The Professional Medical Journal
www.theprofesional.com

DOI: 10.29309/TPMJ/2019.26.11.3155

ORIGINAL PROF-0-3155

ANTIDIABETIC EFFECT OF WITHANOLIDES AND LIRAGLUTIDE ON SERUM INSULIN LEVEL AND PANCREATIC HISTOLOGY IN DIABETIC RATS.

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Article received on:
19/01/2019
Accepted for publication:
13/04/2019

ABSTRACT… Objectives: Type 2 diabetes is characterized by hyperglycemia and occurs as a result of insulin resistance and pancreatic beta cells failure to compensate. Present study was conducted to determine the antidiabetic effect of Withania coagulans and liraglutide on postprandial serum insulin levels and pancreatic histological features in streptozotocin induced diabetic rat. **Study Design:** This Randomized Control Trial. **Setting:** At multidisciplinary lab Islamic International Medical College, Rawalpindi in collaboration with Animal House, National Institute of Health, Islamabad. **Period:** From March 2016 to April 2017. **Material and Methods:** Forty male Sprague daily rats were randomly divided into normal Control Group A (n=10) and Experimental Group (n=30). Experimental group was given streptozotocin (30mg/kg/day) intraperitoneally for 5 days and diabetes was confirmed in experimental group by measuring fasting blood glucose level (mg/dl). Experimental group was divided further into Group B (Diabetic control), Group C (Withania coagulans treated) and Group D (Liraglutide treated). Group C were given Withania coagulans in addition to normal diet and Group D received Liraglutide besides normal diet for 30 days. Second sampling for evaluating postprandial serum insulin level and pancreatic histology was done after 30 days. **Result:** Postprandial serum insulin levels of Withania coagulans treated Group C (5.54 + 0.23 μU/ml) and Liraglutide treated Group D (6.06 + 0.17 μU/ml) were significantly raised in comparison with Diabetic control Group B (3.50 + 0.19 μU/ml), whereas islets of Langerhans cell morphology were markedly improved in Group C and D as compared to Group B. **Conclusion:** Withania coagulans significantly increases postprandial serum insulin level and improves pancreatic beta cells architecture.

Key words: Diabetes, Liraglutide, Pancreatic Histology, Withania Coagulans.

Article Citation: Samad A, Sadiq N, Ayaz H, Rajpoot NN. Antidiabetic effect of withanolides and liraglutide on serum insulin level and pancreatic histology in diabetic rats. Professional Med J 2019; 26(11):1898-1903.
DOI: 10.29309/TPMJ/2019.26.11.3155

INTRODUCTION

Diabetes mellitus is a metabolic disorder described by relative or absolute deficiency or resistance of insulin, leading to hyperglycemia.[1] Diabetes mellitus (Type 2) is a chronic disease in which an individual exhibits a decreased pancreatic beta cell function, insulin resistance and failure to inhibit postprandial secretion of glucagon.[2-3] It has become a global health problem which if left untreated can result in life threatening complications causing serious morbidity and mortality.[4] Studies have shown that insulinotropic function of Glucagon like Peptide-1 (GLP-1) are severely decreased in type 2 diabetes which leads to postprandial and fasting hyperglycemia.[5] Various drugs are commercially available for the treatment of diabetes mellitus which includes sulphonylurea, biguanides and thiazolidinedione. These drugs treats hyperglycemia by up surging the secretion of insulin from pancreatic cells and often results in hypoglycemia. These drugs have various clinical limitations, the most severe among them is the ultimate need for insulin replacement therapy. A new class of drug known as GLP-1 mimetics are in clinical use since 2007, which has shown marvelous results in treating type 2 diabetes mellitus. GLP-1 mimetics are considered over and above the other classes of antidiabetic drugs as they upsurges pancreatic insulin secretion only in response to hyperglycemia and is not associated with the risk of developing hypoglycemia.[6] In comparison with the other classes of antidiabetic drugs, use of GLP-1 mimetics (liraglutide and exenatide)

have shown reduction in hyperglycemia along with improvement in pancreatic beta cells mass.[7] Parenteral administration and high cost are the main shortcoming which limits the use of these GLP-1 mimetics.[8]

