalent to those achieved by the method of competitive binding method.

Semen Analysis: Epididymal contents of the treated rats were obtained after cutting the tail of epididymis, squeezing it gently on clean slide and the sperm progressive motility and cell count were determined [26]. Microscopical examination of the seminal smears stained with Eosin Nigrosin stain were carried out to determine the percentages of sperm viability (ratio of alive/dead) and sperm cell abnormality [27].

Histopathological Examination: Testes of the treated rats were taken and fixed in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H &E) then examined microscopically [28].

Statistical Analysis: Data were expressed as means±S.E. and statistical analysis was carried using computerized SPSS program Version 12). Significance was performed using the least significant difference and paired Student "t" test [29].

RESULTS

Intraperitoneal injection of alloxan (120 mg.kg^{-1} b.wt.) to rats caused significant ($P < 0.01$) decreases in the weight of testes, seminal vesicle and prostate glands. Oral administration of *Zingiber officinale* extract in doses of 250 and 500 mg.kg^{-1} b.wt. to diabetic rats for 65 days significantly increased the weight of testes and seminal vesicle but did not affect the weight of prostate glands as compared to the diabetic control group. *Cinnamon zeylanicum* extract when given to diabetic rats by the same doses and for the same period significantly increased the weight of testes, but did not significantly affect the weight of seminal vesicles and prostate glands as recoded in table 1.

Concerning semen picture, it was found that alloxan decreased the sperm progressive motility, sperm count and viability, but increased the percentage of sperm cell abnormality in diabetic rats as depicted in Table 2. The most frequently seen sperm cell abnormalities in the examined seminal smears of diabetic rats were detached head and coiled tails as demonstrated in fig. 1.

Table 1: Effect of oral administration of extracts of *Zingiber officinale* and *Cinnamon zeylanicum* for 65 days on the weight of sexual organs of male diabetic rats

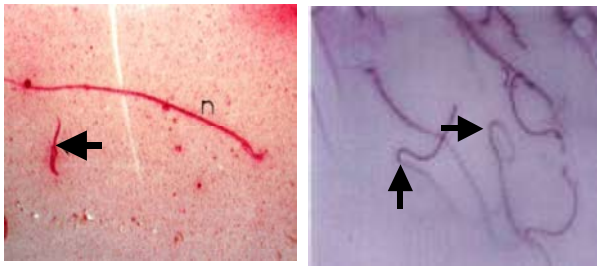
	Mean±SE of sexual organs weight (g)		
Groups and Treatment	Testes	Seminal vesicles	Prostate glands
Normal control Distilled water (1ml)	2.80±0.22	1.75±0.15	0.66±0.01
Diabetic control Alloxan (120 mg/kg)	1.65**±0.12	1.00**±0.14	0.23**±0.01
Zingiber officinale ext. (250 mg/kg)	2.15**±0.33	1.51*±0.16	0.35±0.03
Zingiber officinale ext. (500 mg/kg)	2.40**±0.26	1.65*±0.18	0.39±0.02
Cinnamon zeylanicum ext. (250 mg/kg)	2.25**±0.13	0.87±0.15	0.22±0.04
Cinnamon zeylanicum ext. (500 mg/kg)	2.10**±0.22	1.12±0.12	0.33±0.01

All treated groups were compared to the diabetic control group using Student 't' test
n= 10 rats * Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table 2: Effect of oral administration of extracts of *Zingiber officinale* and *Cinnamon zeylanicum* for 65 days on Semen picture of male diabetic rats

