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Study on the Effect of Genistein, a Soy Isoflavone in Insulin Tolerance in Albino Rat (*Rattus albicans*)

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Abstract: In the recent time, phytoestrogens have attracted much attention. Soy products which contain phytoestrogen genistein and diadzein are becoming increasingly popular in a wide range of food products including infant food. Different food substances tend to influence the blood glucose level through the insulin tolerance of the target cell. The effect of genistein, a soy-based isoflavone was studied on insulin tolerance in inbred albino rats of 03 months of age having an average weight of 110gm. Two different dosages of genistein viz 0.2mg/kgbw/day and 0.4mg/kgbw/day was administered in two different groups respectively. The present investigation reveals that insulin tolerance gradually decreases after administration of genistein.
Keywords: Phytoestrogen, genistein, blood glucose, insulin tolerance.

INTRODUCTION

Worldwide the rates of diabetes and other metabolic diseases have exploded over the last several decades. More than 170 million individuals currently suffer from diabetes across the globe and this number is projected to reach around 366 million by 2030 [1]. This scenario has resulted in significant individual morbidity and mortality [2]. As such every effort must be made to understand the factors underlying this emerging metabolic disorder to mitigate its impact on the individual and society. With obesity and hyper glycaemia reaching alarming proportions in the developed world [3], the role of insulin tolerance, insulin secretion and its sequel is gaining prominence [4]. Metabolism of carbohydrates is an essential factor for establishment and maintenance of normoglycaemia in the blood.

Metabolism of carbohydrate is a complex process highly regulated by both peptide and steroid hormone and is influenced by diet [5]. Diet is a major factor in the study of metabolic disorders like diabetes mellitus. Diet of an individual found to influence the blood glucose level through insulin tolerance of the target cells. In this regard, it is to be mentioned that, in the recent times, phytoestrogens, mainly soy isoflavones have gained increased attention [5, 6]. Phytoestrogens are diverse group of naturally occurring phenolic, non-steroidal compounds that are natural components of certain plant foods such as soybeans, beans, cabbage, grains and hops, and are part of a wider class of polyphenols found in all plants and fungi. Structurally, they are similar to mammalian endogenous estrogen (17β-estradiol) and thus they have the ability to interact with the estrogen receptors (ERα and ERβ) and eventually cause estrogenic or /and antiestrogenic effects through their affinity for and binding to estrogenic receptors [7]. Phytoestrogens are defined by the British Working Group on phytoestrogens of the Committee of Toxicity of Chemicals in Food, Consumer Products and the Environment of the Food Standards Agency as *"any plant substance or metabolite that induces biological responses in vertebrates and can mimic or modulate the actions of endogenous estrogens usually binding to estrogen receptors".* The Working group on Phytoestrogens and Health [8] classified phytoestrogens according to their chemical structures into flavonoids (including isoflavones and prenylflavonoids), coumestans, and lignin's (non-flavonoid phytoestrogens). Among these the major bioactive isoflavones are genistein and diadzein. The most abundant food sources of isoflavone are soybean and soybean products [5]. The use of soy containing infant food is on the rise and as such human are exposed to soy -based food from infant stage. The isoflavone genistein and diadzein that is present in raw beans primarily as genestin and diadzin [9] are heat stable and show substantial carry over through the regular processing methods [10]. Recently concern has been expressed that the exposure to soy isoflavones may pose a developmental hazard to infants [11-15]. On the other hand, genistein and other major isoflavones have been detected in the blood and urine of animals and human. In healthy humans, taking soy-less diet,

plasma concentrations of isoflavones are in the nanomolar range [16]. However, there is a marked increase in the plasma concentrations in the micro molar range after ingestion of isoflavones from soy-milk [17], soy-meal [18]. In populations consuming food rich in isoflavone, plasma isoflavone concentration of 1-4µmol/L have been reported [19-21]. These isoflavones are strikingly similar in chemical structure to mammalian estrogens [22]. When the structures of the isoflavone metabolite and estradiol are overlaid, they can be virtually superimposed. Due to their structural similarity with estrogen, they have an ability to bind to estrogen receptors in various cells and exert estrogenic or anti-estrogenic effects [5]. Genistein, the primary soy-derived isoflavone also has an estrogenic effect of binding to estrogen receptors [23] and inhibiting protein tyrosine kinases [24, 25]. Although the endocrine pancreas is not a classic estrogen target, estrogen receptors are present in the islets of Langerhans [26].

