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Effect of intragastric daidzein administration on blood testosterone level in male Wistar rat.

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Abstract : Daidzein is a phytoestrogen found in many plants consumed by humans and animals. The influence of isoflavone, daidzein on blood testosterone hormone level was studied in sexually mature male Wistar rats. Rats were divided into three groups: Control (received no daidzein), Sham control [received vehicle as ethanol: saline water (1:9)] and Experimental group [received daidzein 15mg/kg body weight (bw)]. Daidzein was administered intragastrically, i.e. by using a cannula inserted via oesophagus into the stomach once a day for 30 continuous days. In animals treated with daidzein blood testosterone hormone level was significantly reduced as compared to the controls after 15 and 30 days. This effect was probably due to the direct inhibitory influence of daidzein on testosterone hormone secretion from testis. Results obtained in the study indicate that daidzein affects testosterone hormone secretion and may therefore be responsible for infertility, cryptorchidism and reduced sperm concentration in Wistar rat.

Key words: daidzein, testosterone, Wistar rat, isoflavone

INTRODUCTION

Daidzein (4'7, dihydroxyflavone) is a phytoestrogen belonging to class of soy isoflavones, a sub-class of flavonoids that have received great attention for their potential on human health benefits. Daidzein aglycone is an isoflavone found at low concentrations in soybeans but at high concentrations in certain soy-derived food. Daidzein is much more abundant in the unprocessed soybean. Some phytoestrogens have been categorized as endocrine disruptors, i.e. exogenous substances that alter the functions of the endocrine system and consequently have adverse effects on the health of the organism, its progeny or its populations. They mimic or block hormones and disrupt normal endocrine functions by altering normal hormone levels, inhibiting or stimulating the production of hormones. They have been classified as natural environmental oestrogens[1] or natural endocrine- active agents[2]. Structurally, daidzein closely resembles 17ß-estradiol and it binds to estrogen receptors (ERs), the stronger affinity being for the ERß isoform[3]. Acting as a natural selective ER modulator, daidzein exerts its estrogen agonist or antagonist action in tissue in a dose dependent manner[4].

There is growing public concern regarding the adverse effects of environmental chemicals with an estrogenic influence on reproductive health. Daidzein and genistein are widely distributed in daily human diet[5]. In a typical Western diet, an average of 0.2 mg/kg isoflavones are consumed daily, whereas a typical Asian diet contains > 1.5 mg/kg isoflavones per day[6], which can raise individual human isoflavone serum levels to 500 Nm[7]. For infants fed soy- based formulas isoflavone intake can reach 9.3 mg/kg bw/day.[8] Phytoestrogens have been part of

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traditional diet in Asia for millennia. The negative effects of isoflavones, particularly on the male reproductive system have also been reported[9]. Previous studies demonstrated that isoflavones may produce male reproductive toxicity. Their main adverse effects on the male reproductive system include the disturbance of sex hormone release,[10,11] interference with the onset of puberty[12], altering penile corpus cavernosum structure weakening erectile function[13,14], suppressing the activity of some steroidogenesis associated enzymes[15] and decreasing the weight and epithelial height of accessory sex organs[11]. A high intake of soy-based food and soy isoflavones is associated with reduced sperm concentration, as demonstrated in animal experiments and human epidemiological studies[16,17].

Dietary isoflavones have caused infertility in male mice[18] and rats.[19] Genistein and ß- sitosterol had adverse effects on the reproductive organ weights in mice[20,21] and rats.[22,19] Reductions in the levels of plasma testosterone, estradiol and progesterone have been observed in rats after exposure to genistein[23,21,24]. Phytoesterol exposure caused an increase in plasma testosterone levels in the European polecat[25] and in the field vole[26].

Keeping the above in mind and considering the fact that there are limited reports on daidzein, the present study was conducted to observe the effects of short term intragastric administration of the isoflavone, daidzein on testosterone hormone in male Wistar rat.

Experimental Design

The experiment was performed according to the guidelines accepted by the Ethics Committee, Ranchi University, Ranchi for Investigations on Animals.

Male and female Wistar rats weighing about 150g were used in the experiment. The animals were kept under standard conditions, at a constant temperature (21±4°C) with a 12-h dark-light cycle. Rats were fed a soy- free diet *ad libitum*. Males were divided into three groups of ten animals each. Two female rats were introduced in each group. Animals in the control group received no daidzein, whereas, rats in the second group received the vehicle, i.e. saline water: ethanol mixture (1:9 v/v). The third group (experimental) received daidzein dissolved in the vehicle in the amount of 15 mg/kg BW. The vehicle and daidzein solutions (Sigma) were administered intragastrically (0.5ml/150g BW) once a day for 30 consecutive days. The males were anesthetized (di-ethyl ether) and their blood serum were collected after 15 and 30 days of the start of the experiment. It was stored (-80°C) until analysis.

