Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

*Article*
**Volume 11, Issue 1, 2021, 8141 - 8155**
https://doi.org/10.33263/BRIAC111.81418155

# Improvements in Insulin Resistance and β-Cells Dysfunction by DDP-4 Inhibition Potential of *Withania somnifera* (L.) Dunal Root Extract in Type 2 Diabetic Rat

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**Received: 20.06.2020; Revised: 16.07.2020; Accepted: 17.07.2020; Published: 19.07.2020**

**Abstract:** The study was aimed to evaluate improvements in insulin resistance and β-cell dysfunction by DPP-4 inhibition potential of *W. somnifera* (L.) Dunal root extract in type 2 diabetic rats. The experimental design was containing the *in-vitro* assay of chosen extract for DPP-4 inhibition, *in-silico* analysis of DPP-4 binding with dominating compound withaferin – A and *in-vivo* assays, respectively. Diabetes induction made through the administration of the corticosteroid [1.0 mg/Kg] and high sucrose diet, which was calculated by HOMA [Homeostasis model assessment]. Whereas the presence of the Withaferin – A (steroidal lactone) in extract (dominating compound of extract) was confirmed by HPLC isolation in comparison to the standard compound. Consequently, the histopathology of the pancreas and antioxidants of renal and hepatic tissues were assayed by standard methods. The chosen extract showed 77.3 % *in-vitro* DPP-4 inhibition and -9.18 to -6.16 KD binding energy performed with active sites of DPP-4. The corticosteroid and high sucrose feeding caused significant changes in HOMA-IR = 3.9 ±40 %, HOMA β % = 65.4±4.12 % and HOMA sensitivity = 25.5±1.2 %. The treatment of extract of WS altered significantly ($P \leqslant 0.001$) to HOMA indices, HbA1c, insulin, and glucose levels. Consequently, significant changes were seen in the histology of pancreas and antioxidants levels in hepatic and renal tissues. Accordingly, the occurrence of withaferin-A was (dominating compound of extract) confirmed from HPLC isolation in comparison to the standard compound. The FT-IR spectra annotated the availability of potent functional groups in the extract. The results illustrated that amelioration of insulin resistance and β-cell dysfunction were conducted as per DPP-4 inhibition potential of *W. somnifera* root extract. Therefore, it can be concluded that *W. somnifera* root extract possesses withaferin -A like bioactive compounds having a capacity of DPP-4 inhibition, which can ameliorate insulin resistance and β-cell dysfunction.

**Keywords:** Insulin resistance; β-cell function; HOMA; Diabetes; Glucose; withaferin –A.



## 1. Introduction

Type-2 Diabetes mellitus (T2DM) or insulin non-dependent diabetes mellitus (INDDM) is mainly caused by β-cell dysfunction and insulin resistance characterized by hyperglycemia and insulin insensitivity[1,2]. Besides this, there are various kinds of therapeutic agents. However, associated side effects limit their uses and effectiveness, which promoting search of new therapeutic agents from dietary sources as well as other conventional knowledge-based remedies[3].

From the last decade, the DPP-4 inhibitors are emerged as the dominating anti-diabetic drugs by enhancing incretin activity, which stimulates to pancreatic endocrinal activity[4]. Whereas, other than the DPP-4 inhibitors are frequently ineffectual at sustaining glycemic homeostasis and causes associated side effects, such as weight gain and episodes of hypoglycemia[3]. Thus, the DPP-4 inhibitors showing relevancy in the present context, but synthetic DPP-4 inhibitors again faced some questionable remarks of cancer linked activities, so that the natural or plant-based DPP-4 inhibitors may have prominent and suitability[5,6].

Despite this, the plant-derived secondary metabolites or bioactive phytocompounds such as unsaturated fatty acids, flavonoids, alkaloids, polyphenols, and others have been reported for benefits to human health including in managing diabetes and other metabolic disorders[8]. I is claimed that phytocompounds are able to manage diabetes by targeting several pathways incorporating inhibition of enzymes such as dipeptidyl-peptidase-4 (DPP-4), α-glucosidase, α-amylase, lipase, aldose reductase, and protein tyrosine phosphatase 1B (PTP1B) and catabolic enzymes of carbohydrate metabolism [8,9]. An additional mode of actions of phytocompounds was also recognized, such as anti-inflammatory, stimulation of hepatic antioxidant enzymes activities, encouragement of glucose transport, and incretin hormones secretion, as well as β-cell cytoprotecting as reported by several researchers from *in vitro*, *in vivo* and experimental animal studies [10].

The chosen material of plant extract, i.e., *Withania somnifera* root or rhizome extract, has also been explored to resolve various metabolic disorders such as neuro-protection, psychiatric problems, diabetes, obesity, hypertension, and reproductive disorders[11]. Additionally, *Withania somnifera* has been known to contain more than 80 types of phytochemicals, whereas the root extract dominating the steroidal lactone is known as withanolide. The Withaferin -A is one of the dominating withanolide explored for several studies and reported as a competent anticancer agent[12].

