ORIGINAL ARTICLE

Comparison of Postprandial Insulin Levels in Withania Coagulans and Liraglutide Treated Diabetic Rats

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ABSTRACT

Objective: To investigate and compare the antidiabetic effect of withania coagulans and Liraglutide on serum postprandial insulin levels in streptozotocin induced diabetes in rats.

Study Design: Randomized Control Trial.

Place and Duration of Study: The study was conducted from 1st April 2016 to 31st March 2017 at Islamic International Medical College in collaboration with National Institute of Health Islamabad.

Materials and Methods: Total 40 male Sprague dawley rats were randomly divided into two groups; Group A (n=10) and Experimental Group (n=30). Group A was given normal diet for 5 days whereas experimental group was given normal diet plus streptozotocin (30mg/kg/day) intraperitoneally for 5 days and diabetes was confirmed in experimental group by fasting blood glucose (mg/dl). Experimental group was further divided into B (Diabetic control), C (Withania coagulans treated) and D (Liraglutide treated). First sampling group A and B was done after 5 days. Group C were given Withania coagulans and Group D were given Liraglutide along with normal diet for 30 days. Second sampling (fasting blood glucose, postprandial glucose and insulin level) was done from group C and D.

Results: Fasting blood glucose of group C (98 mg/dl ± 1.80) was significantly reduced than group D (102 mg/dl ± 2.04). Postprandial glucose and insulin levels of Group D (163 mg/dl ± 3.95 and 6.06 µU/ml ±0.17) were significantly decreased and increased as compared to Group C PPG (183 mg/dl ± 6.30) and insulin level (5.54 µU/ml ± 0.23).

Conclusion: Withania Coagulans significantly improves postprandial insulin levels as compared to liraglutide.

Key Words: *Diabetes, Insulin, Liraglutide, Withania Coagulans.*

Introduction

Diabetes mellitus is a disorder of carbohydrate metabolism in which the body sugar is unable to be oxidized due to lack or improper functioning of pancreatic hormone mainly insulin.[1,2] Type 2 diabetes is a medical condition characterized by hyperglycemia in which glucose is not metabolized due to insulin resistance and pancreatic beta cells can not compensate this insulin resistance.[3,4] Studies have shown that in type 2 diabetes pancreas fails to produce sufficient quantity of insulin in response to glucose stimulation which leads to chronic postprandial and fasting hyperglycemia which causes serious complications leading to excess morbidity and mortality.[5,6]

A number of pharmaceutical preparations are available for treating type 2 diabetes which targets insulin release from pancreas like sulphonylurea. These formulations are found to have a number of clinical limitations, the most serious long term limitation being the eventual need for insulin replacement therapy. These drugs which stimulate insulin secretion from pancreas are also associated with a common side effect-hypoglycemia. A new drug was discovered called Liraglutide (GLP-1 mime tics) which causes insulin secretion from pancreas only in response to hyperglycemia to avoid the risk of producing hypoglycemia and have enjoyed a great deal of success in treating type 2 diabetes.[7,8] Basic mechanism of action of liraglutide is through enhancement of insulin secretion from pancreas and

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can also be taken in combination with insulin sensitizer like metformin.[9,10]

The main drawback of this drug is that these formulations are not available in oral form so it has to be administered parentally. Continuous subcutaneous or intravenous administration of this drug is another challenge faced by patients of type 2 diabetes who take liraglutide.[11] Due to adverse effects of hypoglycemic drugs and exogenous insulin, use of medicinal plant has become a common practice as these are reported to have very less side effects and are easily accessible and affordable. In this category of medicinal plants, Withania Coagulans (paneer doda) belongs to a family Solanaceae is a famous herb known long for its antidiabetic property. This plant is cultivated in India, Pakistan, Iran and Afghanistan.[12,14]

Different compounds of Withania Coagulans have been studied for their different medicinal properties including phenolic compounds, fruit berry extract, seeds, flowers and alcoholic extract. Aqueous extract of Withania Coagulans (aqWC) dried fruits have specially been studied for its antidiabetic property because of presence of active compound called Withanolides in it. Several studies have proved that aqueous extract of Withania Coagulans has shown significant improvement in blood glucose levels and its effects were compared with biguanides and sulphonylureas.[15,16] Studies have also revealed that Withania coagulans treated rats exhibits increase level of serum insulin and results were compared with glibenclamide(sulphonylurea).[17,18] None of the previous studies has attempted to elucidate the level of postprandial insulin level and its comparison with GLP-1 analogues. So the aim of this study was to investigate the effect of aqueous extract of Withania Coagulans (aqWC) and Liraglutide on postprandial insulin, fasting and postprandial blood glucose levels, along with their comparison.