In contrast with antidiabetic drugs, herbal medicinal plants are famous for being free from toxic side effects.[9] Withania Coagulans (Paneer Doda) is herb of family Solanaceae and is renowned for its antidiabetic property. This herb is cultivated in Iran, India, Afghanistan and Pakistan. Various compounds of Withania Coagulans including phenolic and alcoholic extract of seed, flower and fruit berry has been studied for its numerous medicinal properties. Among the various active compounds "Withanolides" has been studied and evaluated for its antidiabetic property.[10] Studies on humans and animals have shown that the use of aqueous extract of Withania Coagulans resulted in significant reduction in HbA1C and Fasting blood glucose levels. These results which were obtained by the use of Withania Coagulans were merely compared with the use of sulphonylureas and biguanides.[11] Moreover different studies in animal model have also shown that the use of aqueous extract of Withania Coagulans (aqWC) significantly increases serum insulin level.[12-13]

Review of literature shows inadequate data regarding studies conducted with an aim to determine the effect of withania coagulans and GLP-1 analogues on postprandial serum insulin levels and on pancreatic histology. So the aim of this study was to determine and compare the effect of aqueous extract of Withania Coagulans (aqWC) and Liraglutide on postprandial serum insulin levels and on pancreatic histological features.

METHODOLOGY

This randomized control trial was conducted from April 2016 to March 2017 in multidisciplinary Lab, Islamic International Medical College in collaboration with animal house NIH, Islamabad. Total 40 male Sprague dawly rats were randomly distributed into two groups; Group A (n=10) and Experimental Group (n=30). Rats of Group A were provided with standard diet for 5 days while rats of experimental group were injected intraperitoneally for 5 days with streptozotocin (30mg/kg/day) in addition to standard diet. Fasting blood glucose levels (mg/dl) of both groups were measured after 5 days and diabetes in experimental group was confirmed.

Experimental group was divided further into three groups i.e. Group B, Group C and GroupD. Group B was diabetic control group and for the next 30 days they were provided with standard diet. Aqueous extract of Withania coagulans (1000mg/kg/day) were given orally to Group C rats in addition to standard diet. Group D rats were injected with liraglutide via Victoza pen at a dose of 0.3mg/kg/day subcutaneously for 30 days along with normal diet. Measurement of serum postprandial insulin levels and pancreatic histology of rats of all groups were done at the end of 30 days.

Blood sample for measuring serum postprandial insulin levels was collected from rat tail vein through 1 ml syringe .For pancreatic histology, rats were dissected and pancreas were removed. Fat from pancreas was cleaned off and pancreas were then stored in jar containing formalin 10% for 24 hours before slides were prepared for microscopic examination. For hematoxylin and eosin (H & E) staining 4 µm tissue sections of pancreas were prepared. Pancreatic tissue sections were deparaffinized in xylene and were hydrated through an ethanol series of 100%, 90%, 80%, 70%, & 50%. Pancreatic tissue sections were then stained with H&E. Sections were then mounted with Canada balsam after washing with xylene and observed on light microscopy. The endocrine portion of pancreatic cells i.e. islets of Langerhans were observed under microscope (Marco polo) using 400 power lenses.

RESULTS

Fasting blood glucose (mg/dl) levels in experimental group rats (131 ± 3.05mg/dl) were significantly higher ($P<0.05$) as compare to normal control group A rats (80 ± 3.19mg/dl). This confirmed diabetes in experimental group rats on day 5.

Serum postprandial insulin levels of normal control Group A (5.9 ± 0.19 µU/ml) were significantly raised (P<0.05) as compare to diabetic control Group B (3.5 ± 0.19 µU/ml). Serum postprandial insulin levels of Withania Coagulans treated Group C rats (5.54 ± 0.23 µU/ml) and Liraglutide treated Group D rats (6.06 ± 0.17 µU/ml) were significantly raised (P<0.05) as compared to the Group B rats (3.5 ± 0.19 µU/ml) as shown in Table-I.

Para-meters	Group A (Control)	Group B (Diabetic)	Group C (WC Treated)	Group D (Liraglutide Treated)
Serum Insulin (µU/ml)	5.9 ± 0.19	3.5 ± 0.19*[a]	5.54 ± 0.23*[b]	6.06 ± 0.17*[c]

Table-I. Comparison of Mean ± SEM of serum postprandial insulin (µU/ml) levels in all four groups (A, B, C, D):

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

Histology of Group A Rats

The histology of pancreatic section from healthy control group A rats showed normal pancreatic architecture with closely packed pancreatic acini. Pale stained Islets of Langerhans are mainly located in peripherally and are embedded within the acini. The islets of Langerhans are surrounded by capsule. The pancreatic lobules are separated by properly arranged interlobular connective tissue septa as shown in Figure-A.

