Do phytoestrogens affect reproductive performance in male rats?

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Abstract

Daidzein and genistein are important soy-borne phytoestrogens that have been implicated in human health. In this study, independent effects of diadzein and genistein were tested on fertility output in male rats. Adult male Wistar rats (90 days old) were subjected to weekly injections of 2, 20, 100 mg/kg BW daidzein or genistein dissolved in DMSO for 8 weeks. Following treatment, control and experimental males were cohabited with sexually mature normal females for 5 days. Female rats were sacrificed and autopsy was performed on day 18 of pregnancy when uterus and ovaries were examined for the numbers of corpora lutea, implantations, dead and live fetuses and resorptions. No significant changes ($p > 0.05$) were observed in the body weight gain of experimental males. Similarly, no changes ($p > 0.05$) were observed in the mating and fertility index. In contrast, significant decrease ($p < 0.05$) was observed in fertility rate, measured by counting numbers of live fetuses in the uterus of females mated with males exposed to either daidzein or genistein. Females mated to daidzein exposed males showed resorptions and a significant decrease ($p < 0.05$) in number of live fetuses per rat. These data demonstrate that significant decrease in fertilization capacity of adult male rats exposed to either daidzein or genistein.

Keywords: Daidzein, Genistein, Fertility, Implantation loss, Rat.

1. Introduction

The phytoestrogens are a group of naturally occurring compounds that are present in plants (1) and in adult mammals they may have protective effects against certain forms of cancer, cardiovascular disease, and osteoporosis and may also prevent undesirable menopausal symptoms (2). Dietary exposure to phytoestrogens is common for both animals and humans. Exposures occur through regular dietary intake or through nutritional supplements of phytoestrogens. The most widely available phytoestrogens are genistein and daidzein, which are abundant in soybeans and other plant products. The estrogenic action of phytoestrogens is based on their ability to activate estrogen receptor (ER) α and β (3,4). In addition, phytoestrogens have been shown to cause both direct and indirect adverse effects on the reproductive tract of animals. These effects include persistent vaginal cornification, hemorrhagic ovarian follicles, premature vaginal opening and compromised fertility in females (5,6).

Abnormalities in reproductive health due to high intake of soy products have also been reported in sheep (7), cows (8), cheetahs (9) and women (10,11). These observations demonstrate that dietary phytoestrogens can have adverse effects on reproductive functions in adult females.

The issue of the effect of low doses of phytoestrogens on male reproduction is controversial. Previous studies reported that administration of phytoestrogens do not cause significant changes in reproductive parameters of rats (12-15), while other studies have shown that low doses of daidzein/genistein caused reproductive toxicity (16-18). The present experiment was carried out to contribute to the discussion of this controversy, so in the present study the rats were administered with different doses of either genistein or daidzein and assessed for fertility output after 60 days.

2. Material and methods

2.1. Chemicals and reagents

Daidzein (>98% purity by HPLC) and Genistein (>98% purity by HPLC) were purchased from Chengdu Biopurify Phytochemicals Ltd. China. All

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other chemicals used in study were of analytical grade and obtained from Sri Sai Ram (SSR) Scientifics, Tirupati, India.

2.2. Animals and treatment

Wistar strain rats (male and female) were purchased from an authorized vendor (M/S Raghavendra Enterprises, Bengaluru, India) and used in the experiments. Each four animals were housed in plastic box cages (18 X 10 X 8 inches) under controlled environmental conditions (12-h light period starting at 6.00 h, temperature 23 ±2°C). Laboratory chow (purchased from Saidurga feeds and foods, Bengaluru, India) and tap water were given *ad libitum*. Care was taken to examine the animals for general physiological symptoms. The study was performed according to ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments in Animals, Government of India (CPCSEA, 2011), reviewed and approved by the Institutional Animal Ethical Committee at S. V. University, Tirupati, India.