Therefore, the present study was assigned to evaluate the effect of DPP-4 inhibition potential of *Withania somnifera* root extract on insulin resistance by homeostasis model assessment and β-cells protection in corticosteroid-induced type 2 diabetes rat through *in-vitro*, *in-vivo* and *in-silico* approaches.

## 2. Materials and Methods

### *2.1. Extract, Drugs, and chemicals.*

The root extract of *Withania somnifera* (L.) Dunal was in powder form provided by the Amsar, Pvt Ltd., Indore [MP], India. The extract characterized with detail, i.e., a solvent used hydroalcoholic, brownish color, bitter organoleptic test, characteristic odor, 5:1 herb ratio, extractive in water NLT 60%, total withanolide: NLT 2.5 % and alkaloids: NLT 0.8%.
Standard drug sitagliptin (2.5mg/kg/day) (Januvia) and dexamethasone oral table-4mg (Dec dak ST), Wockhardt Limited (Merind) Purchased from a local market, Jodhpur [Rajasthan], India.

Withaferin A (purity 95% by HPLC) were procured from Sigma-Aldrich, India. Other chemicals, HPLC solvents, and reagents were purchased from the local supplier up to a chemical grade of Loba Chemie Pvt Ltd. Similarly, the Erba (Transasia) diagnostic biochemical kits were also procured from a local distributor.

*2.2. Phytochemistry of W. somnifera (L.) Dunal root extract by FTIR and withaferin A isolation by HPLC.*

The sample of plant extract was loaded in FTIR spectroscope with a scan range of 400 to 4000 $cm^1$ with a resolution of 4 $cm^{1,}$ and results were interpreted by annotated IR spectra by following the standard method[13].

Whereas, the presence of the withaferin – A confirmed by comparative HPLC isolation by standard protocol[14]. The HPLC Column C18 Syncronis was used and made at detection -230nm. Whereas the HPLC solvent system was prepared by a ratio of methanol: ammonia hydroxide: water [1:0.5:0.5], and the flow rate was 0.5ml/Min.

*2.3. Experimental animals.*

Healthy Wistar rat, weighing of 150 -200 gm were housed in polypropylene cages in standard photoperiod (14 h light: 10 h dark) with controlled temperature (26 ± 1°C) and fed with the standard of laboratory feed (Gold Mohur feed, Hindustan Lever Limited, Mumbai, India) with water *ad libitum*. Animals were maintained as per the guidelines of CPCSEA (The Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment, Forest and Climate Change, Govt. of India (Reg. No. 1646/GO/a/12/CPCSEA valid up to 27.03.23).

*2.4. Induction of type 2 diabetes.*

The corticosteroid, i.e., dexamethasone [1.0 mg/Kg] along with the high sucrose diet feeding, was used for the induction of type 2 diabetes for three weeks as reported by numerous studies[15,16]. The variables were analyzed from the first day to the end of the third week, i.e., food intake, daily water consumption, glucose levels ($7^{th}$, $14^{th,}$ and $21^{st}$ day), insulin concentration, HOMA-IR, HOMA β %, and HOMA sensitivity.

*2.5. In-vitro DPP-4 inhibition assay.*

The DPP-4 inhibition assay was evaluated based on the cleavage of Gly-Pro-p-nitroanilide by DPP-4 enzyme resulting in the generation of a stable chromophore. In brief, DPP-4 inhibition activities of plant extracts were determined by measuring the release of 4-nitroaniline from an assay mixture containing 0.1 M Tris-HCl [pH 8.0] and 2 mM Gly-Pro **p**-nitroanilide (substrate). After incubation at 37°C, the reaction was stopped by the addition of sodium acetate buffer (pH 4.5) and absorbance at 405 nm by using a UV-VIS Spectrophotometer. [17].

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of inhibitor}}{\text{Absorbance of Control}} \text{ x } 100$$

*2.6. Biochemical assessments of serum.*

2.6.1. Glucose, insulin, HbA1C, lipid profile, and toxicity profile assays.

The serum was isolated from blood by following proper standard protocol and proceeded for assessments of assigned parameters. The serum insulin[18], HbA1C[19], glucose[20] levels were assayed by commercially available kits. The lipid profile, i.e., total cholesterol[21], HDL-cholesterol[22], LDL-cholesterol, Triglyceride[23], and VLDL –

cholesterol was calculated by following friedewald's formula as following after estimation of Total cholesterol, triglyceride level and HDL[24].

$$\text{LDL-C (mg/dL)} = \text{TC (mg/dL)} - \text{HDL-C (mg/dL)} - \text{TG (mg/dL)}/5.$$

Subsequently, toxicity parameters i.e. total protein[25], creatinine[26], SGOT[27], SGPT[27], urea[28], uric acid[29] and alkaline phosphatase[30] were also assayed by following routine laboratory protocols by following standard methods.

