ORIGINAL RESEARCH

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Antidiabetic effect of *Withania Somnifera* Root in STZ induced diabetic rats

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Abstract

Objective: To study the antidiabetic effect of *Withania Somnifera* root powder (WSR) in Streptozotocin induced diabetic rats.

Methods: Diabetes in rats were induced by injecting Streptozotocin intraperitoneally (45 mg/kg of body weight) to overnight fasting rats. *Withania Somnifera* root was given to group C (100 mg/ kg of body weight) and Pioglitazone to group D (20 mg/kg of body weight) orally for 4 weeks respectively. Blood glucose level, glycated Hb, insulin, protein tyrosine kinase activity and serum DPP-4 levels were measured.

Results: Significant Improvement in body weight, glycemic profile, dyslipidemia and tyrosine kinase activity were observed in WSR treated rats at week 4 of the study compared to diabetic control rats. Blood glucose level, glycated Hb and Serum DPP-4 levels, were also found to be decreased in WSR treated rats compared to diabetic control rats at the end of study. This is possibly due to augmented serum insulin levels and increased insulin sensitivity after treatment with WSR.

Conclusion: The results from above study it was concluded that *Withania Somnifera* root powder has antidiabetic activity. Further study required to confirm the exact mechanism of action.

Keywords

Antidiabetic, Diabetes, Streptozotocin, *Withania Somnifera*

Imprint

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Introduction

Type 2 Diabetes mellitus is a metabolic disorder of characterised by defect incarbohydrate metabolism due to abnormality in both insulin resistance and insulin secretory ability of beta cell of pancreas causing hyperglycaemia. This chronic increase in glucose concentration is associated with dysfunction, and failure of various organs, especially the eyes, kidneys, heart, and blood vessels [1, 2].The frequency of diabetes is increasing all over the world due to aging, food habit, urbanisation, and physical inactivity. Modern synthetic drugs which has been used since long have several side effects, due to this patients are looking for other options and used of herbs are one of them, which popularity has been increased[1]. For the last few decades, there is cumulative study has reported that plant based drugs help to control various diseases. Also the herb based products are safer and free from any side effects than synthetic products which may be harmful and toxic to the human and environment [3].

Withania Somnifera which iswell-known as Ashwagandha and widely used in indigenous medicine in India [4,5]. Roots of *Withania* have a several bioactive chemical compounds such as flavonoids, tannin, steroidal lactones such withanaloid, withaferin and alkaloids [6,7]. Many studies have reported that the root of *Withania*have been several therapeutic properties, such asimmunomodulatory anti-inflammatory,anti-tumour and antioxidant [8,9]. Recently, *Withania*is used as tea to improve immunity and facilitate detoxification, which helps preserve better health. Ashwagandha roots also reported to not having any toxic effect in studies[10].

Methodology

Experimental animal model

Male Wistar rats were used weighing 150-180 grams to induce diabetes for the present study. Animals were secured from the animal house of the University. Rats were allowed free access to feed on a standard chow diet and water *ad libitum*. The rats were acclimatized underlaboratory condition of temperature (25 ± 2°C) with light dark cycle (14/10hr). Ethical clearance was acquired from University Animals Ethical Committee for present study.

Induction of diabetes

To induce diabetes, a freshly prepared solution of streptozotocin (45 mg/kg of body weight in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to

overnight fasted rats. Nicotinamide at a dose of 230 mg/kg body weight was given 15 minutes prior to STZ injection for the development of stable type 2 diabetes mellitus[11]. After 48 hours of STZ administration, fasting blood glucose (FBG) levels were measured.

Plant material

Fresh roots of WS (WSR) were obtained in the month of March from the local market of Delhi was air dried at room temperature. Dried roots were crushed in an electric grinder machine to a fine powder and stored in an air-tight container, until further use[12].

Grouping of animals

After induction rats were divide randomly into 4 groups with 6 rats each.