Histology of Group B Rats

The histology of pancreatic section from diabetic control group B rats showed disturbance in the pattern of islets of Langerhans and disarrangment of connective tissue septa. Islets cells of langerhans were found with irregular outline, reduced dimensions and shrinkage as compared to group A rats. Some of the islets cells were also found to exhibit degenerative and necrotic changes with eosinophilic deposits, as shown in Figure-B.

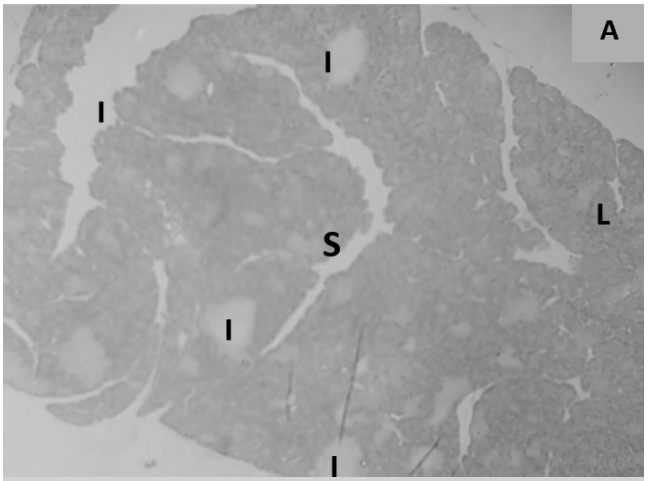

Figure-A. Photomicrograph showing following structures in a pancreatic section of group A rats: Pancreatic lobules with acini (L), spaces of unstained connective tissue septa (S) and Islets of Langerhans (I).

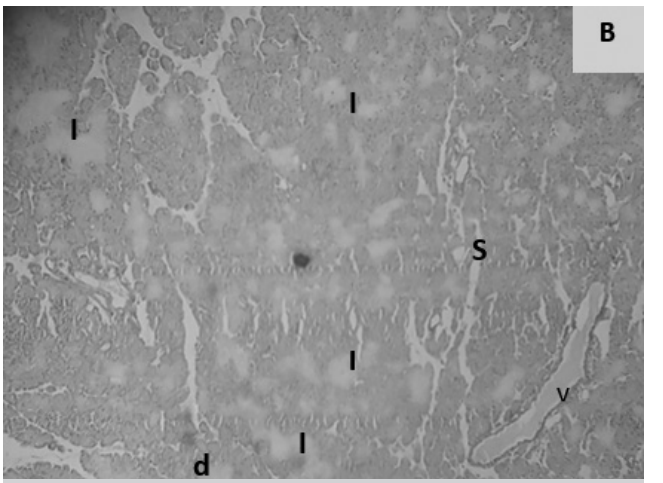

Figure-B. A photomicrograph showing following structures in a pancreatic section of group B rats: Islets of Langerhans (I), connective tissue septa (s), blood vessels (v), degenerative and necrotic changes around islet cell (d).

Histology of Group C Rats

Histology of pancreatic section from withania coagulans treated group C rats showed marked improvement in pancreatic architecure with reduction in histological alteration of islets cells. Islets cells were regenerated considerably, suggesting the presence of stable cells in the islets. Increased dimension and regular outline of islets cells were observed as compared with group B rats pancreatic section. The normal arrangment of connective tissue septa were

observed. No degenerative and eosinophilic deposit were seen.

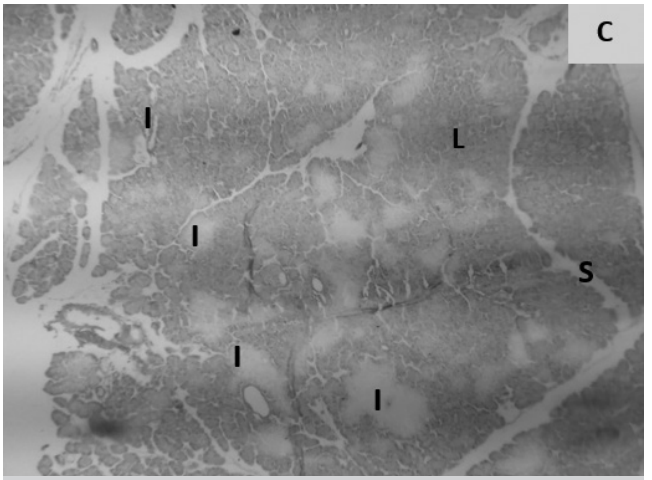

Figure-C. A photomicrograph showing following structures in a pancreatic section of group C rats: islets of Langerhans (I), connective tissue septa (s), pancreatic lobules (L).