*2.6.2. HOMA (Homeostatic model assessment) analysis.*

HOMA-IR and HOMA-β scores and insulin sensitivity were calculated using fasting serum insulin and glucose concentrations measured at the end of the experiment, according to the formula of Matthew *et al*. [31–33].

$$\text{HOMA} - \text{IR} = \frac{\text{Insulin (U/L)x Blood Glucose[mmol/L]}}{22.5}$$

$$\text{HOMA} - \beta = \frac{20 \text{ x Insulin [U/L]}}{\text{Blood Glucose (mmol /L)}} - 3.5$$

$$\text{Insulin sensitivity [IS]} = \frac{1}{[\text{[Fasting Insulin [U/L] xLog [Fasting glucose [mmol/L]]]}}$$

*2.7. Tissue biochemistry for antioxidant assays.*

Liver and kidney tissues were homogenized in PBS (0.1M, pH 7.4), centrifuged at 15,000g for 30 min at $4^0$C, and the supernatant was used for subsequent analysis.

2.7.1. Lipid peroxidation (LPO).

Lipid peroxidation level in the tissues was measured based on the TBA reaction with MDA, a product of MDA formed due to the peroxidation of membrane lipids[34]. The amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient, E =1.56x105) using a spectrophotometer[35].

2.7.2. Super-oxide dismutase (SOD) assay.

The activity of SOD was determined following the pyrogallol auto-oxidation inhibition assay. The rate of auto-oxidation is calculated from the increase in absorbance at 420 nm[36].

2.7.3. Catalase (CAT) assay.

Catalase activity was estimated by following the standard method based on the decomposition of $H_2O_2$, which measured by spectrophotometer at an absorbance at 240 nm [37,38].

2.7.4. Glutathione (GSH) assay.

The estimation of tissue GSH content was measured by the standard protocol of Ellman **[**1959] followed by the –SH group of GSH reacts with DTNB to produce a yellow-colored 2-nitro-5-mercaptobenzoic acid, and the absorbance was taken at 412 nm[36,39].

*2.8. Histopathological studies.*

Histo-pathological slides were prepared by following the routine laboratory method [40,41] after exsanguinations, a portion of the pancreas tissue was washed with ice-chilled phosphate buffer (0.1M, pH 7.4) and fixed in 10% formaldehyde for 24 hours. The tissues were dehydrated in the descending grades of isopropanol, finally cleared in xylene, and then embedded in molten paraffin wax. Sections were cut at the 5-µm thickness, stained with hematoxylin and eosin, and scrutinized under a clinical microscope (Lieca-DM RA, Research Microscope system, Germany) and the slides were scanned using an attached camera.

*2.9. In-silico analysis.*

The withaferin – A PDB file retrieved from PubChem (Pub chem. CID – 265237 with detail of MW: 470.606 g/mol and MF: $C_{28}H_{38}O_6$) and the target DPP-4 enzyme retrieved from NCBI databank (Accession: PDB-1NU6). Molecular docking of Dipeptidyl Peptidase IV (DPP-4) with Withaferin - A was performed to analyze binding affinity and interaction between the molecules using the AutoDock tool with default parameter[42]. Chain A of the DPP-4 protein was prepared for docking experiment. Water molecule and other co-factors were removed from the target molecule, whereas hydrogen atoms were added. The ligand was also prepared and docked into the active/ binding site of the target molecule [43].

*2.10. Statistical analysis.*

Values of biochemical assessments and other data were expressed as mean ± standard error of the mean (S.E.M.) and analyzed for one-way ANOVA and post hoc Dunnett's $t$ test using SPSS 22 trial version for windows.

## 3. Results

The results of *Withania somnifera* (L.) Dunal root extract has shown as following through *in-vitro*, *in-vivo*, *in-slilico*, and phytochemistry analysis.

*3.1. HOMA (Homeostatic model assessment) analysis of insulin resistance and HbA1C, insulin, and glucose levels.*

The corticosteroid and sucrose high diet feeding caused significant ($P \leq 0.001$) elevations in glucose, insulin, and HbA1C.

**Table 1.** Effect of *Withania somnifera* root extract on diabetic status of treated groups by HOMA (Homeostatic Model Assessment) calculation

| Treatment group | Glucose (pmol/l) | Insulin (mmol/l) | HOMA (%β) | HOMA (%S) | HOMA (IR) |
|---|---|---|---|---|---|
| Intact Control | 4.5±0.21 | 80.3±3.10 | 154.4±13.1 | 69.0±2.1 | 1.4±.21 |
| Diabetic Control | 9.9±0.41*** | 187.1±13.2*** | 65.4±4.12*** | 25.5±1.2*** | 3.9±40*** |
| WS Extract | 5.9±0.31### *** | 120.4±6.7### *** | 119.6±7.43### *** | 43.5±1.5### *** | 2.3±1.8### *** |
| Sitagliptin | 5.1±0.32### *** | 103.2±4.3### *** | 142.7±8.12### *** | 52.1±1.9### *** | 1.9±1.4### *** |

Data are presented as means ± S.E.M. ($n$ = 5); *** P≤ 0.001 and * P≤ 0.05; non-significant as compared to the respective control values; ### $P \leq 0.001$; and # P≤ 0.05; non-significant as compared to the respective values of the diabetic control group.

