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Daidzein affects leptin hormone secretion in the male Wistar rat

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Abstract : Daidzein (7, 4 dihydroxy isoflavone) is a naturally occurring isoflavone present in a number of plants, especially in soybeans and soy-derived products. The influence of this isoflavone, daidzein on leptin hormone was studied in sexually mature 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 15, 30 and 60 continuous days. In animals treated with daidzein blood leptin-hormone level was significantly reduced. After 15 days, the effect was less significant ($p<0.05$) as compared to that observed after long duration administration for 30 and 60 days ($p<0.001$).This latter effect was probably due to direct inhibitory influence of daidzein on leptin-hormone secretion from adipocytes. Results obtained in the study indicate that daidzein may affect leptin-hormone responsible for metabolism, energy expenditure and food intake in Wistar rat.

Key words: daidzein, testosterone, Wistar rat, isoflavone

INTRODUCTION

Daidzein is an isoflavone found in high concentrations in soy. It plays an important role in prevention of many diseases and affects a number of metabolic pathways. The isoflavones belong to a large group of compounds classified as phytoestrogens[1], which have a chemical structure similar to estrogen and can bind to estrogen receptors.[2,3] Daidzein has an aromatic ring with two hydroxyl groups (other isoflavones such as genistein, formononetin and biochanin A have one or three hydroxyl groups). This structure enables it to bind to the estrogen receptor (ER). Daidzein has an affinity to both ER subtypes -ER α and ER β, but its affinity for ER β is significantly higher.[2,4]

The soy- derived isoflavone demonstrates pleiotropic action in the organism. It has both estrogenic and anti-estrogenic effects.[5] Adipose tissue has been shown to be a major endocrine system that plays an important role in energy homeostasis, lipid metabolism, immune response and reproduction.[6,7] Phytoestrogens promote, maintain and control the typical distribution of body fat and adipose tissue metabolism, through a still unknown mechanism. Daidzein and genistein were also found to inhibit lipogenesis and stimulate lipolysis in rat adipocytes. This activity was manifested by the direct effect of these compounds on isolated fat cells.[8]

The literature data reveal that the effects of daidzein on metabolism have not been fully elucidated. Many experiments performed to test the direct influence of daidzein and genistein on metabolism of fat cells suggests the possibility of its effect on leptin hormone secretion. This problem seems to be important since the proper leptin secretion constitutes an important factor regulating the energetic status of the whole organism.

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The aim of the present study was to determine the influence of daidzein administration for 15, 30 and 60 days on leptin hormone parameter in mature male rat.

EXPERIMENTAL DESIGN

Animals and measurement

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 10 animals each. Two female rats were introduced in each group. Animals in the control group received no daidzein, whereas, those 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 given intragastrically (0.5ml/150g BW) once a day for 60 consecutive days. The males were anesthetized (di-ethyl ether) and their blood serum were collected after 15, 30 and 60 days of the start of the experiment. It was stored (-80°C) until analysis.

ANALYSIS

Leptin hormone was assayed by Radio immunoassay kit.

RESULT

Results obtained for leptin hormone content of blood serum after daidzein administration at a dose of 15 mg/kg BW for 15, 30 and 60 consecutive days are presented in Table- 1.

A significant reduction was noted in the leptin levels of blood serum after 15 days ($p<0.05$), 30 days ($p<0.001$) and 60 days ($p<0.001$) of daidzein treatment, as compared to the controls. Leptin levels in sham-control animals were not significantly different from those of controls, in all cases.

Table 1 -Effect of intragastric administration of daidzein on serum leptin level (ng/ml) of male Wistar rat

Treatment	15 days	30 days	60 days
Control	0.31±0.01	1.34±0.02	2.24±0.01
Sham Control	0.29±0.02	1.31±0.02	2.26±0.01
Treated	0.26±0.02*	0.29±0.03**	1.89±0.02**

Daidzein was dissolved in saline water: ethanol mixture (9:1 v/v) and was administered intragastrically (0.5 ml; 15 mg/kg BW) for 15 days, 30 days and 60 days.
Values are means ±SEM, Significance of difference from control: *, $p<0.05$; ** $p<0.001$.

DISCUSSION

The present results indicate that intragastric administration of daidzein at a dose of 15 mg/kg BW causes a significant reduction in the serum leptin concentration of the rat. This effect of daidzein was observed after 15 days of treatment although the effect was less significant ($p<0.05$) as compared to that observed after long duration administration for 30 and 60 days ($p<0.001$). An effect of genistein, administered to male rats for a period of three and seven days at a dose of 5 mg/kg BW, in substantially diminishing blood leptin levels, has been reported.[9, 10] However, such an effect was not observed, by these authors, in animals receiving daidzein at the similar dose. Neither genistein nor daidzein consumed by rats affected leptin gene expression in adipocytes isolated from these animals.[9] The present results are different from these and showed significant decrease in blood leptin levels after daidzein administration to rats, in all cases. It can be opined that perhaps a duration of three and seven days was not sufficient for the expression of the effect of daidzein on leptin level of blood in rat, and that a longer period of treatment was required to produce any effect.