	Sperm cell characteristics (Mean±S.E.)			
Groups and Treatments	Motility (%)	Count (10^6/epididymis)	Viability (%)	Abnormality (%)
Normal control Distilled water (1ml)	90.00±1.0	77.67± 0.48	89.0±0.12	3.67±0.18
Diabetic control Alloxan (120 mg/kg)	50.00***±1.03	55.00***±0.33	60.0***±0.16	8.25±0.08
Zingiber officinale ext. (250 mg/kg)	64.00***±2.30	64.67***±0.43	65.0***±0.10	4.33± 0.18
Zingiber officinale ext. (500 mg/kg)	85.0***± 0.22	72.67***±0.28	75.0***±0.13	3.76± 0.18
Cinnamon zeylanicum ext. (250 mg/kg)	60.00***±0.12	58.00***±0.35	66.0***±0.15	5.43± 0.18
Cinnamon zeylanicum ext. (500 mg/kg)	65.00***±0.20	59.00***±0.30	72.0***±0.13	4.24± 0.24

All treated groups were compared to the diabetic control group using Student 't' test
n= 10 rats *** Significant at $P < 0.001$



(A) (B)

Fig. 1: Seminal smear obtained from a diabetic control rat given alloxan showing detached head (A) and coiled tails (B). (Eosin Nigrosin stain X 60).

Table 3: Effect of oral administration of extracts of *Zingiber officinale* and *Cinnamon zeylanicum* for 65 days on blood glucose and insulin hormone levels in male diabetic rats

Groups and Treatments	Glucose level (mg/dL)	Insulin level (µU/ml)
Normal control Distilled water (1ml)	95.80±3.5	6.40±1.5
Diabetic control Alloxan (120 mg/kg)	225.50**±4.6	3.66**±1.2
Zingiber officinale ext. (250 mg/kg)	212.97**±5.9	4.90*±1.4
Zingiber officinale ext. (500 mg/kg)	195.90**±6.2	5.10**±1.2
Cinnamon zeylanicum ext. (250 mg/kg)	216.22*±7.3	4.00±1.4
Cinnamon zeylanicum ext. (500 mg/kg)	218.50 *±8.5	4.10±1.3

All treated groups were compared to the diabetic control group using Student 't' test

n= 10 rats * Significant at P < 0. 05 ** Significant at P < 0. 01

Table 4: Effect of oral administration of extracts of *Zingiber officinale* and *Cinnamon zeylanicum* for 65 days on serum testosterone hormone in male diabetic rats

Groups and Treatments	Testosterone level (ng/dL)
Normal control Distilled water (1ml)	7.73±0.1400
Diabetic control Alloxan (120 mg/kg)	3.30**±0.13
Zingiber officinale ext. (250 mg/kg)	5.18**±0.11
Zingiber officinale ext. (500 mg/kg)	6.46**±0.14
Cinnamon zeylanicum ext. (250 mg/kg)	5.46**±0.13
Cinnamon zeylanicum ext. (500 mg/kg)	5.64**±0.15

All treated groups were compared to the diabetic control group using Student 't' test

n= 10 rats ** Significant at P < 0. 01

Oral administration of *Zingiber officinale* or *Cinnamon zeylanicum* extract (250 and 500 mg.kg^{-1} b.wt.) for 65 days to diabetic rats induced significant (P < 0.001) increases the sperm progressive motility, sperm count and viability as well as decreases in the percentage of sperm cell abnormality.

As shown in table 3, glucose and insulin hormone levels in serum of diabetic rats were 225.5±4.6 mg/dL and 3.66±1.2 µU/ml versus 95.8±3.5 mg/dL and 6.40±1.5 µU/ml of normal rats, respectively. Oral administration of *Zingiber officinale* extract (250 and 500 mg.kg^{-1} b.wt.) for 65 days to diabetic rats significantly (P < 0. 01) decreased