According, the calculations of diabetes status by homeostatic model assessment shown significant alterations, i.e., HOMA-IR (Homeostasis Model Assessment-Insulin Resistance) = 3.9±40 %, HOMA β % = 65.4±4.12 % and HOMA sensitivity = 25.5±1.2 %. Whereas, the treatments of *Withania somnifera* root extract and sitagliptin significantly improved glucose levels, HbA1C, insulin, and HOMA indices by improving β-cell function (Table 1).

*3.2. Lipid profile.*

The lipid profile parameters, i.e., total cholesterol, HDL-cholesterol, LDL- cholesterol, VLDL-cholesterol, and triglyceride were elevated significantly ($P \leq 0.001$) in the diabetic control group which were also significantly ($P \leq 0.001$) reduced by treatments of *Withania somnifera* (L.) Dunal root extract and sitagliptin (Figure 1).

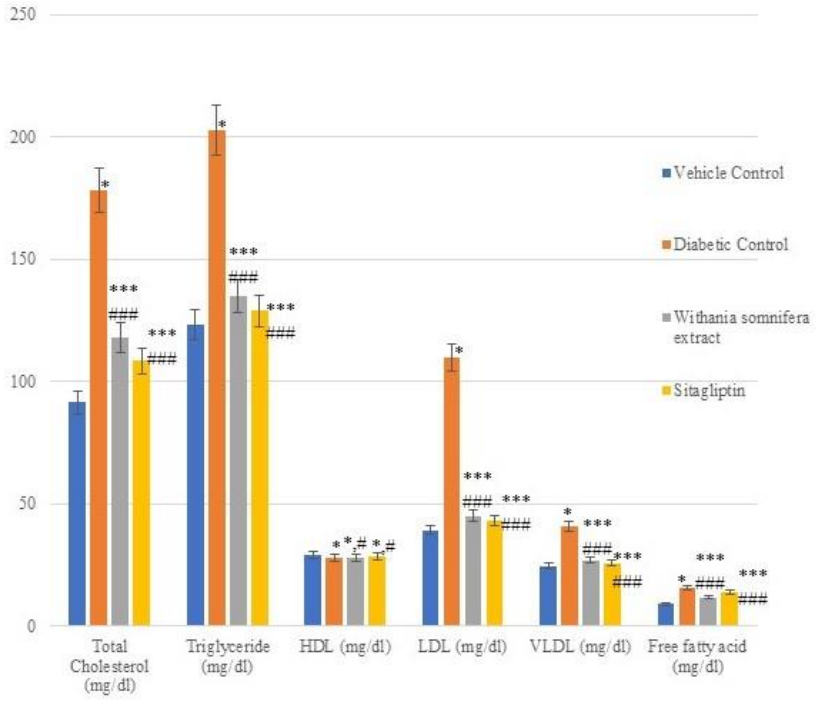



**Figure 1.** Lipid profile of *Withania somnifera* (L.) Dunal root extract and sitagliptin treated groups.

All data are represented as means ± S.E.M. ($n$ = 5); *** $P \leq 0.001$ and *$P \leq 0.05$; * non-significant as compared to the respective control values; ### $P \leq 0.001$ and # $P \leq 0.05$; # non-significant as compared to the respective values of the diabetic control group.

*3.3. Histopathology of the pancreas.*

The corticosteroid administration with high sucrose diet caused detrimental histopathological changes in the pancreas of a diabetic control group with degenerative changes in an endocrinal part of β – cells (peripheral) with different degrees of degenerations in nucleus up pyknosis, necrosis and apoptosis along with vacuolization in islets and exocrine area of acinus (Figure 2B) in comparison to vehicle control (Figure 2A). Besides this, the treatments of *Withania somnifera* root extract and sitagliptin promoted regenerative changes in pancreatic histomorphology in treatment groups (Figure 2 C and 2 D).

