International Journal of Advance Research in Science and Engineering
Volume No.07, Issue No.04, April 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

Studies on the effect of Daidzein on some biochemical parameters of male Wistar rat

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ABSTRACT

Daidzein is a phytoestrogen and is found in many plants consumed by humans and animals. The influence of isoflavones,daidzein on some hormones and metabolic parameters in sexually mature Wistar rats was studied. Rats were divided into three groups: Control (receivedno daidzein), sham control [received vehicle as ethanol: saline water (1:9)] and experimental group [received daidzein 15mg/kg body weight(bw)]. The tested compound was administered intragastrically, i.e. by using a cannula inserted via oesophagus into the stomach once a day for 30 days. Simultaneously, in animals treated with daidzein (15 mg/kg BW) blood leptin-hormone was significantly reduced). This later effect was probably due to direct inhibitory influence of daidzein on leptin-hormone secretion from adipocytes. Several metabolic parameters were determined in blood like glucose, protein, triglyceride, cholesterol, LDL, VLDL. Blood glucose level showed significant increase after daidzein treatment. A reduction was observed in the parameters such as total cholesterol, HDL, LDL and VLDl, triglyceride. Results obtained in the experiment indicate that daidzein may affect leptin-hormonesresponsible for metabolism, energy expenditureand food intake in Wistar rat.

Keywords: daidzein,ethanol, leptin, metabolism, Wistar rat

I. 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. Many epidemiological studies suggest that high dietary intake of soy is associated with lower incidence rates of certain forms of cancers (**Setchell,1998; Messina and Messina,2010**). Indeed, Asian women, whose diet is rich in isoflavones, have a low incidence of breast cancer as well as of other hormone-associated problems, such as osteoporosis and menopausal symptoms (**Somekawa *et al*; 2010, Messina *et al*, 2004).**

Daidzein aglycone is an isoflavones found at low concentration in soybeans but at high concentrations in certain soy-derived food. In contrast, daidzin, the glycoside form of the aglycone Daidzein is much more abundant in the unprocessed soybean. Structurally, Daidzein closely resembles 17ß-estradiol and indeed it binds to estrogen receptors (ERs) the stronger affinity being for the ERß isoform (**Kuiper *et al;* 1998**). Acting as a natural selective ER modulator, Daidzein exerts its estrogen agonist or antagonist action in tissue and dose dependent manner (**Satchell, 2001**).

The thyroid is a gland located in the lower part of the neck enfolded around the front of the trachea. As its primary function the thyroid gland produces thyroid hormones, which primarily function to regulate basic metabolic rate. The thyroid gland produces three hormones: calcitonin, which is involved in calcium regulation, and 3, 5, 3'-tri-iodothryonine (t_3) and 3,5, 3, 5'- tetra – iodothryonine. Iodine is an essential component of both t_3 and t_4, contributing 59% and 65% of their molecular weights, respectively.

In physiological conditions the human thyroid weighs between 15 and 20 g. However, in response to prolonged iodine deficiency, the thyroid gland can increase approximately 5-fold to the size of an apple, a condition recognized as goitre. If serum iodine levels are low or the thyroid is not able to incorporate enough iodine, the anterior pituitary secretes a hormone called Thyroid stimulating hormone (TSH). When in excess, the secretion of TSH compensates by increasing the size of the thyroid and thereby allowing the gland to work more efficiently. For this reason, the primary cause of goitre and hypothyroidism around the world is inadequate iodine intake. The adult iodine recommended daily dose (RDA) is 150mg, so one teaspoon (approximately 4-5g) of iodine is all a person requires in a lifetime. However, approximately one billion people are considered to be iodine deficient worldwide. Iodine deficiency is a large problem in many world regions, especially in the Himalayas, the Andes, the south of Italy, and in the central part of Africa. In the US approximately 50% of the population uses iodized salt which is why the population uses iodized salt. Which is why the iodine intake of the US population is considered adequate. Some subsets of the population, such as women of child bearing age, still have marginal iodine intakes, however, and thyroid problems can also develop in populations consuming adequate amounts of iodine. Thyroid abnormalities in these situations are often due to presence of dietary goitrogens, such as glucosinolates in cruciferous vegetables which interfere with utilization of iodine or functioning of the thyroid gland. This could be case of flavonoids which are abundant in green vegetables and seasonal fruits and, due to their wide spread occurrence are regularly ingested by humans. Flavonoids are polyphenol compounds that are ubiquitous in nature and are categorized, according to their chemical structure, in to flavones, flavones, flavanones, isoflavones, catechins,anthocyanidins and chalcones. Isoflavones are a group of flavonoids, which can act as antioxidants, due to ability of trap a singlet oxygen and as estrogen with protective functions.