Histology of Group D Rats

The histology of pancreatic section from group D rats showed marked increase in the size of the preexisting islets. The pancreatic lobules containing acinar cells were divided by properly arranged septa. Islets cells were well preserved with normal pancreatic architecture, as shown in Figure-D.

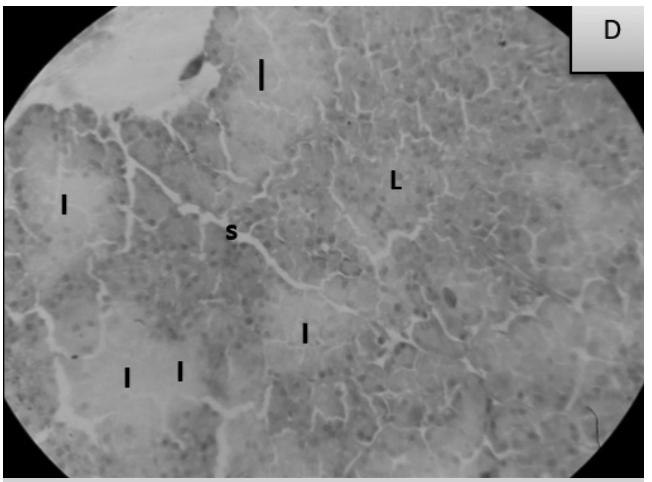

Figure-D. A photomicrograph showing following structures in a pancreatic section of group D rats: Islets of Langerhans (I), connective tissue septa (s), pancreatic lobules (L).

DISCUSSION

Diabetes is an area of concern worldwide which results in various life threatening complications. Management of type 2 diabetes includes oral hypoglycemic drugs and numerous parenteral drugs (Insulin and GLP-1 mimetics) which treats hyperglycemia by increasing insulin secretion from pancreatic cells. However use of these drugs often results in hypoglycemia. In our study we hypothesized that the use of Withania Coagulans a natural occurring herb, will enhance serum postprandial insulin levels and will improve the pancreatic histology in streptozocin induced diabetic rat. We compared the result of serum postprandial insulin level and pancreatic histology of withania coagulans treated rats with normal control, diabetic control and liraglutide treated rats. Results of our study showed that postprandial insulin levels in withania coagulans treated rats were significantly raised as compare to diabetic control group. Pancreatic architecure in withania coagulans treated group was also markedly improved.

Shimoda et al explored GLP-1 levels and pancreatic histology after treating the diabetic rats with GLP-1 analogue.[14] The improvement in pancreatic morphological feature were similar to our study results with difference that in the present study aqueous extract of withania coagulans and liraglutide were used instead of GLP-1 analogue alone. Findings of improvement in pancreatic islets cells architecture in current study is similar to the study results carried out by Yasir et al with a difference that in addition to evaluating the effect of aqueous extract of withania coagulans on pancreatic morphology, we also evaluated its effect on serum postprandial insulin levels. Moreover Yasir et al study was based on using a combination therapy of herbs i.e withania coagulans and acacia arabica lamk.[15]

Hoesseni et al conducted a study on diabetic rats and used curcumin as antidiabetic drug which showed a marked improvement in pancreatic architecture (restoration of size and number of islets cells).[16] All these improvement in pancreatic histology are in line with the findings of current study. Mechanism of action of the active ingredients used in both studies may be similar which resulted in improvement of pancreatic architecture. Findings of the current study is

parallel to the findings of study conducted by Davies et al on type 2 diabetic patients, who evaluated the efficacy of liraglutide on serum insulin level and pancreatic beta cells functions (measured by HOMA-B). Davies et al estimated serum insulin pancreatic function by HOMA-B method whereas in the present study pancreatic histology were done to estimate pancreatic islets cells morphological parameters.[17] In present study immunohistochemistry of pancreas tissue was not done due to lack of resources. In future studies can be done to explore the effect of withania coagulans on morphological features of intestinal L cells which releases glucagon like Peptide-1.

CONCLUSION

The aqueous extract of withania coagulans is effective and comparable to liraglutide in controlling hyperglycemia in streptozotocin induced diabetic rats. Use of withania coagulans upsurges serum postprandial insulin level by enhancing pancreatic islets cell mass, the same action which is seen by the use of liraglutide. Therefore, orally administrable Withania coagulans can be used as a better treatment option in place of liraglutide as it will not only save the patients from troublesome of parenteral administration, paying high cost but will also protect them from the adverse effects caused by liraglutide usage.

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4	Noor Nasir Rajpoot	Final drafting.	