PHYTOESTROGEN EFFECTS ON THE REPRODUCTIVE SYSTEMS OF JUVENILE MALE SPRAGUE-DAWLEY RATS.

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ABSTRACT

Phytoestrogens, bisphenol A, and exogenous 17β-estradiol have all been implicated as endocrine disrupting chemicals. In this study, juvenile male Sprague-Dawley rats (PND 22) were fed with phytoestrogens (soya extract), bisphenol A, and 17β-estradiol separately by oral gavage to determine the potential effect on the morphology of their reproductive organs and their hormonal levels. After three weeks of treatment (PND 43), all animals were sacrificed and the blood and testes collected. All the three treatment groups showed histological differences in testes morphology compared to the control. Soya extract and bisphenol A treated groups showed a decrease in circulating estradiol levels while the 17β-estradiol treated group showed elevated circulating levels of estradiol. Only the soya extract treated group showed elevated levels of circulating testosterone. The results of the present study showed that, phytoestrogens, bisphenol A, and 17β-estradiol can alter the histological structure of the testes and influence circulating steroidal hormone levels.

INTRODUCTION

Phytoestrogen are naturally occurring compounds found in soya bean and the similarity of it structure to endogenous oestrogen make phytoestrogen exert hormonal or anti-hormonal effects toward hormone-dependent disease or as a dietary alternative to hormone replacement therapy. Its plays important role in numerous aspect of human health in preventing disease such as breast cancer (Parkin, 1989; Muekies et al., 2000), prostate cancer (Adlercreutz, 1995), other types of cancer such as gastric, colon, endometrial (Adlercreutz, 2002), cardiovascular disease (Nagata et al., 1998; de Klein et al., 2002; Vanharanta et al., 1999) and osteoporosis (Nelson et al., 2002; Messina 2000).

Although phytoestrogen is significantly important in treatment cancer but it also shows reproductive toxicity effect on the development of reproductive organ as well as estrogenic properties to animal reproductive system. Studies have shown estradiol benzoate (Hossaini et al, 2003), 17β-estradiol (Kato et al, 2005) causes decrease in testis weight, sperm production, generation of the semiferous tubules (Hossaini et al, 2003) and decrease in reproductive capability (Nagao et al, 1999) in rats.

Soya bean products are a daily part of a typical Asian diet and it extract is rich in phytoestrogens. It has been highlighted that the average daily consumption of phytoestrogens in most Asian countries is between 25 mg to 50 mg a day (Becker et al., 2005). Since soya bean is commonly used throughout the world as one of the main daily diet it is therefore the objective of the project to investigate the effect of phytoestrogen to the reproductive system especially the gonads as well as the hormonal system, mainly testosterone and estradiol of animal that consume plants or food that contains high level of phytoestrogen.

PROJECT OBJECTIVES

1. To determine the effect of the phytoestrogen on the sex hormones in the juvenile male rats.

2. To evaluate the potential of phytoestrogen in stimulating sex hormones in the rats.

MATERIALS AND METHODS

Two kg of soya beans (*Glycine max*) were obtained from the local market, authenticated, and ground to powder. The soya powder was then oven dried for 3 days at 55^0C to eliminate any moisture and extracted with HPLC grade methanol. The freeze dried extract was analyzed by Liquid Chromatography Mass Spectrometry (LCMS) according to the procedure by Seelan (2005).

Thirty juvenile male Sprague-Dawley rats were collected on post natal day (PND) 21 from the animal breeding centre of the Faculty of Medicine, University of Malaya. The animals were divided into 5 groups, with each group containing six animals. The groups were (i) control 1 (Tween 80), (ii) control 2 (corn oil), (iii) soya extract, (iv) bisphenol A, and (v) 17β-estradiol. Animals were maintained on rat feed supplied by Gold Coin Feedmills Pte. Ltd. Malaysia, and given distilled water *ad libitum*. The animals were kept in an animal holding room with 12 hours photoperiod (0700 – 1900 hours), and a temperature of 26 - 29°C with a relative humidity of 40 - 50%. Treatment commenced on PND 22 after allowing the animals to adjust to their new environment.