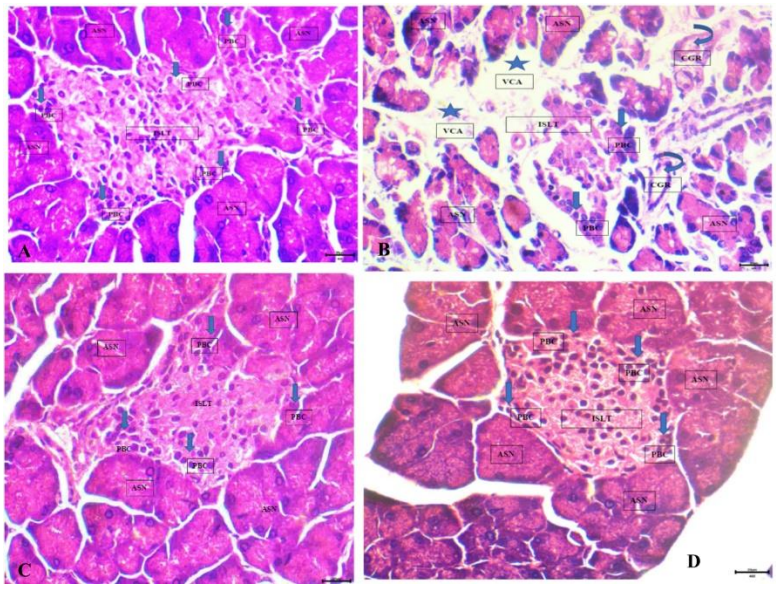
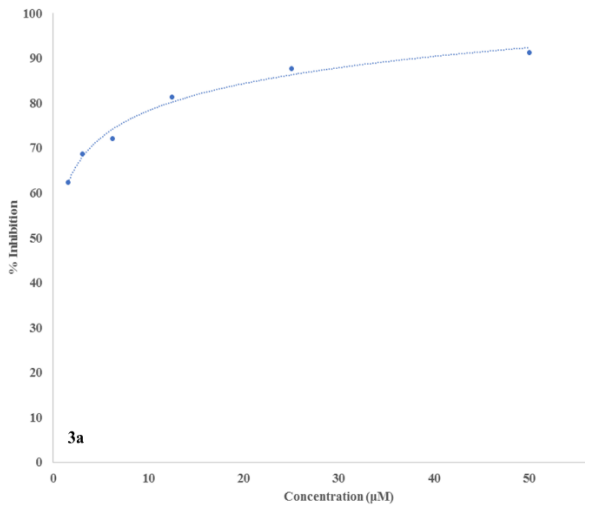



**Figure 2.** (A) Histo-architecture of Intact Control Pancreas (400x HE): The arrows are indicating the proper cellular mass of peripheral β-cells (PBC) distribution in islet (ISLT) and morphology along with arranged acini (ASN); (B) Histo-architecture of T2DM control pancreas (400 x HE): The arrows are indicating peripheral β – cell, rounded arrow showing congestion with RBC and star showing the vacuolated area in pancreatic islet (ISLT) of abnormal morphology of nucleus and disarranged acini (ASN); (C) Histo-architecture of *Withania somnifera* (L.) Dunal root extract-treated pancreas (400x HE): The arrows are demonstrating recovery of the peripheral β – cells in islet (ISLT) and increased cellular mass with arranged acini; (D) Histo-architecture of Sitagliptin treated pancreas (200x HE): The sign of arrows is pointing out the recovering part of the damaged and degenerated area of peripheral β – cells (PBC) and compact arrangement of acini.

*3.4. In-vitro DPP-4 assay.*

The results of *in-vitro* assay of chosen extract shown significant inhibition of DPP-4 up to 77.3 % in comparison to standard drug considered up to staring of plateau (Figure 3a & 3b).



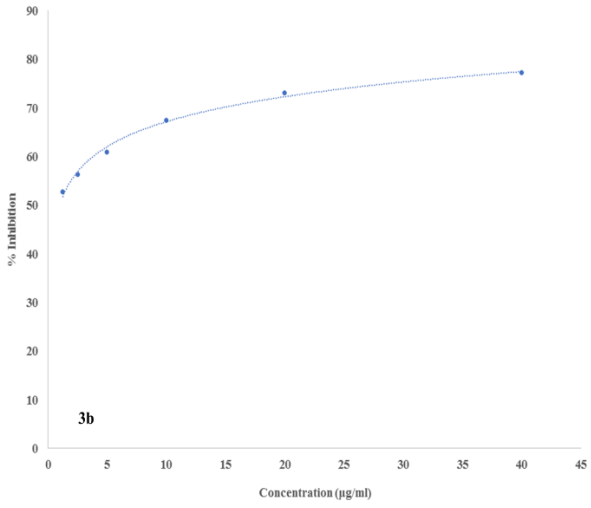




**Figure 3a.** *In-vitro* assessment of DPP-4 inhibition potential of sitagliptin (Equation-y = 8.7485, ln(x) + 58.134; $R^2$ = 0.986, IC50=0.39µM).

**Figure 3b.** *In-vitro* assessment of DPP-4 inhibition potential of *Withania somnifera* (L.) Dunal root extract (Equation- y = 8.7485, ln(x) + 58.134; $R^2$ = 0.9863, IC50=0.39µM).