Isoflavones are transformed by bacteria in the intestinal flora during digestion. It is only once this transformation has been completed that the isoflavones can be absorbed and exert their beneficial effects in the body. The individual intake of flavonoids however, varies considerably depending on the type of diet consumed. Asian individuals have based their dietary style on rice,vegetables and beans for centuries, whereas western countries have a greater intake of cereals, meat and derivatives; this explains the differences in flavonoidplasma levels detected in different populations. There are considerable regional differences in soy protein and isoflavones consumption, with Asian population consuming an estimated 25-30mg of isoflavones aglycone equivalents daily, with 10% of the population consuming more than 100 mg isoflavones daily. Isoflavones intake in the US is several fold lower than in Asia, with intake estimate ranging from 0.15-3 mg per day. In addition to dietary pattern, there is currently an extensive range of flavonoid supplements on the market.

Suppliers of such supplements recommended daily flavonoid intake in amount that are many times higher than those doses which can normally be achieved from a flavonoid –rich diet. Another example is the isoflavones-based nutraceuticals (e.g. :- pills, tablets, extracts) which are one of the most widely tested and usedflavonoids supplements so for. The declared content of isoflavones is extremely variable, ranging from 50 to 500 mg and different daily doses are recommended. However, at present, no specific dosage of isoflavones has been established to exert a beneficial effect.

1.1 GOITROGENIC EFFECTS OF FLAVONOIDS

As for other naturally derived substances, flavonoids may exhibit anti-thyroid and goitrogenic activity. Excessive soy intake has been reported as a risk factor for the development of goitre, in both iodine- deficient rodents and infants fed soy-flour based formula without iodine fortification. Animals fed soy diet require almost twice as much iodine compared with animals not fed soy. Indeed, several experimental studies demonstrate that isoflavones can inhibit the catalytic activity of thyroid peroxidase (TPO). Inhibition of TPO- catalysed reactions results in decreased levels of circulating thyroid hormones, which lead to increased secretion of TSH provide a growth stimulus to the thyroid, and it has been proposed that a prolonged stimulus can select for clones of follicular cells with the potential for transformation. TPO is the heme-containing enzyme found on the apical membrane of thyroid follicular cells that catalyse the two reaction required for thyroid hormone synthesis: iodination of thyrosyl residues on thyroglobulin and sub –sequent oxidative coupling to yield t3 and t4 (Ferreira et al: 2002). Studied the in-vitro effect of various flavonoids on thyroid type 1 iodothyronine deiodinase activity was significantly inhibited by isoflavones, quercetin and catechins.

In a recent study in rats (Chandra and De, 2010), decreased activity of TPO and 5-deiodinase was reported in response to dietary green tree extracts. Serum t3 and t4 levels were found to be significantly reduced and associated with a significant increase a serum TSH levels. The authors concluded that green tea extract at high dose could impair thyroid function. Furthermore, isoflavones have been reported to exert, at high concentrations, goitrogenic effects in humans. A significant correlation between circulating isoflavones concentration in blood and thyroid function (Milerova and co-worker,2006).Keeping the above in mind the presents study was conducted to observe the effects of short term intragastric administration of the isoflavones, daidzein on certain hormones and biochemical parameters of male Wistar rat.

II.EXPERIMENTAL DESIGN:

II.1 Animals and measurement

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

Male and female Wistar rats weighing about 150gm were used in 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 and females were divided in to three groups (n=10). Animals in the control group

IJARSE
ISSN: 2319-8354

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, respectively. The vehicle and daidzein solutions (Sigma) were given intragastrically (0.5ml/150gm of BW) once a day for 30 consecutive days. The animals were anesthetized (di-ethyl ether)and their blood serum were collected and stored(- 80˚C) until analysis.

II.2ANALYSIS

Serum hormone concentrations were assayed by ChemiLuminescent Immuno Assay method: thyroid hormones, total triiodothyronine(t3) and thyroxin(t4) by competitive Chemi Luminescent Immuno Assay and TSH by ULTRASENSETIVE SANDWITCH CHEMI LUMINESCENT IMMUNO ASSAY) by the method of (Stagnaro-Green*et al*., 2011). Serum triglycerides were measured by the Enzymatic Colorimetric Method (NCEP ATP 3 GUIDELINES) and Serum cholesterol (Total) by CHOD POD Method. HDL- cholesterol was assayed in the serum after separation of high density lipoproteins using Enzyme selective protection method. LDL- cholesterol was assayed by Homogenous Enzymatic Colorimetric Assay. Total protein was determined by Biuret Method of Henry et al; (1974). Estradiol / Estrogen hormone were assayed by the method of Muse and Wilson (1986). Testosterone hormone was determined by Fully Automated Bidirectional Interfaced Chemi Luminescent Immuno Assay.

III. RESULTS

Daidzein given at a dose of 15 mg/kg BW for 30 daysslightly reduced the lipid metabolism in male adult rat. The serum concentration of t_3 was slightly reduced and t_4 was slightly increased by daidzein administration.TSH levels were significantly higher in daidzein treated animals as compare to the control (Table 1). The serum concentration of testosterone was reduced by the effect of daidzein. Whereas, it had slight effect on Estradiol/oestrogen concentration in male adult rat. A significant reduction was noted in the blood serum leptin- hormone level in daidzein treated animals.