All the compounds were administered by oral gavage daily from PND 22 up to PND 43. The dosage given was 100 mg/kg bw, which was calculated using a 50 mg/ml stock for all the compounds. Treatment was carried out between 8.00 am to 10.00 pm everyday till sacrifice. Body weight was recorded every 3 days.

All 30 rats were sacrificed between 8.00 am to 10.00 pm on PND 43 of the experiment. The blood collected was placed in a glass Vacutainer containing EDTA as an anti-coagulant for estradiol and testosterone measurement. At necropsy, the testes were excised from the animals and weight.
The measurement of estradiol and testosterone was conducted using enzyme immunoassay (EIA) kits. The assays were both measured using an EIA reader.

All statistical analysis was conducted using the SPSS 10.0 (SPSS Inc., Illinois, USA) computer program.

RESULTS AND DISCUSSION

Table 1**:** Phytoestrogen content measured in soya bean extract by LCMS

Phytoestrogen	**Levels detected in extract (µg/ g dry weight)**
Daidzein	10.1
Genistein	28.3
Daidzin	9.4
Genistin	16.4
Coumesterol	-

Table 2: Phytoestrogen content measured in rat feed by LCMS

Phytoestrogen	**Levels detected in rat feed (µg/ g dry weight)**
Daidzein	0.002
Genistein	0.006
Daidzin	0.05
Genistin	0.09
Coumesterol	-

Table 3: Mean weight gained by rats, mean absolute and relative testis weight, mean level of circulating estradiol, and mean level of circulating testosterone in control and treated groups

	Treatment				
	Control (Corn Oil)	17β-Estradiol	Control (Tween 80)	Bisphenol A	Soya Extract
Body weight gain (g)	78.67 ± 10.96	38.11 ± 13.80**	80.48 ± 9.10	79.47 ± 15.99	106.70 ± 16.11*
Right testis weight (g)	0.67 ± 0.20	0.24 ± 0.06**	0.87 ± 0.11	1.13 ± 0.37	1.60 ± 0.22**
Relative Right Testis Weight	0.55 ± 0.14	0.27 ± 0.05**	0.65 ± 0.09	0.79 ± 0.18	0.98 ± 0.09**

(g)/100 g body weight					
Estradiol Level (ng/ml)	79.91 ± 27.11	648.14 ± 74.93**	122.44 ± 90.37	12.35 ± 13.88*	2.54 ± 2.97*
Testosterone Level (pg/ml)	45.59 ± 33.16	49.66 ± 13.98	135.77 ± 83.28	101.47 ± 119.33	254.15 ± 100.17**

Data are presented as mean ± S.D

*: Difference between treatment compared to control was statistically significant, $P \leq 0.05$.

**: Difference between treatment compared to control was statistically significant, $P \leq 0.01$.

This study has shown that soya extract, BPA, and 17β-estradiol do have an impact on male reproductive organ morphology as well as on the steroidal hormonal system. All the effects shown have different degrees of severity which vary greatly between each treatment group.

Soya extract when given orally caused a statistically significant increase in body weight, testis weight, and circulating testosterone levels. It also caused morphological changes in the testis and a decrease in circulating estradiol levels. The increase in testosterone level is a novel finding as previous studies have reported a statistically significant decrease in testosterone levels in laboratory animals treated with phytoestrogens (Strauss et al., 1998; Sharpe et al., 2002; Wisniewski et al., 2003; Cline et al., 2004). The study conducted by Sharpe and colleagues (2002), also reported an increase in Leydig cells in the testis which produce testosterone of their laboratory animals fed with a soya based milk formula.

Rats treated with BPA showed a significant decrease in the level of circulating estradiol. BPA also showed a slight decrease in circulating levels of testosterone; however the results were not statistically significant. This is in line with previous results (Takahashi and Oishi, 2003; Kato et al., 2006). These findings correlate with the study done by Murono et al., (2001) using cultured Leydig cells which showed that BPA does not decrease ambient testosterone levels in cultured Leydig cells.

17β-estradiol treated rats showed, as expected, a statistically significant rise in circulating estradiol levels compared to the control, but showed no significant difference in circulating testosterone levels. This data correlates with other studies conducted on rats exposed to 17β-estradiol neonatally, which showed no significant difference in circulating testosterone levels (Kato et al., 2006).

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