Group A: Healthy control (normal saline)

Group B: Diabetic control (normal saline)

Group C: Diabetic treated with WSR (100 mg/kg of body weight) [12]

Group D: Diabetic treated with Pioglitazone (20 mg/kg of body weight)

Glycemic parameters and hormonal assays:

Rats were fasted overnight and blood was drawn from retro orbital plexus on day 1 and afterwards at week 4 of the study for the estimation of Glycosylated hemoglobin and plasma was separated from blood for the estimation of Glucose. Serum was separated for the estimation of lipids, Insulin and Dipeptidyl Peptidase-4 using commercially available kits: plasma glucose (Fortress diagnostics, United Kingdom), HbA1c (Fortress diagnostics, United Kingdom), serum triglycerides (Fortress diagnostics, United Kingdom),-Insulin (Mercodia rat ELISA kit, Sweden) and DPP-4 (Bioassay technology laboratory rat ELISA kit, China) respectively.

Insulin Receptor (IR)-binding assay: At the end of the study ratswere sacrificed and their organs including liver and adipose tissues were dissected for the estimation of protein tyrosine kinase activity. These tissues were collected and solubilized by lysis buffer. IR-binding assay was performed using Rayto 2100c microplate ELISA reader (Rayto, China). The amount of phosphorylated IR was quantified by sandwich enzyme-linked immunosorbent assay (ELISA). The absorbance was measured at 450 nm through ELISA plate reader.

Histopathology of Pancreas

At the end of the study, all the rats were sacrificed and their pancreas were dissected out and fixed in 10 % buffered formalinsolution for further processing. Tissues were processed and embedded in paraffincassettes. 5µ sections were cut using rotary microtome (A.O. Spencer). These slideswere subjected to heat at 60 ° C for 10 minutes for melting of paraffin section and further processed for Heamatoxylin and Eosin staining. All the tissues weremicroscopically examined and compared among the four study groups.

Results

Effect of WSR on Body weight and Serum TG level

The effect of the WSR on body weight in the diabetic rats is given in Table 1. Treatment of diabetic rats with WSR and reference drug Pioglitazone causes increase in body weight,but the increase stayed less than the normal rats, however in the diabetic rats, there was a substantialdecline in the body weight over the same period.

Triglyceride (TG) levels of the diabetic rats were significantly higher when compared with the normal rats though after treatment TG comes close to healthy normal rats (Table 1).

Parameters	Time points	Group A	Group B	Group C	Group D
Body Weight	Week 0	225.1±4.32	215.41±5.74	228.12±8.41	244.48±24.20
	Week 4	255.41±21.14	2.17±7.58	235.18±5.99	2.48.32±21.47
Serum TG (mg/dl)	Week 0	62.33±4.12	66.25±5.55	91.25±4.71	84.65±4.53
	Week 4	65.45±6.84	115.12±8.87*	68.75±4.69#	67.15±3.67#

Values are mean ± S.D. (n = 6). ($p < 0.001$)

* = group A vs. Group B, C, D, #= Group B vs. Group C, D

Effect of WSR on glucose and insulin level

The effects of the WSR on the blood glucose in diabetic rats are showed in Figure 1. Glucose levels were considerablyhigher in diabetic rats as compared to healthy rats. Oral administration of WSR lowered the increased level of blood glucose as compared to diabetic rats and their glucose levels were close to the normal control.

Insulin level was found to be decreased in diabetic rats which was ameliorated to near normal level in treated rats (Figure 2).

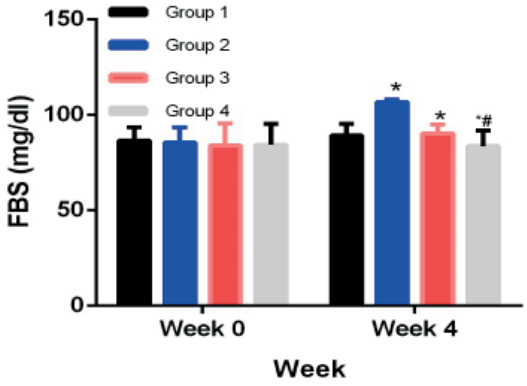

Figure 1. Showing Blood glucose level at week 0, 4 after treatments with WSR and Pioglitazone. Values are mean ± S.D. (n = 6). (p < 0.001), * = group A vs. Group B, C, D, #= Group B vs. Group C, D