Leptin is a hormone that plays an important role in regulation of food intake, body weight and energy status

of the organism. [9, 11] Daidzein has been found to inhibit lipogenesis and stimulate lipolysis in rat adipocytes. This activity is manifested by the direct effect of daidzein; on isolated fat cells.[8] This direct influence of daidzein on metabolism of fat cells suggests the possibility of its effect on leptin secretion.

It has been found that inhibition of some enzymes in adipocytes substantially abates leptin secretion in spite of unchanged expression of its gene.[12]

The results clearly show an inhibitory effect of daidzein on leptin level of blood under the present experimental conditions. It may be suggested that this effect of daidzein was possibly due to its ability to restrict glucose transport due to conformational changes in GLUT 4, as reported for genistein.[13] The effect may also be a result of restricted glucose transport through GLUT 1. Hence, inhibition of glucose transport in adipocytes could be inhibiting secretion of leptin. Such an effect has been reported for genistein in case of rat adipocytes.[14] Further, it has also been demonstrated that GLUT 1 levels are decreased after treatment with daidzein.[15] GLUT expression is dependent on the concentration of compounds used and their inhibition constant. Compounds that block glucose transport also decrease glycosylation levels, and as a result, GLUT levels are lower.[16] This observation demonstrates that effects of daidzein on adipocytes are responsible for decreased leptin release and this significantly diminishes the blood leptin level.

The inhibition of glucose transport and/or glycolysis in adipocytes strongly prevent the secretion of leptin.[17] These observations allow to suppose that daidzein induced drop of leptin secretion in the present study could be due to inhibitory influence of this phytoestrogen on glucose transport.

The decreased blood leptin level observed in the present experiment after 15, 30, and 60 continuous days administration of daidzein to mature male rats may also have resulted from the direct influence of daidzein on fat tissue. Daidzein has been reported to inhibit lipogenesis and stimulate lipolysis in rat adipocytes.[18,19] This direct influence of daidzein on metabolism of fat cells suggests the possibility of its effect on leptin secretion.

Phytoestrogens are endogenous estrogens. They can act as weak estrogens and bind to the estrogen receptor in various tissues.[20] It has been demonstrated that the appetite repressing action of estrogen causes reduction in food intake.[21] Dietary phytoestrogens decrease feed intake and hence decrease body weight and abdominal fat mass.[22] Phytoestrogens are capable of inducing apoptosis of adipocytes suggesting that, at least, part of the weight loss is due to destruction of fat cells which could result in better maintenance of weight loss. [23] The present observation that dietary daidzein significantly ($p<0.001$) depressed serum leptin concentration after continuous administration for 15 to 60 days allows to believe that the effect of this phytoestrogen daidzein may be due to its direct influence on adipocytes which are the main source of leptin.[9] Phytoestrogens such as daidzein and genistein inhibit enzyme activity in adipocytes which decreases leptin secretion.[12]

It has been documented that factors augmenting cAMP content in adipocytes decrease blood leptin concentration[24,25] and reduce its release from fat cells.[26,27,28] Lipolysis is enhanced by the activation of protein kinase A (PKA) in the presence of increased c AMP in fat cell.[29] An inhibitory action of genistein on cAMP phosphodiesterase has been found in adipocytes[30], neural cell line[31] and hepatocytes[32]. Further research in this direction is needed to ascertain if daidzein exerts a similar effect on adipocytes. It was demonstrated that different lipolytic agents, including cAMP phosphodiesterase inhibitors, substantially reduced secretion of leptin from isolated adipocytes.[26] Thus, it may be possible that the inhibition of cAMP phosphodiesterase by daidzein contributes to restriction of leptin secretion.

Results obtained in the present study clearly indicate an inhibitory effect of dietary daidzein on leptin secretion in male Wistar rat. It is suggested that this inhibitory effect of daidzein may be due to the restriction of glucose metabolism. The decrease in serum leptin could be the effect of daidzein action on adipocytes such as inhibition of glucose transport, restriction of insulin action or inhibition of cAMP phosphodiesterase activity. The mechanisms involved in the expression of these effects, by daidzein, remain yet to be investigated.

CONCLUSION

The results obtained in this study show that intragastric administration of daidzein for 15 to 60 days

significantly affects leptin hormone levels in male rats. This compound essentially decreased leptin concentration in blood serum. Mechanisms responsible for the detected activity of daidzein require further investigations.

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