*3.5. Antioxidant assessment of liver and kidney.*

Lipid peroxidation, SOD, Catalase, GSH, and GPx were significantly ($P \leq 0.001$) altered in tissues of liver and kidney in diabetic rats. The treatments caused significant ($P \leq 0.001$) alterations in the status of free radicals as promoted recoveries shown in damaged and degenerated tissues of the pancreas (Figure 4).

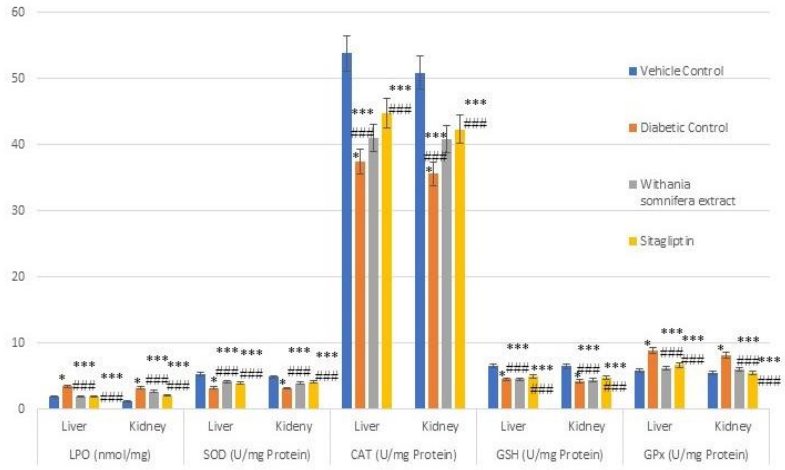



**Figure 4.** Effect of *Withania somnifera* (L.) Dunal root extract on antioxidants status in hepatic and renal tissues of treated groups.

Data are presented as means ± S.E.M. ($n$ = 5); *** $P \leq 0.001$ and * $P \leq 0.05$; non-significant as compared to the respective control values; ### $P \leq 0.001$ and # $P \leq 0.05$; h non-significant as compared to the respective values of the diabetic control group.

*3.6. Toxicity profile of Withania somnifera (L.) Dunal extract and sitagliptin treatments.*

The treatments of *Withania somnifera* (L.) Dunal extract and sitagliptin revealed non-significant alterations in levels of urea, uric acid, creatinine, SGOT, SGPT, alkaline phosphatase, and total proteins in comparison to the vehicle control group (Table 2).

**Table 2.** Effect of *Withania somnifera* root extract on toxicity profile of treated groups.

| Treatment group | Urea (mg/dl) | Uric Acid (mg/dl) | Creatinine (mg/dl) | Alkaline Phosphatase (U/L) | SGOT (U/L) | SGPT (U/L) | Protein (mg/dl) |
|---|---|---|---|---|---|---|---|
| Intact Control | 26.13±1.1 | 1.8±.3 | 1.02±.01 | 102±6.2 | 104.1±5.1 | 85.3±4.2 | 5.7±.21 |
| Diabetic Control | 37.18±2.1 ** | 3.1±.2 ** | 1.50±.03 ** | 203±8.1 ** | 148.1±8.3 ** | 145.2±3.4 ** | 6.1±.41 * |
| WS Extract | 33.18±2.2 *, # | 2.7±.2 *, # | 1.11±.02 *, # | 145±9.2 *, # | 128.9±5.3 *, # | 113.2±2.5 *, # | 5.8±.32 *, # |
| Sitagliptin | 34.13±1.3 *, # | 2.9±.1 *, # | 1.20±.02 *, # | 163±6.2 *, # | 133.5±8.2 *, # | 119.3±2.4 *, # | 5.9±.41 *, # |

Data are presented as means ± S.E.M. ($n$ = 5); *** P≤ 0.001 and * P≤ 0.05; non-significant as compared to the respective control values; ### $P \leq 0.001$; and # P≤ 0.05; non-significant as compared to the respective values of the diabetic control group.

*3.7. In-silico assessment.*

In the current study, a total of 10 binding modes of the Withaferin - A were analyzed. The binding energy of the molecule varied from -9.18 to -6.16 (Figure 5). The lowest binding

energy shows the highest binding affinity between receptor and ligand. Therefore, the highest binding affinity conformation of Withaferin - A was used to analyze interaction with DPP-4 protein. Interaction of LYS-71, ASN-74, GLU-91, ASN-92, THR-94, PHE-95, ASP-96, ILE-102, ASN-103, and ASP-104 was analyzed around 4Å of Withaferin - A (Figure 6).

