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TOXICITY AND BIOCHEMICAL EFFECT OF DAIDZEIN IN WISTAR RAT

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ABSTRACT

Daidzein, a phytoestrogen was used for estimation of acute toxicity (LD50) and elect of daidzein on biochemical parameters protein, triglyceride and cholesterols in serum of Wistar rats. LD50 was estimated by log-dose/ probit regression method. Five sets containing 10 rats each were taken and serially diluted doses were given orally with the help of gavage tube. The dose were control or 0, 50,100,150 and 300 mg/kg body weight(BW) for Daidzein. The rats were observed for 96 hrs and then mortality was noted for further calculation. LD50 of the daidzein has been estimated (150mg/kg bw) and 1/10th of LD50 dose was introduced for sub-chronic 60 days treatment and effect was observed after 15,30 and 60 days exposure. Saline water and ethanol (1:9 v/v) were used as vehicle. The vehicle and daidzein solution (Sigma) were given intragastrically (0.5ml/15mg/kg BW) once a day for 60 continuous days. Biochemical analysis showed an increase in blood serum glucose concentration. A decrease was observed in the total lipids. No significant effect was noted in total blood protein content. This alteration in biochemical parameter may result in imbalance between protein synthesis and utilization.

Keywords: Daidzein, LD50, Protein, Toxicity, Wistar Rat

I.INTRODUCTION

Endocrine disruptors are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological and immune effects in human beings and wildlife animals. This has been discussed since the beginning of the nineties (**Bolt and Degen, 1997**). Initially, this discussion had been concentrated on plasticizers, polychlorinated organic compounds and other artificial chemicals. But after sometime, the influence of naturally occurring and hormonally active estrogenic compounds in human food has been considered. By the order and regulation of government, the impact of artificial environmental chemicals with hormonal/ endocrine effects has been differentiated with that of natural food ingredients (**Bolt *et al*; 2001**). This has introduced new interest in biological activities of phytoestrogen, a substance found in certain plants which can produce effects like that of hormone oestrogen when ingested in to the body. Daidzein is a naturally occurring compound found exclusively in soybeans and other legumes and structurally belongs to a class of

compounds known as isoflavones. Significant part of human exposure to natural phytoestrogens is, therefore, dependent on the type of diet.

Minimally processed food provide wide nutritional advantages over industrial processed foods. Factory processed foods have been compromised by the addition of hormones, additive, preservatives, unnatural genetic material or other chemical or heat treatments that alter or destroy the natural healthy enzymes, fatty acids vitamins and minerals known to be health protective to animals (**Meserole,2002**). There are huge health benefits of fresh food and medicinal plants in contrast to that have been processed or stored for long time durations and therefore have altered chemical and nutrient profiles. The presence of beneficial nutrients and active constituents in the diet may contribute the ultimate prophylactic approach in disease risk reduction and anti-ageing medicine **(Iwu, 1986).**For human beings soybean proteins are an ideal source of dietary protein of vegetable origin just like biologically active cereal protein (**Messina, 1999**). Soybean proteins and isoflavones (genistein and daidzein) have shown to reduce the risk of cardiovascular diseases by lowering blood pressure, blood cholesterol and triglycerides (**Sagara*et al;* 2004, Mcveigh*et al;* 2006**). Several studies have shown that soybean or soy derivatives such as daidzein and genistein reduced LDL-Cholesterol (bad cholesterol) in the arteries thus lowering the incidence of atherosclerosis (**Wagner, 2003**) and are also connected to the reduction of diabetes mellitus (**Villegas *et al*; 2008**). Isoflavones act as anticancer agents, suppressing the growth of hormone dependent cancers such as prostate and breast cancers (**Messina *et al*; 2006, Allresed*et al*; 2004, Kurahashi et*al*; 2007**). Soy isoflavones in diet inhibit bone loss and increase bone mineral concentration in menopausal women (**Ma *et al*; 2008**). People that consume large amount of dietary soybean have most of the health benefits of isoflavone content and soy assigned to its high protein **(Omoni and Aluka, 2005**).

In whole animal models and in experimental systems in vitro, phytoestrogens appear capable of acting both as partial agonists and as antagonists, the primary effects of which are mediated via interaction with the oestrogens receptors. Phytoestrogen-rich diets have the potential to exert adverse as well as beneficial effects in humans (**Cassidy, 1996).** This is related to the hormonal activity, which is therefore relevant for the assessment of associated risks to humans. Very few studies are published on the toxicological determination of LD50 of daidzein and its effects on mammals.

Therefore, the present study was aimed at observing the lethal toxicity and biochemical effects of daidzein after short and long exposure on glucose, protein, triglycerides and cholesterol contents of serum in Wistar rat.