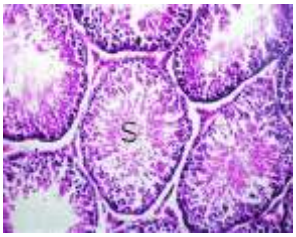


Fig. 2

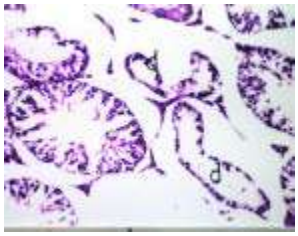


Fig. 3

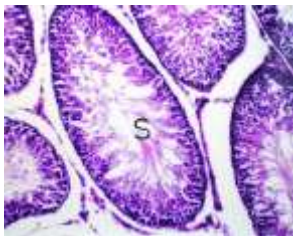


Fig. 4

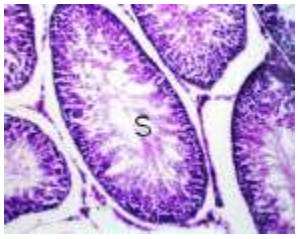


Fig. 5

Fig. 6

Fig. 7

Fig. 2: Testis of a control rat showing normal histological structure of active mature functioning seminiferous tubules(S) associated with complete spermatogenic series.

Fig. 3: Testis of a diabetic rat showing marked degeneration (d) of most seminiferous tubules with absence of spermatogenic series in tubular lumen.

Fig. 4: Testis of a rat given of *Zingiber officinale* extract at 250 mg.kg ^{1} b.wt. for 65 days showing normal histological structure of most seminiferous tubules.

Fig. 5: Testis of a rat given *Zingiber officinale* extract at 500 mg.kg ^{1} b.wt. for 65 days showing normal histological structure of most seminiferous tubules.

Fig. 6: Testis of a rat given *Cinnamon zeylanicum* extract at 250 mg.kg ^{1} b.wt. for 65 days showing mild degeneration of most seminiferous tubules with normal spermatogenic series.

Fig. 7: Testis of a rat given *Cinnamon zeylanicum* extract at 500 mg.kg ^{1} b.wt. for 65 days showing normal histological structure of most seminiferous tubules. (H&EX 40)

serum glucose concentration and increased insulin level as compared to the diabetic control group. *Cinnamon zeylanicum* extract when given to diabetic rats at both doses and for the same period significantly ($P < 0.05$) reduced serum glucose level, but did not affect the level of insulin hormone as compared to the diabetic control group.

Data in table 4 show that intraperitoneal administration of alloxan (120 mg.kg^{-1} b.wt.) to normal rats induced a significant ($P < 0.01$) decrease in serum testosterone hormone to 3.3±0.13 ng/dL versus to 7.7±0.14 ng/dL in the normal control rats. Oral administration of *Zingiber officinale* or *Cinnamon zeylanicum* extract at 250 and 500 mg.kg^{-1} b.wt. for 65 days to diabetic rats caused significant ($P < 0.01$) increases in serum testosterone levels as compared to the diabetic control rats.

Histopathological examination of the testes of normal rats showed normal histological structure of active mature functioning seminiferous tubules associated with complete spermatogenic series as demonstrated in fig. 2. The testes of alloxan –diabetic rats revealed marked degeneration of most seminiferous tubules with absence of spermatogenic series in tubular lumen as shown in fig.3. Microscopic examination of the testes of rats given *Zingiber officinale* extract (250 and 500 mg.kg^{-1} b.wt.) revealed normal histological structure of most seminiferous tubules with normal spermatogenic series as illustrated in fig 4 and fig.5. The testes of rats given orally *Cinnamon zeylanicum* extract at 250 mg.kg^{-1} b.wt. showed mild degeneration of most seminiferous tubules associated with normal spermatogenic series as demonstrated in fig 6. Examination of the testes of rats given *Cinnamon zeylanicum* extract at 500 mg.kg^{-1} b.wt. revealed normal histological structure of most seminiferous tubules with normal spermatogenic series as demonstrated in fig. 7.

DISCUSSION

The obtained results showed that intraperitoneal administration of alloxan (120 mg.kg^{-1} b.wt./day) to normal male rats for 3 days decreased the weight of testes, seminal vesicle and prostate glands, induced marked testicular degeneration, lowered semen quality and quantity, increased blood glucose and decreased serum insulin and testosterone levels. It is well known that diabetes is positively associated with lowered male fertility and sexual dysfunction [2]. Recently, Sandra *et al*. [30] concluded that the neuropathy and vascular insufficiency which caused by diabetes may be related to sexual dysfunction.