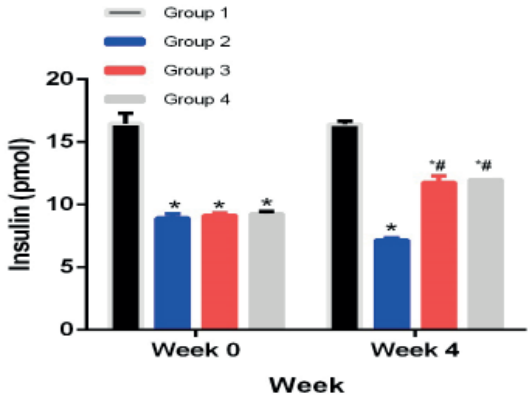

Figure 2. Showing Insulin level at week 0, 4 after treatments with WSR and Pioglitazone. Values are mean ± S.D. (n = 6). (p < 0.001), * = group A vs. Group B, C, D, #= Group B vs. Group C, D

Effect of WSR on Serum HbA1c level

The effects of the WSR on the HbA1c in diabetic rats are showed in Figure 3. HbA1clevels were significantlyincreased in diabetic rats as related to healthy rats. Treatment with WSR lowered the HbA1clevel significantly in compared to diabetic rats (Figure 3).

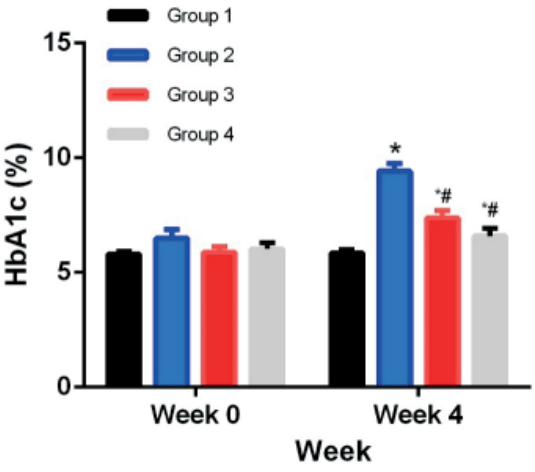

Figure 3. Showing HbA1c level at week 0, 4 after treatments with WSR and Pioglitazone. Values are mean ± S.D. (n = 6). (p < 0.001), * = group A vs. Group B, C, D, #= Group B vs. Group C, D

Effect of WSR on DPP-4 activity

In the present study we found an increase in DPP-4 activity in diabetic rats which after treatment with WSR level of DPP-4 was decreased significantly and the results were comparable to Pioglitazone treated rats (Figure 4).

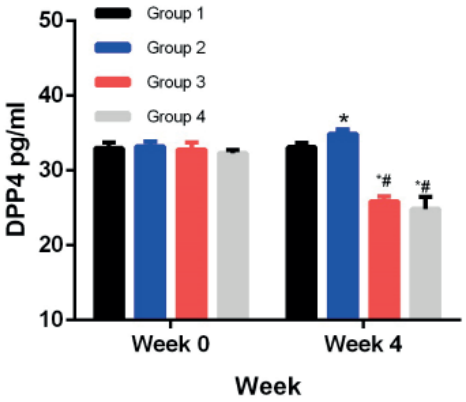

Figure 4. Showing DPP-4 level at week 0, 4 after treatments with WSR and Pioglitazone. Values are mean ± S.D. (n = 6). (p < 0.001), * = group A vs. Group B, C, D, #= Group B vs. Group C, D

Effect of WSR on tyrosine kinase activity

After treatment with WSR, we have observed increase in tyrosine kinase activity in liver and adipose tissues respectively compared to the diabetic rats and the results were comparable to Pioglitazonetreated rats.

