Genistein, a Soy Isoflavone Acutely Alters Insulin Tolerance in Albino Rat (Rattus albicans)

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ABSTRACT: Globally, over the last few decades, there is marked increase in the rates of hyperglycaemia and other metabolic diseases. Of late, phytoestrogens have gained much attention owing to its extensive dietary availability. Consumption of soyfood products which contain phytoestrogen genistein and diadzein are increasing. Overtime, different food substances tend to influence blood glucose level through the insulin tolerance of the target cells. In the present investigation, 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 were administered in two different groups of rats respectively. The present investigation reveals that, genistein in the above dosages, acutely alters insulin tolerance in the treated rats.

Key words: Phytoestrogen, genistein, blood glucose, insulin tolerance.

1. INTRODUCTION

The last several decades have witnessed a global surge in the rates of diabetes and other metabolic diseases. It is estimated that, worldwide, more than 170 million individuals currently suffer from diabetes and this number is projected to reach a staggering 366 million by 2030 (Wild *et al*., 2004). As, obesity and hyperglycaemia are increasing at an alarming rate in the developed world (World Health Organisation, Geneva 2000), the role of insulin tolerance, insulin secretion and its sequel is gaining prominence (Wilcox, 2005). 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, have gained much attention owing to its extensive dietary availability (Clarkson *et al*., 1995). 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, classified phytoestrogens according to their chemical structures into flavonoids (including isoflavones and prenylflavonoids), coumestans, and lignans (non-flavonoid phytoestrogens) (Hughes *et al*., 2003). Among these soy isoflavones like genistein and diadzein have gained increased attention (Bhathena *et al*., 2002, Liu *et al*., 2006). This is perhaps, because of increased consumption of soybeans and soyfood products (Messina and Messina, 1991). The most abundant food sources of isoflavone are soybean and soybean products (Franke *et al*., 1991; Bhathena *et al*., 2002). 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 genistin and diadzin (Murphy *et al*., 1982) are heat stable and show substantial carry over through the regular processing methods (Miksicek *et al*., 1995). Recently concern has been expressed that the exposure to soy isoflavones may pose a developmental hazard to infants (Setchell, 1985, Clarkson *et al*., 1995, Irvine *et al*., 1995, Sheehan *et al*., 1995). 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 (Morton *et al*., 1994). However, there is a marked increase in the plasma concentrations in the micromolar range after ingestion of isoflavones from soy-milk (Xu *et al*., 1994), soy-meal (King *et al*., 1998). These isoflavones are strikingly similar in chemical structure to mammalian estrogens (Setchell and Adlercreutz, 1988). 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 (Bhathena *et al*., 2002). Genistein, the primary soy-derived isoflavone also has an estrogenic effect of binding to estrogen receptors (Kuiper *et al*., 1997) Although the endocrine pancreas is not a classic estrogen target, estrogen receptors are present in the islets of Langerhans (Nadal *et al*., 2000).

In this regard few data exist on whether genistein has a direct effect on pancreatic beta cells (Liu *et al*., 2006). Several earlier studies have shown that genistein stimulates insulin secretion from pancreatic beta cell line (Ohno *et al*., 1993) and cultured islets (Sorenson *et al*., 1994, Jonas *et al*., 1995) whereas other studies have found an inhibitory effect on insulin secretion (Jones *et al*., 1994, Persaud *et al*., 1999). Therefore, it is still unclear whether genistein, at physiological dose, can affect pancreatic beta cells. The present investigation was conducted to assess the acute 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.

2. MATERIAL AND METHOD

2.1. Test Animal: Albino rats (*Rattus albicans*) of 03 months age and weighing 100-110gm 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 guidelines of the Institutional Animal Ethical Committee (I.A.E.C.), Gauhati University (*vide* Ref. No. IAEC/PER/2017/RF/BBC/AS/2017-2).

2.2. 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(E_2) 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 = 04 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 food 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. Post administration of insulin load, blood glucose was measured at 15min, 30 min, 45 min, 60 min and 120 min respectively using a glucometer.

2.3. Statistical Analysis: Data are expressed as mean ± S.E.M. Level of statistical significance was determined by performing t-test ($p<0.05$) using statistical package SPSS.

3. RESULT

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.