Materials and Methods

This Randomized Control Trial was carried out at Physiology department and Multidisciplinary research laboratory, Islamic International Medical College, Rawalpindi in collaboration with NIH animal house after approval from Ethics Review Committee.

Rats weighing between 200-300 grams were included in the study. Rats were first allowed to get acclimatized for one week in the NIH Animal house in 50-70% humidity at a room temperature of 24$\pm$2°C with a 12 hour light and dark cycle.

A total 40 male Sprague dawley rats were taken and randomly divided into two groups; Group A (n=10) and Experimental Group (n=30). Group A was given normal diet for 5 days whereas experimental group was given normal diet plus streptozotocin (30mg/kg/day) intraperitoneally for 5 days. After 5 days, diabetes was confirmed in experimental group by measuring and comparing Fasting blood glucose levels (mg/dl) with group A. Experimental group was then further divided into three groups i.e. B (Diabetic control), C (Withania coagulans treated) and D (Liraglutide treated). First sampling of the experiment was done after 5 days from group A and B which included postprandial glucose (mg/dl), serum insulin (µU/ml). Group C rats were given normal diet along with aqueous extract of Withania coagulans (1000mg/kg/day) orally mixed in drinking water for 30 days. Group D rats were given normal diet along with Liraglutide (Victoza pen) drug (0.3mg/kg/day) subcutaneously for 30 days. After 30 days of treatment, second sampling of the experiment was done from group C and D which included fasting blood glucose (mg/dl), postprandial glucose (mg/dl), and serum postprandial insulin (µU/ml).

The dried fruits of Withania Coagulans were identified and authenticated (# IIMC 03) by herbarium section of National Agriculture Research Center (NARC) Islamabad. Aqueous extract was prepared in Multi-disciplinary research labortatory IIMC at a dose 250mg/ml and was used for experimental work. Liraglutide belongs to GLP-1 mimetics was administered subcutaneously at a dose of 0.3mg/kg body weight for 30 days to diabetic rats.

Sample for blood glucose was collected from rat tail vein with 1 ml syringe. Blood glucose levels were assessed using the EASY GLUCO Ultra plus Auto Coding meter by Isotech Co.Ltd., Korea. Through Intracardiac sampling 2 mL of blood was withdrawn .The blood was saved in labeled gel tubes and kept in a laboratory ice box at 2-8 $^{\circ}$C until they were analyzed for insulin estimation (ng/mL) which was done using Sandwich-ELISA method. Statistical analysis was done applying the SPSS 21. Results were documented as mean $\pm$ SEM. Comparisons among the two groups was analyzed using the independent

sample t-test and correlation among the variables was done using Pearson's correlation coefficient. P value of <0.05 was considered significant for both analyses.

Results

Fasting Blood Glucose (FBG) mg/dl

Fasting blood glucose (mg/dl) levels in group B rats (131 ± 3.05mg/dl) were significantly higher (P<0.05) than group A rats (80 ± 3.19mg/dl). While on comparison FBG levels in group C rats (98 ± 1.80mg/dl) and group D rats (102 ± 2.04) were significantly lower (P<0.05) than group B rats (131 ± 3.05mg/dl). Comparison of Mean ± SEM of FBG mg/dl levels in all four Groups (A, B, C, D) are shown in Table I.

Postprandial Glucose (PPG) mg/dl

Comparison of Mean ± SEM of PPG (mg/dl) in all four Groups (A, B, C, D) is displayed in Table I. Group B rats showed increased PPG (330± 15.95mg/dl) levels which were significantly higher (P<0.05) than Group A rats (143 ±5.34mg/dl). Significantly lower (P<0.05) PPG levels of group C rats (183± 6.30mg/dl) and Group D rats (163 ±3.95 mg/dl) were observed on comparison with Group B (330± 15.95mg/dl) rats.