Table 1. The influence of daidzein on serum hormones in mature male rats

Blood serum	**Control**	**Sham control**	**Experimental Group**
Total Triiodothryonine (T_3), ng/dl	82±0.004	81±0.003	78±0.002
Total Thyroxin(T_4),μg/dl	4.2±2.74	3.2±2.76	4.3±2.50

Thyroid Stimulating Hormone(TSH),μ/ml	0.72±0.015	1.72±0.010	2.68±0.016
Estradiol/Oestrogen(E2), pg./ml	10±0.32	18±0.22	13±0.19
Testosterone, ng/dl	21.4±0.10	7±0.15	7±0.14
Leptin, ng/ml	0.34±0.18	0.32±0.26	0.29±0.11

Daidzein was dissolved in saline water: ethanol mixture (1:9 v/v; 0.5 ml/150g BW) and was administered intragastrically for 30days. Values are means ±SEM for 10 rats.

Table 2. The influence of daidzein on metabolic parameters in mature male rats

Blood serum	**Control**	**Sham Control**	**Experimental**
Glucose (mg/dl)	92.3±0.14	95.8±0.22	132.7±0.26
Total Protein(gm./dl)	6.5±0.22	7.2±0.23	6.4±0.25
Triglycerides (mg/dl)	104±0.09	104±0.05	98±0.08
Total Cholesterol (mg/dl)	167±0.14	94±0.17	67±0.11
Cholesterol HDL(mg/dl)	48±0.28	34±0.31	31±0.22
Cholesterol LDL (mg/dl)	35±0.25	48±0.28	20±0.30
Cholesterol VLDL (mg/dl)	35.6±0.22	20±0.25	19.6±0.28

Daidzein was dissolved in saline water: ethanol mixture (1:9 v/v; 0.5 ml/15mg/kg BW) and was administered intragastrically for 30days. Values are means ±SEM for 10 rats.

Dietary daidzein administration at a dose of 150 mg/kg bwsignificantly increased the blood glucose level. The other parameters such as total protein, triglyceride, total cholesterol and cholesterol (HDL,LDL and VLDL) showed reduction after daidzein administration.

IV. DISCUSSION

Daidzein administered at a dose of 150 mg/kg BW for 30 days caused a substantial decrease in blood leptin concentration. Leptin is a hormone that plays an important role in regulation of food intake, body weight and energy status of the organism (Ahima and Flier, 2000). Blood leptin levels were reduced in adult male rats consuming a diet enriched in isoflavones (Lephart *et al.*,2004).

The decreased blood leptin levels observed in the present experiment may also have resulted from the direct influence of daidzein on fat tissue. It is well documented that isoflavones decreased adipose tissue weight in mature male mice (Penza et al., 2006) and in male rats (Lephart *et al*., 2004).

Triiodothyronine and thyroxin are essential hormones in the regulation of the energy balance of an organism, so we investigated the influence of daidzein on these thyroid hormones. The tested compound did not alter blood concentrations of total thyroid hormones. Several studies provide similar results. The tested compound did not effect on TSH,$T_{3,}$ or T_4levels in rats receiving genistein in a diet for 20 weeks (Chang and Doerge,2000).

The literature data provide evidence that consumption of diets containing soya protein (and phytoestrogens which are tightly associated with proteins) reduced total cholesterol and LDL-cholesterol (Carroll, 1991) and augments HDL-cholesterol (Anthony et al., 1996). However, it is not clear whether these beneficial effects are evoked by soya protein, phytoestrogens or other compounds (Kurzer and Xu, 1997). In the present study also a decrease was noted in the triglyceride, total cholesterol and cholesterol (HDL, LDL and VLDL) after intragasric daidzein administration for 30 days. Our results indicate that pure phytoestrogen, daidzein is responsible, at least in antiestrogenic effect of soy containing diets. This observation, of course, does not exclude that other compounds of vegetable diets may have also similar beneficial influence on blood cholesterol.

Results obtained in our experiment clearly indicate that daidzein significantly affectsblood glucose and protein levels. Triglyceride content was substantially reduced by the effect of daidzein. Similar effect was previously observed in ovariectomized rats consuming a diet enriched in genistein (substitute of daidzein) (Nogowski *et al.*,1998).

V. CONCLUSIONS

The results obtained in this experiment prove that 30- days- long intragastric administration of daidzein may change leptin, glucose, thyroid hormone levels and parameters of lipid metabolism in male rats. This compound essentially decreased blood leptin concentration and reduced the triglyceride content in blood serum. Mechanisms responsible for the detected activity of daidzein require further investigations.

VI. ACKNOWLEDGEMET

Grateful acknowledgements aremade to Dr. A. Dutta, Department of Zoology, Ranchi University,Ranchi, for his valuable suggestions and guidance during the completion of this work. Sincere thanks are also expressed to the Head, Department of Zoology, Ranchi University, Ranchi, for his help and support.

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