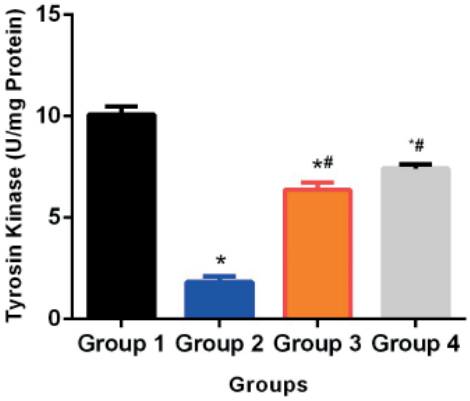

Figure 5. Showing TK (Liver) level at week 0, 4 after treatments with WSR and Pioglitazone. Values are mean ± S.D. (n = 6). (p < 0.001), * = group A vs. Group B, C, D, #= Group B vs. Group C, D

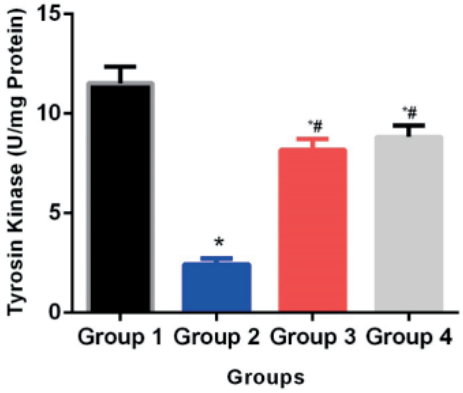

Figure 5. Showing TK (adipose tissue) level at week 0, 4 after treatments with WSR and Pioglitazone. Values are mean ± S.D. (n = 6). (p < 0.001), * = group A vs. Group B, C, D, #= Group B vs. Group C, D