In this regard few data exist on whether genistein has a direct effect on pancreatic beta cells [6]. Several earlier studies have shown that genistein stimulates insulin secretion from pancreatic beta cell line [27] and cultured islets [25, 28] whereas other studies have found an inhibitory effect on insulin secretion [29, 30]. Therefore, it is still unclear whether genistein, at physiological dose, can affect pancreatic beta cells. The present investigation was conducted to assess the chronic effect of genistein on the rise and fall of blood glucose level in different time interval through intraperitoneal insulin tolerance test (IPITT). The objective of the present investigation is to assess the insulin tolerance of the albino rats through the blood glucose level at different time interval of the IPITT.

MATERIALS AND METHODS

Test Animal

Albino rats (*Rattus albicans*) of 03 months age and weighing 100-110gm were taken from the Animal Housing facility, Dept of Zoology, Gauhati University were used for the experiments. Rats were given standard animal diet comprising of wheat bran, maize bran, flour and oil cake along with vitamins and minerals (Agrimin forte). Animals had access to water *ad libitum*. The animals were acclimatized for 07 days prior to treatment with natural dark and light periods at room temperature. All animal experimentation was done in accordance with the institutional ethical guidelines.

Intraperitoneal Insulin Tolerance Test:

The IPITT was conducted following the standard MMPC Intraperitoneal Insulin Tolerance Test protocol. Rats were fasted for 04 hours only by taking away food, while giving them access to water *ad libitum.* The rats were divided into five different groups**.**

Group I: Normal control group (rats received no test chemical);
Group II: Vehicle control group (rats received olive oil injection);
Group III: 17-beta estradiol treated group @ dose of 0.2mg/kgbw/day;
Group IV: Genistein treated group @ dose of 0.2mg/kgbw/day;
Group V: Genistein treated group @ dose of 0.4mg/kgbw/day
(n=5 in each group).
Duration of treatment = 28 days.

Rats were assigned to groups (control and treated) randomly and the weight of each group was not statistically different from the other. Rats in each of the experimental groups were fasted for 04 hours by only taking away while giving them access to water *ad libitum*. Blood sample was drawn from tail of 04 hours fasted rats and fasting blood glucose level was measured. This was used as baseline (t=0). Insulin was injected intraperitoneally at a dose of 0.5U/Kg bw, using a 27G needle. Blood glucose was measured at 15min, 30 min, 45 min, 60 min and 120 min respectively of post administration of insulin load using a glucometer.

STATISTICAL ANALYSIS

Data are expressed as mean ± S.E.M. Level of statistical significance was determined by performing t-test (at 95% confidence level) using statistical package SPSS.

RESULTS

During the 2 hour IPITT, the blood glucose recorded showed a clear trend across all experimental groups. The rats witnessed a fall in blood glucose immediately after exogenous insulin administration indicating insulin tolerance i.e. normal sensitivity to insulin. This was eventually followed by an elevation of blood glucose level which continued till 120 minute.

Immediately after 15 minutes of intraperitoneal administration of exogenous insulin load, fall in blood glucose level has been observed in rats of all the experimental groups. In Group I i.e. control group of rats the blood glucose level falls from a basal range of 86.80mg/dl to 77.60mg/dl($p<0.05$) in 15 minutes, from 77.60 mg/dl to 73.00 mg/dl($p<0.05$) in 30 minutes; from 73.00 mg/dl to 68.00 mg/dl($p<0.05$) in 45 minute. This was followed by a gradual elevation in blood glucose level from 68.00 mg/dl to 71.00 mg/dl($p<0.05$) in 60 minute and upto 82.80 mg/dl in 120 minutes($p<0.05$). Similar trend was also exhibited by Group II i.e. the olive oil treated (vehicle control group) with blood glucose level falling from basal range of 88.40 mg/dl to 67.00 mg/dl($p<0.05$) in 45 minutes followed by a gradual increase from 67.00 mg/dl to 82.40 mg/dl($p<0.05$) in 45 minutes to 120 minutes. This trend indicates normal insulin tolerance of the target