ANALYSIS

Testosterone hormone was determined by Fully Automated Bidirectional Interfaced Chemi Luminescent Immuno Assay.

RESULTS

Results for the effects of daidzein on serum testosterone level of rat after 15 and 30 days administration are presented in Table 1. & Figure 1.

Serum testosterone levels in animals administered daidzein intragastrically for all durations tested, were significantly ($P<0.001$) lower than their respective controls and sham-controls. No significant difference was observed between serum testosterone concentration of sham-operated animals and control animals

Table 1: Effect of intragastric administration of daidzein on serum testosterone level (ng/dl) of male Wistar rat

Treatment	15 days	30 days
Control	22.43 ± 0.08	22.7 ± 0.02
Sham control	22.29 ± 0.04	21.54 ± 0.08
Treated	7 ± 0.10*	7.5 ± 0.09*

Daidzein was dissolved in saline water: ethanol mixture (9:1 v/v) and was administered intragastrically (0.5 ml; 15 mg/kg BW) for 15days and 30 days.

Values are means ±SEM

Significance of difference from control:*, p<0.001.

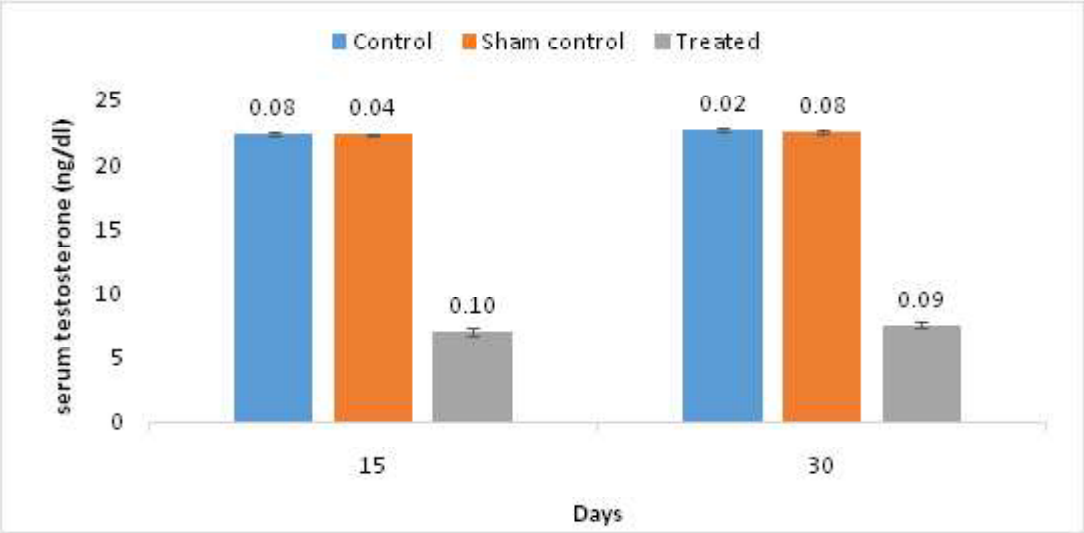

Figure 1: Effect of intragastric administration of daidzein (15mg/kg BW) for 15 days and 30 days on serum testosterone level (ng/dl) in male Wistar rat.

Significance of difference from control:*, p<0.001.

DISCUSSION

Studies for over the last 50 years reported an increased incidence of human male reproductive disorders like cryptorchidism, hypospadias, testicular cancer and low semen quality[27]. The rapid increase in disorder of the reproductive function suggests the involvement of environmental or life style factors. Exposure to endocrine disruptors appears to be an important cause.

Endocrine disrupting chemicals are exogenous agents that interfere with synthesis, secretion, transport, metabolism, binding action or elimination of natural blood -borne hormones that are present in the body and are responsible for homeostasis, reproduction and developmental processes. These endocrine disruptors are highly heterogeneous in structure and widespread in our environment. Less attention has been paid to the action of natural plant-derived endocrine disruptors (phyto estrogens) which are non-steroidal compounds having the ability to bind to estrogen receptors.

Daidzein, the major soy isoflavone glucoside is present at high concentration in soybeans and is a major source of xenoestrogen exposure in humans and animals. The results of the present study indicate an inhibitory effect of daidzein on the testosterone hormone level in the serum of Wistar rat when administered at a dose of 15 mg/kg body weight for a period of 15 days. Study on the long term administration of daidzein, at this dose, for over 30 days showed the same inhibitory action of daidzein on this hormone. The present result clearly shows that this isoflavone i.e. daidzein, under the present experimental conditions, is able to alter the testosterone level in the blood serum of this animal. This could have potentially detrimental effects on the fertility and reproductive functions of the animal.