II.RESEARCH DESIGN

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

Daidzein (7, 4 dihydroxy isoflavone) is a naturally occurring isoflavone present in a number of plants, especially in soybeans and soy-derived products. Daidzein, a phytoestrogen has been considered for this toxicology study. The toxicological study has been done on Wistar rats which are related to human being and easy to handle in

laboratory. Wistar rats have been selected from inbreed colony. Healthy adult rats of almost equal size and weight (145±5) irrespective of sexes were selected randomly. The rats were maintained at 25±5˚c temperature. They were provided food pellets and clean water for survival.

II.1DETERMINATION OF LD_{50}

The LD_{50} was determined by log-dose /probit regression live method (**Finney, 1971**). Five sets containing 10 rats each were taken and serially diluted doses were given orally with the help of gavage tube. The dose were control or 0, 50,100,150 and 300 mg/kg BW for Daidzein. The rats were observed for 96 hrs and then mortality was noted for further calculation. A graph has been plotted between empirical probit and log dose and then LD_{50} has been calculated with the help of regression live and computerized calculation.

II.2SUB-CHRONIC TREATMENT

LD_{50} of the daidzein has been estimated (150mg/kg bw) and $1/10^{th}$ of LD50 dose was introduce for sub-chronic 60 days treatment and effect are observed after 15^{th},30^{th} and 60^{th} days exposure, saline water and ethanol (1:9 v/v) used as vehicle. The vehicle and daidzein solution (Sigma) were given intragastrically (0.5ml/15mg/kg bw) once a day for 60 days. The animals were anesthetized (di-ethyl ether) and their blood serum were collected and stored (-80˚c) until analysis.

II.3 ANALYSIS

The serum total protein was estimated by Biuret method described by **Henry *et al.*, (1974**). 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.

III. TABLES AND GRAPHS

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
—	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

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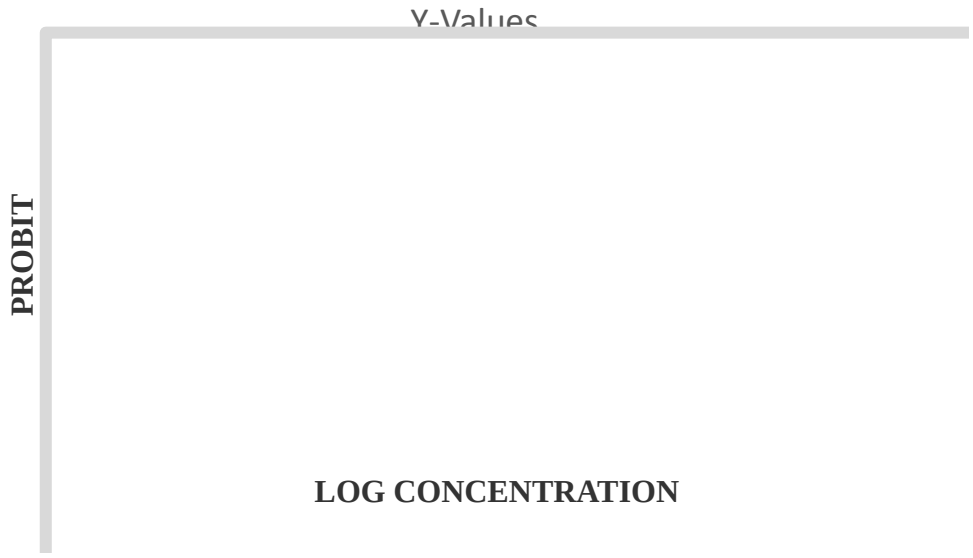
Table 1. Transformation of percentage to probit

S. No.	Concentration mg/kg body weight	Concentration mg/l	Number of animals		Percent mortality	Log concentration	Empirical probit
			Exposed	dead			
1.	300mg/kg bw	6.81	10	10	100	0.833	8.09
2.	150mg/kg bw	1.69	10	5	50	0.227	5.00
3.	100mg/kg bw	0.75	10	3	30	-0.124	4.48
4.	50mg/kg bw	0.18	10	2	20	-0.74	4.16
5.	0 mg/kg bw	0	10	_	_	_	_

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



Plot of log –concentration versus probit from Table-2 for calculation of LD50 of daidzein administered intragastrically.