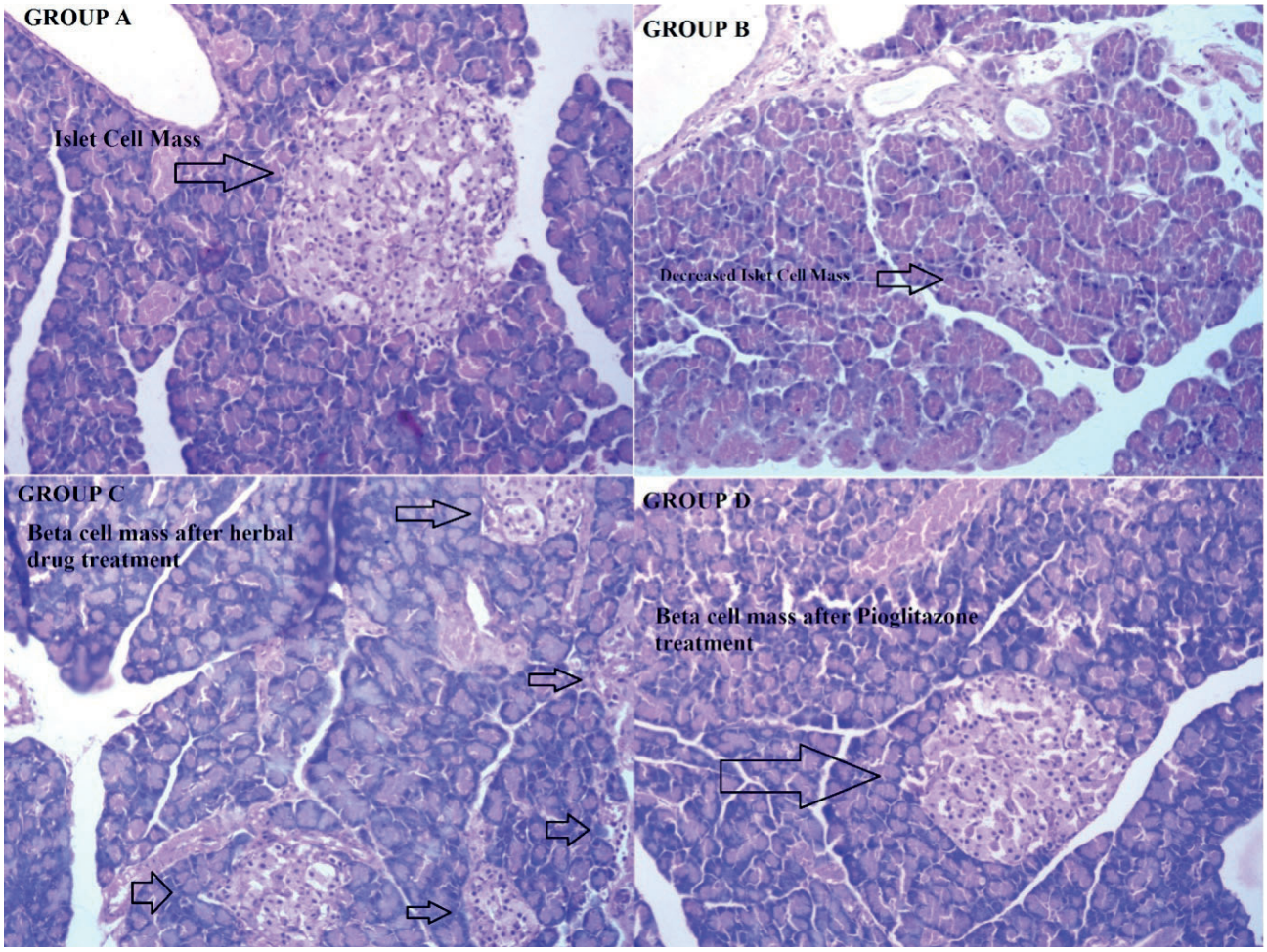

Fig 6- Showing effect of WSR treatment on the pancreatic beta cell islets in all the 4 study groups.

Discussion

In diabetes due to the partial damage of pancreatic beta cells, cause decrease in insulin secretion as we observed and so tyrosine kinase levels also found to be decreased in diabetic rats compared to healthy controls. After oral treatment with WSR for 4 weeks a significant rise in the level of serum insulin and also activity of protein tyrosine kinase in liver and adipose tissue homogenates compared to diabetic rats (Figure 5). In agreement with previous activity, we have also observed reduced tyrosine kinase activity in diabetes[13,14]. This diminution in tyrosine kinase activity is probably due to hyperglycemia which triggers protein kinase C causingincrease production of insulin receptor which preventphosphorylation of beta subunit of tyrosine kinase [15,16]. Reduced insulin level is also one of thereason to diminishthe binding of insulin to its tyrosine kinase receptors that eventuallyleads to increased glucose levels in blood. Further Insulin also responsible for increasing activity of protein tyrosine kinase. After treatment with WSR, we found that a significant increase in plasma concentration of insulin which potentiated increased glucose uptake probably via improving the insulin pathway(Figure 1,2) [17]. In preceding study, it has been reported that *Momordica charantia* binds to IR and activates the insulin signallingpathway, which ultimatelydecreases the blood glucose levels in diabetic animal[18]. Our results are in agreement with previously reported studies that also shows that the treatment with herbs increases tyrosine kinase activity and so improvesglycemic profile.

In the present study we also investigatedthe level of DPP-4, and found a substantialincrease in serum DPP-4 levels in diabetic rats. Studies have statedthat serum DPP-4 levels was found to be altered in diabetic conditions[19]. Treatment with WSR cause suppression of DPP-4, that leads toincreasein incretin levels and in return of this incretin stimulate beta cells of pancreas to increased insulin secretion as shown by our study (Figure 4). Histopathology of pancreases also showed increased Beta cell islets in WSR treated rats (Figure 6) compared to diabetic rats. Previously, Zhang et al. has reported that treatment with alogliptin restored the β-cell mass, and insulin secretion in STZ model

of mouse [20]. Increased beta cell mass cause increase secretion of insulin in blood that also accountable for augmented uptake of triglycerides from the blood into the muscles and adipose tissues and reducedthe rate of lipolysis consequently lowers the level of plasma fatty acid [21]. Hence we summarized that WSE has substantial effect on controlling diabetes via ameliorating the level of glucose, insulin secretion and plasma fatty acid level (Table 1). It also significantly reduced the serum DPP-4 levels and improving the alter levels of tyrosine kinase which is involved in establishing a smooth insulin signalling.

As the findings of the present study are form in-vitro rat model, the evidence to formulate any clinical evidence is lacking.[22] It is expected that the upcoming and ongoing developments in the field of tissue engineering may foster research in this area.[23]

Conclusion

The results from above study it was concluded that *Withania Somnifera* root powder has antidiabetic activity. Further study required to confirm the exact mechanism of action.

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