Adverse effects of phytoestrogens have been observed in experimental animals but almost all of them focused on the effects of genistein and less attention has been paid to study the action of daidzein on reproductive functions. For example, the adverse effects of phytoestrogen, genistein in causing infertility in male mice were observed long back[18]. It has been shown that exposure to genistein adversely affects the reproductive organ weights in mice[20,21] and rats [19,22].

Reduction in the plasma testosterone, estradiol and progesterone levels, after genistein exposure have been shown in rats[21,23]. However, such reports are lacking for daidzein.

The present results are new and show that the isoflavone daidzein is capable of causing an effect on the testosterone level in the plasma, of this animal. These results are different from those observed for the European polecat, *Mustela putorius*,[26] in which it was demonstrated that

phytoesterol exposure increased the plasma testosterone levels.

Studies have reported that certain foods containing isoflavones, particularly infant formula, may have potential adverse effects on male reproductive function. Humans and animals are frequently exposed to products that contain low levels of isoflavones[28]. There are commercially available isoflavone containing products that have estrogen – like effects such as milk with added genistein and soy-based infant formulas[29,30].

Daidzein is found in many natural products that are marketed as promoting health and used to treat various diseases such as asthma, cancer, menopause, osteoporosis, atherosclerosis[31] as well as many commercial pet foods[32] and soy based infant formulas[33,29].

Isoflavones cause infertility in many animal species like sheep[34], the California quail[35], the mouse[36,18], the cheetah[37] and the rat[19]. Dietary phytoestrogens may have similar effects on the development and fertility of other species, including humans. But human data are very limited. There is paucity of studies available to evaluate the effects of soy and phytoestrogens on fertility or reproductive hormone in human males. Associations between exposure to phytoestrogens and hormonal disorders in children have been described[30].

The present results clearly demonstrated that dietary daidzein administration was able to decrease testosterone synthesis just after a period of 15 days in rats. These results are similar to those obtained by Zhu and Cowerkers[38] who reported a suppression of testosterone secretion by daidzein (30 μ mol /l for 24 hrs) in cultured testes and Leydig cells of neonatal mouse testes. These authors also suggested adverse effects of high doses of daidzein on Sertoli cells in neonatal mouse testes. A similar effect for genistein in impairing early testosterone production in fetal mouse testes *in vitro* has also been reported[39].

Genistein at a dose of 213 mg/kg bw/day from gestational day 7 to PND 13 also reduced plasma testosterone levels in rat offspring[40]. A reduction in plasma testosterone level was detected in primary Leydig cells by daidzein treatment in *Anser anser*[41,42]. The findings show a potential harmful effect of daidzein exposure on testis steroidogenesis function during the early neonatal period.

The results of the present study clearly define the association between daidzein exposure and reduced testosterone production in Wistar rat, under the tested experimental conditions. The mechanism of this action is still unclear. It has been suggested that testosterone production can be inhibited by exogenous compounds by suppression of steroidogenic enzymes, P450 scc, 3ß-HSD and P450c17ß[43-46]. It has been reported that genistein suppresses StAR, P450 scc, 3ß-HSD and P450C17ß expression and decreases testosterone production in fetal testis[39]. The observations in these studies may explain why testosterone levels in the testis or plasma are decreased. The expression levels of STAR and steroidogenic enzymes have been evaluated in daidzein- treated Leydig cells, in order to verify the mechanistic activities of daidzein on factors associated with steroid synthesis. The results showed decline in m-RNA and protein expression levels of STAR, P450scc, 3ß- HSD and P450C17. These results have been reported to be consistent with those of organ culture[47]. Testosterone is synthesized from cholesterol.

The conversion of cholesterol to testosterone involves series of key steroidogenic proteins, such as STAR protein, cytochrome p450 scc and 3ß-HSD[48,49,50]. The rate limiting step in the synthesis of testosterone is the transfer of cholesterol to pregnenolone via P450scc; StAR mediates this rate-limiting step in steroidogenesis[51]. The conversion of pregnenolone to progesterone is mediated by 3ß-HSD. The enzymatic action is essential for the production of all active steroid hormones[52].

Phytoestrogens including daidzein can modulate the endocrine system by altering the gene expression or activity of the enzymes involved in steroidogenesis[53]. The key enzymes involved in steroidogenesis have been reported to be important targets for phytoestrogens[54]. Isoflavones including daidzein can exert inhibitory feedback on the hypothalamus-pituitary gonadal axis[55].

The present results confirm the inhibitory effect of dietary daidzein on testosterone levels of blood in Wistar rat. It is suggested that this effect of daidzein may be exerted via the alteration of the activity of the enzymes involved in steroidogenesis. Further research in this direction will elucidate the complete mechanism involved in producing such an effect.

CONCLUSION

The results obtained in this study prove that 15 and 30 days intragastric administration of daidzein affects

testosterone hormone level in male Wistar rats. This compound essentially decreased testosterone concentration in blood serum. Mechanisms responsible for the detected activity of daidzein require further investigations.

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