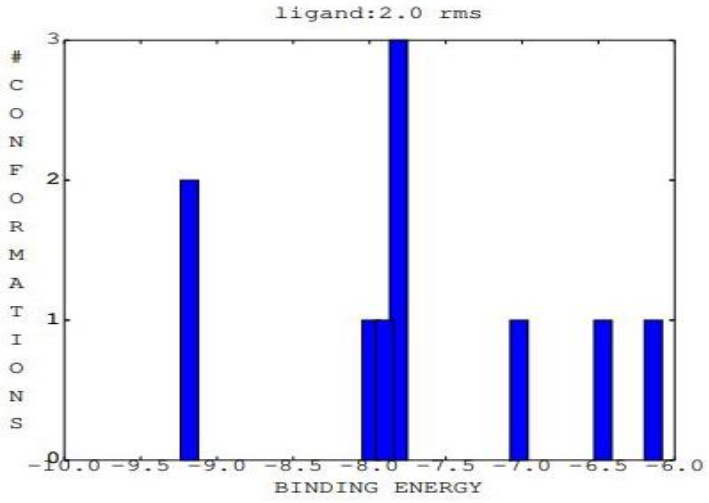



**Figure 5.** Binding energy of Withaferin -A and active sites of DPP-4.

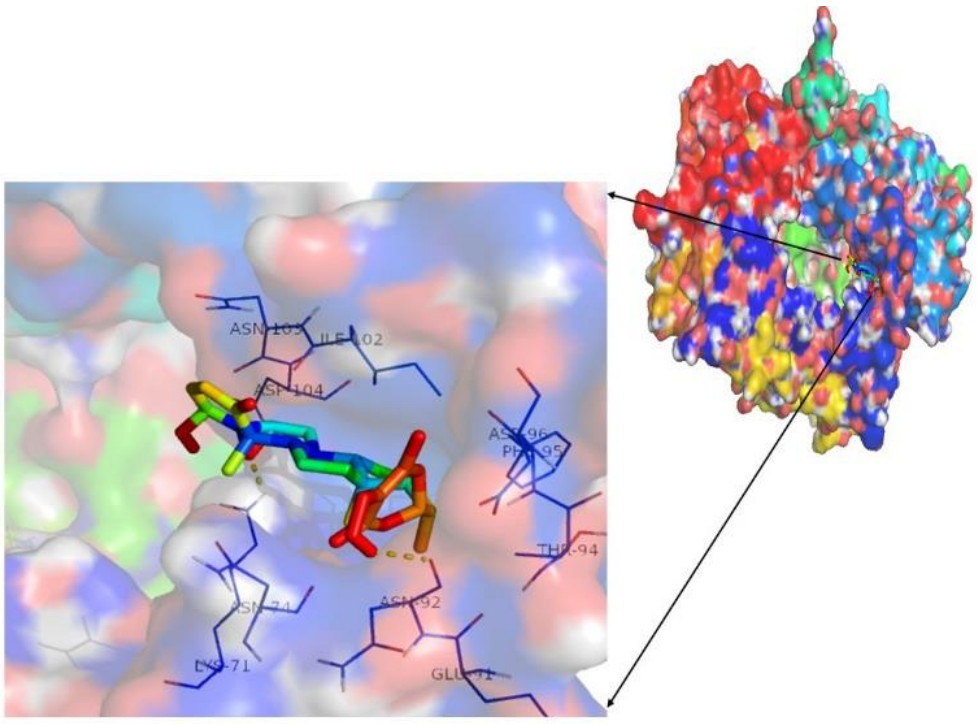



**Figure 6.** Confirmation of Withaferin -A and DPP-4.

*3.8. Phytochemistry of extract by FT-IR and HPLC isolation.*

The phytochemistry of chosen extract [*Withania somnifera* root extract] shown s availabilities of significant interactive functional groups with different picks of FT-IR spectra. The IR spectra (KBr, ῡ, $cm^{-1}$) showed the presence of the functional groups, i.e., OH, C=O, $CH_2$ ($SP_2$), $CH_3$ ($SP_3$), C=C, and C-O. The peak of 3277.81 indicating OH group, 2325.10 for C=C (stretching), 1845.03 for C=O, 1011.35 C-O, and 1675.74 C=C (bending) as occurs in dominating withanolide (Withaferin – A) (Figure 7).

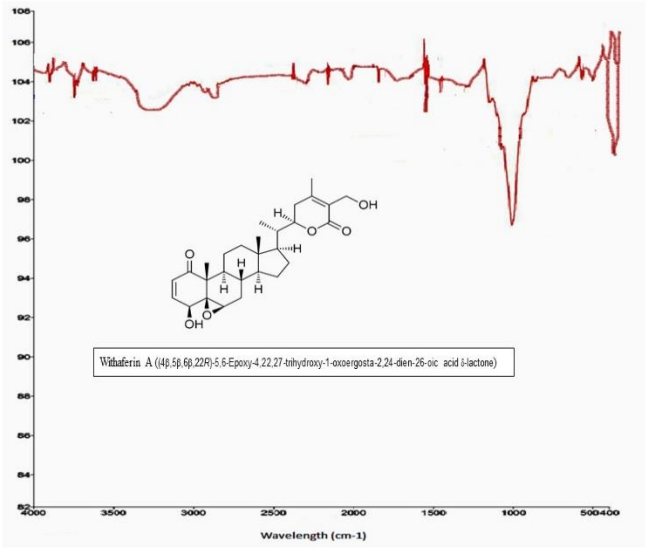



**Figure 7.** FT-IR spectra of *W. somnifera* (L.) Dunal root extract has shown availability of potent functional groups.