cells of the subject animals. However, in the Group III i.e. estradiol (E2) treated rats; the blood glucose level shows an insignificant fall from basal level of 115.60 mg/dl to 112.30 mg/dl($p>0.05$) in 15 minutes, to 108.40 mg/dl in 30 minutes($p>0.05$), to 88.40 mg/dl in 45 minutes. This was followed by a significant increase in blood glucose level to 91.20 mg/dl in 60 minutes and upto 101.80 mg/dl($p<0.05$) in 120 minutes. The blood glucose recorded in E2 treated group at 60 minute and 120 minute in the post-insulin administration period is significantly high in comparison to that of corresponding blood glucose level in control and vehicle control groups. This insignificant fall in blood glucose level after insulin administration and higher elevation of blood glucose level in the later time of the test period indicates a state of altered insulin tolerance in the treated animals. A similar trend has been exhibited by the blood glucose level recorded during the two hour IPITT in the genistein treated groups. In the genistein treated group IV animals, the blood glucose level shows an insignificant fall from basal level of 101.60 mg/dl to 98.20 mg/dl($p>0.05$) in 15 minutes, to 96.30 mg/dl in 30 minutes($p>0.05$), to 98.50 mg/dl in 45 minutes. This was followed by a significant increase in blood glucose level to 99.40 mg/dl($p<0.05$) in 60 minutes and upto 100.00 mg/dl($p<0.05$) in 120 minutes. In the genistein treated Group V the blood glucose level of treated rats show an insignificant fall from basal level of 106.60 mg/dl to 103.00 mg/dl($p>0.05$) in 15 minutes, to 99.02 mg/dl($p>0.05$) in 30 minutes, to 100.05 mg/dl in 45 minutes. This was followed by a significant increase in blood glucose level to 102.35 mg/dl($p<0.05$) in 60 minutes and upto 105.00 mg/dl($p<0.05$) in 120 minutes. The elevation of blood glucose level in group IV and Group V rats at 60 minute and 120 minute interval were significantly higher than their corresponding elevated level in control group ($p<0.05$). The trend of blood glucose recorded in Group IV and Group V exhibits a similar trend with that of estradiol treated rats indicating a gradual loss of insulin tolerance in the target cells.

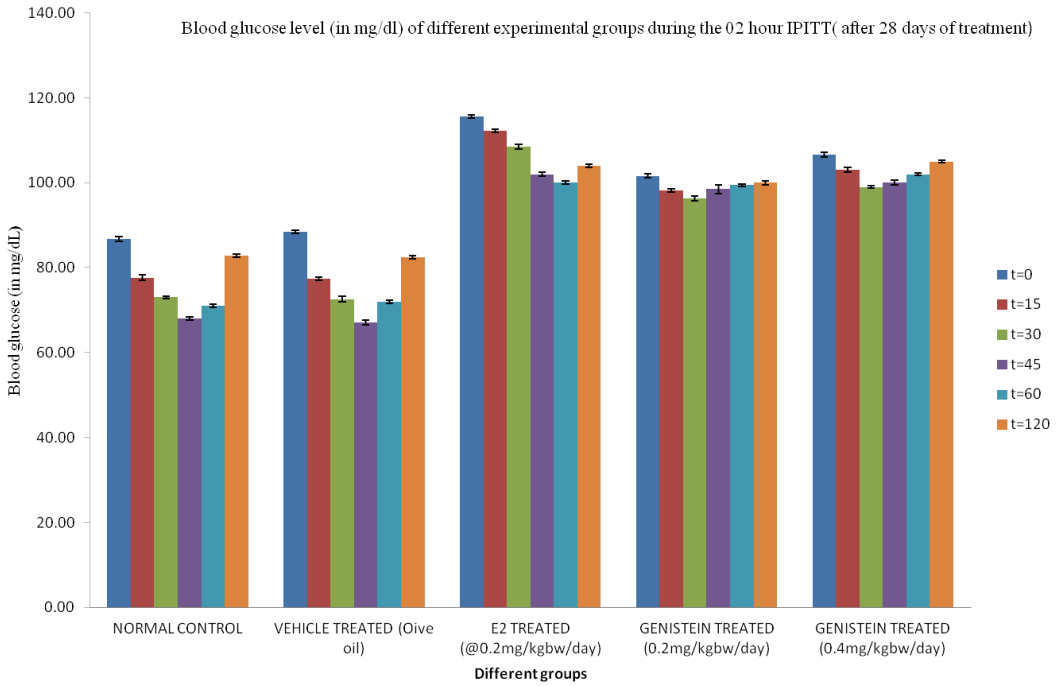
Table-I: Blood glucose level (in mg/dl) during IPITT (after 28 days of treatment)