TABLE III.The influence of daidzein on metabolic parameters in mature rats

Parameter	Treatment	15 day	30 day	60 day
Total protein(gm./dl)	Control Treatment	6.4±0.20 6.3±0.23	6.5±0.22 6.4±0.25	6.6±0.24 6.3±0.27
Glucose(mg/dl)	Control Treatment	107.4±0.10 116.8±0.22	92.3±0.14 132.7±0.26	81.3±0.11 139.2.±0.24
Triglyceride (mg/dl)	Control Treatment	98±0.05 44±0.02	104±0.09 98±0.08	109±0.08 77±0.05
Total Cholesterol(mg/dl)	Control Treatment	56±0.03 51±0.05	167±0.14 67±0.11	67±0.13 56±0.10
HDL Cholesterol(mg/dl)	Control Treatment	20±0.20 15±0.15	48±0.28 31±0.22	51±0.33 23±0.28
LDL Cholesterol(mg/dl)	Control Treatment	20±0.22 16±0.28	35±0.25 20±0.30	22±0.28 17±0.25
VLDL Cholesterol(mg/dl)	Control Treatment	19.6±0.16 8.8±0.22	35.6±0.22 19.6±0.28	15.4±0.15 10.4±0.23

Daidzein was dissolved in saline water: ethanol mixture (1:9 v/v; 0.5 ml/15mg/kg BW) and was administered intragastrically for 15day, 30day and 60 days simultaneously. Values are means ±SEM for 10 rats.

IV.RESULTS

In present investigation the mortality rates of rats were determined by introducing the daidzein orally. To identify the lethality of daidzein in different concentrations/doses ranging from 50 mg/kg BW to 300 mg/kg BW were used. The mortality in percentage at different doses after introducing daidzein to the mammalian model Wistar rat is presented in TABLE1.

The data was computed according to Probit Analysis Method (Finney, 1971) and LD50 value was determined. The animals exposed to different concentrations of daidzein, showed no mortality at 0 mg/kg BW/ control group, 20 percent mortality at 50 mg/kg BW, 30 percent mortality at 100mg/kg BW, 50 percent mortality at 150 mg/kg BW, and 100 percent mortality at 300mg/kg BW observed.

Dietary daidzein administration at a dose of 15 mg/kg bw significantly increased the blood glucose level. The other parameters such as triglyceride, total cholesterol and cholesterol (HDL, LDL and VLDL) showed reduction after daidzein administration. No significant effect was observed in total protein content.

V. DISCUSSION

The animals exposed to different concentrations of daidzein, showed 50 percent mortality at 150 mg/kg BW.The computation of percent mortality against different log concentration of the phytoestrogen yielded a sigmoid curve. The LD50 value obtained from sigmoid curve is 150 mg/kg BW for 96 hours. The probit mortality of the Wistar rat were calculated from percent mortality. When the probit mortality was plotted against Log concentrations of the phytoestrogens, a straight line was obtained. The LD50 value obtained from this straight line graph is 150mg/kg BW. LD50 of daidzein was noted as 150mg/kg BW.

The graphical representation of percent mortality versus Log concentration and probit Mortality versus Log concentration of daidzein showed a typical sigmoid curve and a straight line respectively which are in agreement with the principle of probit Analysis (**Finney,1971**).

In the present investigation, the obtained LD50 value is 150mg/kg BW. This value is in agreement with LD50 value reported previously by (**Kishida T *et al*; 2008**).

Every pesticide may vary greatly in its toxicity and persistence. The evaluation of toxicity of a test chemical is a sensitive phenomenon, which can be influenced by several factors such as size (**Jayantha Rao, 1982**), nutritional status (**Pal and Kushwah, 1981; Das and Garg, 1981),** species specificity (**Gouda et al;1981; Jacob et al; 2006; Janardhan et al; 1987**), animal weight (**Pickering et al; 1962**), its developmental stage, time of exposure and temperature (**Macek et al; 1969**). Thus, various factors influence the LD50 values. These factors are manifold and dependent upon the given set of experimental conditions (**Russell andOverstreet, 1987**). The LD50 values can also significantly vary between animals of the same basic strain obtained from different suppliers (**Russell and Overstreet, 1987),** according to the purity of the chemical (**Hoand Hoskins, 1986)** and to the sex differences (**Overstreet et al; 1979**).

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 andXu, 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 for15 days, 30 days and 60 days respectively. 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 affects blood glucose levels and total cholesterol level. Triglyceride content was substantially reduced by the effect of daidzein. No significant effect was observed on total protein content of the blood. Similar effect was previously observed in ovariectomized rats consuming a diet enriched in genistein (substitute of daidzein) (**Nogowski *etal*.,1998**).

VI.CONCLUSIONS

In the present investigation, the obtained LD50 value is 150 mg/kg BW. The results obtained in this experiment prove that 15 days, 30- days and 60 days sub-chronic intragastric administration of daidzein may change glucose level and parameters of lipid metabolism in male rats. This compound essentially decreased blood total cholesterol concentration and reduced the triglyceride content in blood serum. Mechanisms responsible for the detected activity of daidzein require further investigations.

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