Table I presents the results of the IPITT conducted on experimental group I (normal control), group II (vehicle control i.e. olive oil treated group) group III (E_2 treated group at the dose of 0.2mg/kgbw/day), group IV (genistein treated at the dose of 0.2mg/kgbw/day) and group V (genistein treated at the dose of 0.4mg/kgbw/day) at the end of 04 days study. During the 2 hour IPITT, the blood glucose exhibited a trend across all experimental groups. The rats witnessed a fall in blood glucose immediately after administration of exogenous insulin load which was followed by an eventual elevation of blood glucose which continued until 120 minutes. In group I the blood glucose level drops significantly from 85.00 mg/dl (at 0 minutes) to 75.00 mg/dl after 15 minutes of insulin administration ($p<0.05$), to 71.20 mg/dl in 30 minutes ($p<0.05$) and 66.00 mg/dl in 45 minutes ($p<0.05$). This was followed by an elevation in blood glucose to 70.00 mg/dl in 60 minutes and 81.20 mg/dl in 120 minutes. Similar trend was exhibited by the group II with blood glucose dropping from 87.00 mg/dl (at 0 minutes) to 74.80 mg/dl in 15 minutes ($p<0.05$), 70.60 mg/dl in 30 minutes ($p<0.05$), 67.00 mg/dl in 45 minutes ($p<0.05$) and then eventually rising to 71.40 mg/dl in 60 minutes ($p<0.05$) and 82.00 mg/dl in 120 minutes ($p<0.05$). However, the blood glucose in group II did not show any significant difference with that of group I in any of the time points. In group III, the blood glucose at each of the time points are significantly higher than that of group I ($p<0.05$). In 0 minute the blood glucose was 91.00 mg/dl which decreased to 84.40 mg/dl in 15 minutes($p<0.05$), 77.40 mg/dl in 30 minutes($p<0.05$), 72.80 mg/dl in 45 minutes($p<0.05$) and then rose to 78.00 mg/dl in 60 minutes($p<0.05$) and 87.40 mg/dl in 120 minutes($p<0.05$) which is significantly higher when compared to that of blood glucose in group I in each of the corresponding time points($p<0.05$) indicating a trend of decreased insulin tolerance in the subject animals of the group III. Group IV and group V shows a similar trend with that of group III. In group IV, the blood glucose at 0 minutes 88.00 mg/dl decreased significantly to 81.40 mg/dl in 15 minutes post insulin administration ($p<0.05$), 75.60 mg/dl in 30 minutes ($p<0.05$), 70.00 mg/dl in 45 minutes ($p<0.05$) and then gradually elevated to 75.60 mg/dl in 60 minutes ($p<0.05$) and 84.80 mg/dl in 120 minutes ($p<0.05$). The blood glucose at each of the time points in group IV was significantly higher than the blood glucose in group I ($p<0.05$) again indicating a decreased insulin tolerance. In group V the blood glucose in 0 minute was 89.60 mg/dl which decreased significantly to 82.20 mg/dl at 15 minutes after insulin administration ($p<0.05$), followed by a gradual fall to 76.20 at 30 minutes ($p<0.05$), 71.20 mg/dl at 45 minutes ($p<0.05$) and eventually increasing to 76.60 mg/dl in 60 minutes and 85.60 mg/dl in 120 minutes. The blood glucose in group V in each of the time points was significantly higher than that of group I $p<0.05$) indicating its similar trend of decreased insulin tolerance with that of group III and group IV. 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 (in mg/dL) during the 02 hour IPITT in different experimental groups (after 04 days treatment).
Data presented as Mean±S.E.M. (n=5)
[*] Significantly different from blood glucose (in mg/dL) compared to O min of each corresponding group ($p<0.05$).
[#] Significantly different from blood glucose (in mg/dL) compared to corresponding time interval in Group I i.e. Normal control ($p<0.05$).

Groups	Blood glucose (in mg/dL)					
	0 min	**15min**	**30 min**	**45 min**	**60min**	**120 min**
Group I Normal control	85.00±0.71	75.00±0.71*	71.20±0.58*	66.00±0.71*	70.00±0.55*	81.20+0.58*
Group II Vehicle control (Olive oil treated)	87.00±0.71	74.80±0.58*	70.60±0.60*	67.00±0.71*	71.40±0.51*	82.00+0.73*
Group III E2 treated (0.2mg/kgbw/day	91.00±1.14#	84.40±0.93*#	77.40±0.93*#	72.80±0.58*#	78.00±0.71*#	87.40+0.93*#
Group IV Genistein treated (0.2mg/kgbw/day	88.00±0.71#	81.40±0.93*#	75.60±0.93*#	70.00±0.71*#	75.60±0.51*#	84.80+0.80*#

Group V Genistein treated (0.4mg/kgbw/day	89.60±0.51$^{\#}$	82.20±0.37$^{*\#}$	76.20±1.24$^{*\#}$	71.20±0.58$^{*\#}$	76.60±0.68$^{*\#}$	85.60+0.66$^{*\#}$

4. DISCUSSION

Result from the present study reveals that in the control rats, initially after administration of exogenous insulin, the concentration of insulin in the blood increases causing hypoglycaemia 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 E_2 treated group illustrate a subnormal biologic response of insulin indicating a state of decreased insulin tolerance (Jeffrey *et al*., 1983). Our observation supports the findings of Magdalena *et al*. (2006). They observed that exposure to E_2 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 insulin tolerance was altered, which is also evident from our findings. May *et al*. (2006) reported that estradiol acts at least in part through ER alpha and increase insulin production. 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 genistein at the given dosages results in decrease of insulin tolerance in the target cells of the treated subjects in a manner similar to that of E_2. This, perhaps, may be attributed to the estrogenic effects of genistein (Kuiper *et al*., 1997). It can be stated that, our results, are to a certain extent in concordance with some earlier observations (Liu *et al*., 2006 and Lu *et al*., 2008). They found that dietary soy isoflavones and genistein 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.

5. CONCLUSION

From the present investigation, it has been observed that there is a gradual decrease of insulin tolerance in the target cells of the genistein treated rats. This decline in insulin tolerance is similar to that of E_2 treated rats indicating the estrogenic effect of genistein. Insulin tolerance also exhibits a time-dependant response as the insulin tolerance tends to fall with the increase in the time of post-insulin administration period. However, further studies are essential 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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