Young adult rats (90 days old with body weight of 250±10 g) were randomly divided into 8 groups, each consisting of six animals. Corresponding controls were maintained for each chemical treatment (daidzein/genistein). Rats in the first and second groups served as control for diadzein and genistein respectively and were subjected to weekly injections of 50 µL of DMSO for 8 weeks. Animals in 3rd, 4th and 5th groups were injected weekly with doses of 2, 20, 100 mg/kg BW daidzein and rats in 6th, 7th and 8th groups received 2, 20, 100 mg/kg BW genistein for 8 weeks. Phytoestrogens were dissolved in DMSO and administered as intraperitoneal injections in 50 µL volume. The dose selection was done based on previous studies (19-21). From the first day of treatment period rats were observed daily for clinical signs of toxicity (salivation, rhinorrhoea, lachrymation, ptosis, squinted eyes, convulsions, stupor, tremors), postural changes (erection of fur, exophthalmia) and nonsexual behavior (such as cleaning of face, self grooming, climbing in the cage). The body weight of the animals was recorded on the day of initiation of the treatment and also on the day of cohabitation.

2.3. Reproductive performance and fertility output of rats

Control and experimental males were mated with sexually mature normal females presenting at least three regular cycles confirmed by the analysis of daily vaginal smears. Females in the pro-estrus stage in the morning were cohabited with male rats for 5 days. Successful mating was confirmed by the presence of copulatory plugs/presence of spermatozoa in the vaginal smear in the following morning (08:00-08:30 h). The day on which evidence of copulation identified, was termed day zero of gestation (GD 0). Autopsy was performed on day 18 of pregnancy and when uterus and ovaries were examined for the number of corpora lutea, implantation sites, live and dead fetuses and embryo resorptions. The mating index (%) [(number of sperm positive females/ number of pairing) x 100], fertility index (%) [(number of pregnant females/number of sperm positive females) x 100], pre-implantation loss (%) [(number of corpora lutea - number of implantations)/number of corpora lutea x 100] and post-implantation loss (%) [(number of implantations - numberof live fetuses)/number of implantations x 100] were then determined.

2.4. Statistical analysis

Data are represented as mean ± S.D. The statistical difference between the control and experimental groups was determined by using One-way Analysis of Variance (ANOVA) followed by Dunnet's test. Difference between the mean were considered to be significant when 'p' value is less than 0.05 was achieved.

3. Results

The rats were observed for responses with respect to overall appearance, body position, activity, co-ordination or gait, and behavior. No significant changes in lacrination, urination, respiration, vocalization, postural or gait abnormalities were observed in any of the control and treated rats. All the animals were apparently normal and no unusual behaviors (viz., head flicking, head searching, biting, licking, self-mutilation, circling, and walking back-wards) were observed in any of the rats. Significant differences in the mean body weight gain were not observed in either of the controls and treated groups (authors' unpublished data).

No treatment related mortality was observed in the rats during the study and none of the rats were excluded from experimentation. Rats from controls and experimental groups exhibited signs of sexual motivation as soon as the female was introduced, including chasing the female, noising, anogenital sniffing, genital grooming, passing under each other's body and attempted clasping and mounts. Though visual observations on male sexual behavior are not quantified, they made numerous attempts to mount the female.

The reproductive performance of rats, were determined by considering the end points such as

mating index, fertility index, number of implantations per rat and pre- and post-implantation losses (Table 1 and 2). Significant changes were not observed in the mating index and fertility index of rats of either control or experimental group and no significant differences were also observed in the mean number of corpora lutea in rats cohabited with either controls or treated males. The mean number of implantations in females mated with 100 mg daidzein or genistein exposed male rats was decreased significantly. The pre- and post-implantation losses were increased in females mated to daidzein or genistein treated males. A significant (p<0.05) reduction in the number of live pups per rat was observed in females mated with experimental males. Several resorptions were observed in rats mated with daidzein exposed males.

Table 1. Fertility output of male rats exposed to daidzein

Parameters	Control	2 mg	20 mg	100 mg
Mating index (%)	100 (6/6)	100 (6/6)	100 (6/6)	100 (6/6)
Fertility index (%)	100 (6/6)	100 (6/6)	100 (6/6)	100 (6/6)
No. of Corpora lutea/rat	13.01 ± 1.26	12.84 ±1.26 (-1.30)	13.51 ±1.32 (3.84)	13.77 ±1.32 (5.84)
No. of Implantations/rat	11.67±1.12	10.84 ±0.99 (-7.11)	10.51 ±1.01 (-9.94)	9.67*±0.71 (-17.13)
Pre-implantation loss (%)	10.29	15.57	22.20	29.77
No. of Live fetuses/rat	10.84±1.01	9.68 ±0.92 (-10.70)	9.01*±0.89 (-16.88)	8.34*±0.81 (-23.06)
Post-implantation loss (%)	7.11	10.70	14.27	13.75
Number of Resorptions	0	1	3	5

Values are mean ± S.D of 6 individuals.
Values in the parentheses are percent change from that of control.
Values are significantly different from control at *p< 0.05.