Concerning *Zingiber officinale* extract, the obtained results showed that its oral administration at 250 and 500 mg.kg^{-1} b.wt. for 65 days to male diabetic rats increased the weight of testes and seminal vesicle, decreased blood glucose and increased serum insulin and testosterone levels associated with an improvement of sperm motility and quantity as well as an alleviation testicular degenerative changes that seen in the testis of diabetic rats. These findings agree with those reported by Khaki *et al* [17] and Morakino *et al*. [31] who concluded that *Zingiber officinale* may be promising in enhancing healthy sperm parameters. The authors attributed the improvement of reproductive functions of male rats by *zingiber officinale* to its antioxidant and androgenic activities. The alleviation of testicular lesions seen in diabetic rats after oral administration of *Zingiber officinale* extract that reported in this study may be explained by the previously reported antioxidant and androgenic effects of *Zingiber officinale* and/or by its antidiabetic activity. The reported antidiabetic activity of *Zingiber officinale* that evident by hypoglycemia and hyperinsulinemia of *Zingiber officinale*, in this study, was similar to that obtained by Akhani *et al.* [32]; Kadnur and Goyal [33]**;** Ojewole [34] and Nammi *et al*. [35]. These authors concluded that the ethanol extract of *Zingiber officinale* roots produces an antidiabetic effect and increases insulin secretion in streptozotocin-induced diabetic rats. The improvement of male reproductive function by *Zingiber officinale* reported in this study could be explained by its direct cytoprotective activity on testicular tissue that evident by amelioration of testicular lesions or indirectly via increasing serum testosterone or by lowering glucose level and increasing insulin level in the serum.

With regard to *Cinnamon zeylanicum* extract, the present data revealed that its oral administration at the large dose (500 mg.kg^{-1} b.wt.) decreased blood glucose level associated with increases in serum insulin and testosterone levels and improvement of sperm motility and quantity as well as alleviation of testicular degenerative changes which seen in the testes of diabetic rats. These findings are partially similar to those reported by Khan *et al*. [18] who concluded that intake of 3 or 6 gram of *Cinnamon zeylanicum* reduced the fasting serum glucose in people with type 2diabetes. The hypoglycemic effect *Cinnamon zeylanicum* extract that reported herein

may be due to its hyperinsulinemia that evident in this study. The improvement in fertility parameters that caused by large dose of *Cinnamon zeylanicum* extract could be attributed to its previously reported antioxidant activity [19]. The authors concluded that *Cinnamon zeylanicum* extracts have an antioxidant effect in CCL4 - intoxicated rats. Yang *et al*. [16] concluded that the natural antioxidants can protect DNA and other molecules from cell damage induced by oxidation and can improve sperm quality and increase reproductive efficiency of men. Moreover, Jedlinska *et al*. [3] reported that intake of antioxidants and vitamins A, B, C and E can increase stability of testicular blood barrier and protect sperm DNA from oxidative stress caused by active free radicals. In addition, the enhancement of fertility properties which produced by *Cinnamon zeylanicum* extract could be explained by its direct effect on the testes causing an increase in testosterone secretion which reported in this study.

In conclusion, oral administration *Zingiber officinale* extract at 250 and 500 $mg.kg^{-1}$ b.wt. and *Cinnamon zeylanicum* extract at 500 $mg.kg^{-1}$ b.wt. to diabetic male rats for 65 days increase the weight of testes and seminal vesicles, improve semen quality and quantity; reduce blood glucose level and increase serum insulin and testosterone levels. It also alleviates the degenerative lesions which seen in the testes of diabetic rats. Therefore, this study recommends that intake of *Zingiber officinale* roots and *Cinnamon zeylanicum* bark as a drink may be useful for diabetic patients who suffer from sexual impotency as their extracts produce antidiabetic activity and exhibit fertility enhancing properties in male diabetic rats.

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