Serum Insulin Levels (µU/ml)

Serum insulin levels of Group B were observed to be (3.5 ± 0.19 µU/ml) which were significantly reduced (P<0.05) as compared to the group A (5.9 ± 0.19 µU/ml) rats. While serum insulin levels of group C rats (5.54 ± 0.23 µU/ml) and Group D (6.06 ± 0.17 µU/ml) rats were significantly raised (P<0.05) as compared to the Group B (3.5 ± 0.19 µU/ml) rats. Comparison of Mean ± SEM of Serum Insulin (µU/ml) levels in all four Groups (A, B, C, D) is shown in Table I.

Table I: Comparison of Mean ± SEM of Fasting Blood Glucose (FBG) mg/dl, Postprandial glucose (PPG) mg/dl and Serum Insulin (µU/ml) levels in all four Groups (A,B,C,D):

Parameters	Group A (Control)	Group B (Diabetic)	Group C (WC treated)	Group D (Liraglutide treated)
FBG (mg/dl)	80 ±3.19	131 ± 3.05*[a]	98 ± 1.80*[b]	102 ± 2.04*[c]
PPG (mg/dl)	143 ±5.34	330± 15.95*[a]	183± 6.30*[b]	163 ±3.95*[c]
Serum Insulin	5.9 ± 0.19	3.5 ± 0.19*[a]	5.54 ± 0.23*[b]	6.06 ± 0.17*[c]

Withania coagulans **(**WC)

*=P < 0.05 is considered statistically significant.

*[a]= Group A vs B

*[b]= Group B vs C

*[c] =Group B vs D

Discussion

Type 2 diabetes is a major health problem with serious complications results in substantial health-care costs. Treatment of type 2 diabetes includes parenteral therapy (Insulin and GLP-1 mimetics) and oral hypoglycemic drug which included sulphonylurea, metformin and thiazolidiendiones. In the present study antidiabetic effect of withania coagulans and liraglutide on fasting, postprandial blood glucose and serum postprandial insulin level was evaluated along with their comparison.

Jaiswal et al have concluded that hypoglycemic potential of withania coagulans fruit may be due to activation of insulin gene expression through CREB (Calcium Responsive Element Binding protein) responsible for exocytosis of stored insulin from pancreatic beta cells.[13] Current study also seconds this fact concluded by by jaiswal et al as postparandial insulin level was raised after use of withania coagulans extract.

Fasting and postprandial glucose levels in the current study were also similar to the results of hamatha et al ho gave aqueous extract of withania coagulans for 7 days instead of 30 days as were in present study.[12] Their study revealed the antidiabetic and antihyperlipidemic effect of aqueous extract of Withania coagulans although serum postprandial insulin levels were not explored.

Results of the present study were also similar to results obtained by Shukla et al who concluded that along with blood glucose and insulin level, treatment of aqueous extract of Withania coagulans (500mg/kg/wt) has showed marked effect on increasing carbohydrate metabolizing enzymes i.e. glucokinase and phosphofructokinase.[14]

Serum insulin level were significantly increased in the results of present study which were inconsistent with the results of experiment done by Bharti SK et al who investigated the effect of aqueous extract of withania coagulans in poloxomer-induced diabetic rats. The study mentioned that administration of aqueous extract of withania coagulans (200mg /kg body wt) to diabetic rats for 5 weeks causes significant decrease in serum fasting insulin level and insulin resistance (measured by HOMA-IR) on comparison with those in diabetic rats.[15] This dissimilarity between the results of two studies could be due to the reason that in present study

postprandial insulin level was measured whereas Bharti SK et al estimated fasting serum insulin level.

Results of the present study were also similar to the Study conducted by Davies et al on type 2 diabetic patients who evaluated the efficacy of liraglutide on serum insulin levels.[19]

Upadhyay and vandata concluded that powder of Withania coagulans (10 g) when taken orally by diabetic patients showed marked improvement in fasting and postprandial glucose level which was similar to the results in present study in which aqueous extract of Withania coagulans (1000mg/kg) were administered to the diabetic rats.[20]

Another human study carried out by Alam A et al showed that administration of 150 ml of water containing 10 seeds of Withania coagulans twice a day showed reduction in serum level of fasting and postprandial glucose which were similar to the results of present study carried out in streptozotocin induced diabetic rats.[18] In current study because of cost and availability issue immunohistochemistry of pancreas tissue was not done. Further studies need to be conducted on aqueous extract of Withania Caogulans dried fruits on microscopic morphological features of intestinal L cells which release glucagon like Peptide-1.