Table 2. Fertility output of male rats exposed to genistein

Parameters	Control	2 mg	20 mg	100 mg
Mating index (%)	100 (6/6)	100 (6/6)	100 (6/6)	100 (6/6)
Fertility index (%)	100 (6/6)	100 (6/6)	100 (6/6)	100 (6/6)
No. of corpora lutea/rat	12.01 ± 1.21	12.66 ± 1.12 (5.41)	12.83 ± 1.15 (6.82)	13.17 ± 1.25 (9.65)
No. of implantations/rat	11.17 ± 1.07	11.66 ± 1.12 (4.38)	10.33 ± 1.03 (-7.52)	9.33* ± 0.89 (-16.47)
Pre-implantation loss (%)	6.99	7.89	19.48	29.15
No. of live fetuses/rat	10.33 ± 1.03	10.66 ± 1.02 (3.19)	9.01 ± 0.83 (-12.77)	8.01* ± 0.76 (-22.45)
Post-implantation loss (%)	7.52	8.57	12.77	14.14
Number of Resorptions	0	0	0	0

Values are mean ± S.D of 6 individuals.
Values in the parentheses are percent change from that of control.
Values are significantly different from control at *p< 0.05.

4. Discussion

Successful fertility depends on various factors including sperm quality and quantity, hormones, antioxidative status. Bioavailability of testosterone is not only important for the maintenance of structural integrity of testis and accessory sex organs and maintenance of spermatogenesis (22) but also essential for expression of secondary sex characters (23). Significant decrease in circulatory testosterone levels were observed in rats after exposure to either daidzein or genistein (19, 24-25).

Further, administration of daidzein and genistein resulted in decreased spermatogenesis, decrease in daily sperm production, epididymal spermatozoon concentration and sperm quality (26-28). Decrease in sperm production associated with deteriorated sperm quality observed in rats exposed to diadzen and genestien (authors' unpublished data) may be responsible for fertilization dysfunction. Whether the reduced fertility observed in rats exposed to both daidzein and genistein resulted from low sperm numbers, decreased motile and viable spermor depressed sexual desire cannot be determined from the present data. The presence of copulatory plugs in all the females may indicate that sexual desire is not depressed in males.

Male rats exposed to phytoestrogens were able to impregnate the unexposed female and the number of live pups/rat was observed to be comparatively in lower number as compared to control male rat. This is well supported by the observations of Mestrich (29) who reported that infertility occurs when the sperm count falls significantly below normal. We have also reported earlier that the fertilization capacity of male also depends on the sperm quality, such as motility, viability and morphology (30). Females mated with control males had higher implantation. The foetal loss may occur both before and after implantation. The pre-implantation loss (29%) induced by diadzen or genistein treatment of males is due to a toxic effect on spermatids and spermatogonia, whereas no significant post - implantation loss was observed in females mated with experimental males. The cause of this manifestation is probably due to fertilization of oocytes with damaged spermatozoa.

Finally, the current study presents suppressed fertility output in male rats exposed to either daidzein or genistein, at levels of exposure that mimic those found in human food. Whether male human beings may be susceptible to such effects reminds unknown. Only individual effects of phytoestrogens were considered herein. The biological actions of one chemical may be influenced by the presence of another. It may happen that several congeners of these compounds are produced by the same genus. Thus, there is an urgent need to study the effect of phytoestrogen mixture for determining the associated risk to male reproductive health. As the consumption of soy food is popularized in recent years due to its nutritive value, it is important to conduct further in depth and more comprehensive studies on the molecular mechanisms underlying the undesirable effects of isoflavones on reproduction particularly in men.

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