Time (in min) ↓	Blood glucose level (in mg/dl)in various experimental groups.				
	NORMAL CONTROL	OLIVE OIL TREATED	E2 TREATED (@0.2mg/kgbw/day)	GENISTEIN TREATED(@ 0.2mg/kgbw/ day)	GENISTEIN TREATED(@ 0.4mg/kgbw/ day)
O min	86.80 ± 0.58	88.40 ± 0.51	115.60 ± 0.40	101.60 ± 0.40	106.60 ± 0.51
15 min	77.60± 0.68*	77.40 ± 0.51*	112.30 ± 0.37	98.20 ± 0.37	103.00 ± 0.37
30 min	73.00 ± 0.32*	72.60 ± 0.24*	108.40± 0.58	96.30 ± 0.55	99.02± 0.49
45 min	68.00 ± 0.32*	67.00 ± 0.55*	88.00 ± 0.55*	98.50 ± 0.51*	100.05 ± 1.05*
60 min	71.00 ± 0.32*	72.00 ± 0.32*	91.20 ± 0.37*#	99.40± 0.40*#	102.35± 0.24*#
120 min	82.80 ± 0.37*	82.40 ± 0.24*	101.80 ± 0.37*#	100.00 ± 0.37*#	105.00± 0.49*#

Data are presented as mean ± S.E.M

* Significantly different from blood glucose at 0 minutes after 04 hour fasting ($p<0.05$)

Significantly different from blood glucose at corresponding time interval in the control group ($p<0.05$)



Data are presented as means ± S.E.M

Fig-1: Graphical representation of blood glucose level (in mg/dl) during IPITT (after 28 days of treatment)

DISCUSSION

It has been suggested that glucose metabolism is highly regulated by peptide hormone insulin and is influenced by diet [5]. In the present study, in the control rats, initially after administration of exogenous insulin, the concentration of insulin in the blood increases causing hypoglycemia by increasing the rate of glycolysis and cellular glucose uptake in the target cells. This increased activity may in-turn lead to stress of the hormone receptors thereby decreasing the activity of insulin which is evident by rise in blood glucose level. The results of E2 treated group illustrate a subnormal biologic response of insulin indicating a state of loss of insulin tolerance which also supports similar findings in earlier works [31, 32]. They observed that longer exposure to E2 induced an increase in pancreatic beta cell insulin content in an estrogen-receptor-dependent manner. They found that the animals developed chronic hyperinsulinemia and their glucose and insulin tolerance were altered, which is also evident from our findings. It has been reported that estradiol acts at least in part through ER alpha and increase insulin production [33]. This increase in insulin production may eventually lead to chronic hyperinsulinemia and gradual loss of insulin tolerance. Interestingly, in the present study, it has been observed that geinstein at the given dosages results in loss of insulin tolerance in the target cells of the treated subjects in a manner similar to that of E2. This may be attributed to the estrogenic effects of geinstein [23]. Finally, it can be pointed out that our results are to a certain extent in concordance with some earlier observations [6, 34]. They found that dietary soy isoflavones and geinstein acutely stimulates insulin secretion. However, this may result in altered insulin tolerance assisted with elevation of blood glucose levels indicating a sub-normal biological response of insulin hormone.

CONCLUSION

From the present investigation, it has been observed that there is a gradual loss of insulin tolerance in the target cells of the genistein treated rats. This loss in insulin tolerance is similar to that of E2 treated rats indicating the estrogenic effect of genistein. Insulin tolerance also exhibits a time-dependant response as the insulin tolerance tends to falls with the increase in the time of post-insulin administration period. However, further investigations are necessary in this regard to establish the correlation between exogenously administered insulin with the endogenous insulin during the intraperitoneal insulin tolerance test.

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