Conclusion

Withania coagulans reduces fasting and postprandial blood glucose levels while increasing serum insulin levels. This can be used as a better treatment option for type 2 diabetes because of its oral intake and lack of adverse effects usually caused by liraglutide.

REFERENCES

1. Tierna LM Jr., Mephee SJ, Papadakis MA. Diabetes mellitus and hypoglycemia. Current medical diagnosis and treatment. 41st ed. New York: McGraw Hill, 2002; 1203-44.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care. 2004; 27(5): 1047-53.
3. Russell-Jones D, Vaag A, Schmitz O, Sethi BK, Lalic N, Antic S et al. Liraglutide vs insulin glargine and placebo in combination with metformin and sulfonylurea therapy in type 2 diabetes mellitus (LEAD-5 met+ SU): a randomised controlled trial. Diabetologia. 2009; 52(10): 2046-55.
4. Morris DH, Khunti K, Achana F, Srinivasan B, Gray LJ, Davies MJ et al. Progression rates from HbA1c 6.0–6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis Diabetologia. 2013; 56(7): 1489-93.
5. White WB, Pratley R, Fleck P, Munsaka M, Hisada M, Wilson C, Menon V. Cardiovascular safety of the dipeptidyl peptidase-4 inhibitor alogliptin in type 2 diabetes mellitus. Diabetes, Obesity and Metabolism. 2013; 15(7): 668-73.
6. Kaneto H. Pancreatic β-cell failure in type 2 diabetes mellitus. Expert Review of Endocrinology & Metabolism. 2016; 11(1): 1-2.
7. Ismail-Beigi F. Glycemic management of type 2 diabetes mellitus. New England Journal of Medicine. 2012; 366(14): 1319-27.
8. DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. Annals of internal medicine. 2000; 133(1): 73.
9. Kolterman, O.G et al. Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with T2DM. J. Clin. Endocrinol. Metab. 2003; 132(5): 3082-3089.
10. Nielsen, L.L.; Young, A.A.; Parkes, D.G. Pharmacology of exenatide (synthetic exendin-4): A potential therapeutic for improves glycemic control of T2DM. Regul. Pept. 2004; 117: 77-88.
11. Pabreja, K., et al. "Molecular mechanisms underlying physiological and receptor pleiotropic effects mediated by GLP-1R activation. British journal of pharmacology.2014; 171(5): 1114-1128.
12. S.Hemlatha et al. Chansouria; Hypolipidemic activity of aqueous extract of Withania coagulans Dunal in albino rats. Phytotherapy Research.2006; 20 (7): 614– 617.
13. Jaiswal D, Rai PK, Watal G. Antidiabetic effect of Withania coagulans in experimental rats.Indian Journal of Clinical Biochemistry. 2009; 24(1): 88-93.
14. Shukla K et al. Hypolipidemic and antioxidant activity of aqueous extract of fruit of Withania coagulans (Stocks) Dunal in cholesterol-fed hyperlipidemic rabbit model.Indian Journal of Experimental biology. 2014; 56: 870-875.
15. Bharti SK et al. Antidiabetic effect of aqueous extract of Withania coagulans flower inPoloxamer-407 induced type 2 diabetic rats. Journal of Medicinal Plants Research. 2012; 6(45): 5706-13.
16. Shukla K, Dikshit P, Shukla R, Gambhir JK. The aqueous extract of Withania coagulans fruit partially reverses nicotinamide/streptozotocin-induced diabetes mellitus in rats. Journal of medicinal food. 2012; 15(8): 718-25.
17. Datta A etal. Antidiabetic and antihyperlipidemic activity of hydroalcoholic extracts of Withania coagulans Dunal dried fruit in experimental rat models. Journal of Ayurveda and integrative medicine. 2013;4 (2): 99-04.
18. Alam A, Siddiqui MY, Hakim MH. Clinical efficacy of Withania coagulans Dunal and Trigonella foenum-graecum Linn. in Type 2 Diabetes mellitus. Indian journal of traditional Knowledge. 2009; 8(3): 405-409.
19. Davies MJ et al.Efficacy of liraglutide for weight loss among patients with type 2 diabetes: the SCALE diabetes randomized clinical trial. Jama. 2015; 314(7): 687-99.
20. Upadhyay BN, Gupta V. A clinical study on the effect of Rishyagandha (Withania coagulans) in the management of Prameha (Type II Diabetes Mellitus). AYU (An international quarterly Journal of research in Ayurveda). 2011; 32(4): 507-12.