Whereas, the isolation of the withaferin-A from HPLC was confirmed by the selectivity of the method through analysis of standard compound and extract. The peaks of the standard Withaferin-A and the extract were identified by comparing their retention time and spectra with the standard (Fig. 8A and Fig. 8B).

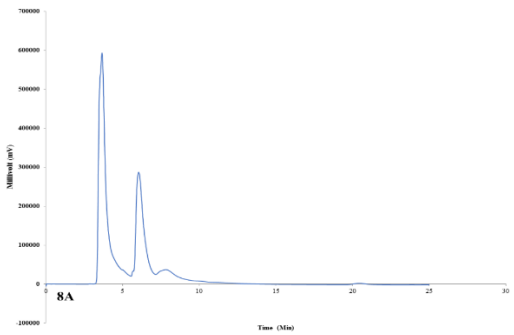



**Figure 8A.** Isolation of withaferin-A by HPLC from *W. somnifera* (L.) Dunal root extract; HPLC Column C18 Syncronis; Detection -230nm, Solvent system; Methanol: Ammonia hydroxide: water (1:0.5:0.5) and flow rate; 0.5ml/Min.

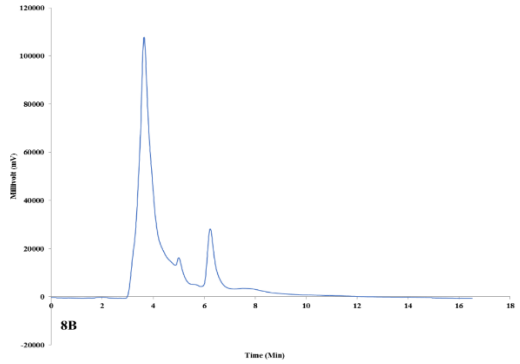



**Figure 8A.** Isolation of withaferin-A by HPLC from *W. somnifera* (L.) Dunal root extract; HPLC Column C18 Syncronis; Detection -230nm, Solvent system; Methanol: Ammonia hydroxide: water (1:0.5:0.5) and flow rate; 0.5ml/Min.

*3.9. Discussion.*

In the present study, insulin resistance and other homeostatic model assessment indices were seen abnormal with the defined diabetic status along with hyperglycemia, increased glycated hemoglobin, and hyperinsulinemia in a corticosteroid-induced diabetic animal model. In this context, it is claimed that corticosteroids promote gluconeogenic activities in the liver along with enhancing specific gene expression, and moreover, the excess content of sucrose promotes misleading to feedback mechanism of glycemia[35,44]. Accordingly, the several studies explaining that β-cells maintain their responsiveness in the face of insulin resistance through increased insulin secretion in response to meals as well as through a chronic response by increasing β-cell mass whereas if insulin resistance and β-cell dysfunction occur then promoting type 2 diabetes mellitus[15,45–47]. Besides this, glucolipotoxicity explaining that

pancreatic β-cells exposed to excess glucose and lipid contents provoked specific gene expression can recompense to the slow deterioration of the efficient β-cell mass in type 2 diabetes[48,49]. Further, it is explained that the differences of energy metabolism between normal and pathologic situations and linked variations of vital genes expression in β-cells should help explain the mechanism of glucolipotoxicity in β-cells.

Thus, the assessment of the insulin resistance and the β-cell dysfunction could permit the targeting of pharmacologic treatment based on the predominant metabolic alterations. The treatments of sitagliptin and *W. somnifera* (L.) Dunal extract caused significant alterations in HOMA indices of β-cell function and insulin resistance and HbA1C. This kind of activity may be conducted by following DPP-4 inhibition potential of sitagliptin, and *W. somnifera* extract as evaluated by *in-vitro*, *in-vivo*, and *in-silico* assessments. It is well established that plant extract possessing bioactive phytocompounds may serve as novel DPP-4 inhibitors or DPP-4 inhibitors like molecules as proven by several studies[Kempegowda et al., 2018; Modak et al., 2007; Sharifuddin et al., 2015] DPP-4 inhibitors are working on promoting longevity of GLP-1 released from gut cells and stimulate pancreatic insulin secretion[51–53]. Consequently, it is claimed the phytocompounds having potency to reduce insulin resistance by scavenging of free radicals through antioxidant potential. Supportively, it was seen that glucose levels significantly reduced, which may be following through hypoglycemic activities of possessing phytocompounds, promoting the uptake of glucose at peripheral tissues or inhibition of carbohydrate metabolism as reported by several studies[41]. Additionally, the treatments of WF extract and sitagliptin caused significant reductions in lipid profile, which may be following by inhibition of HMG – CoA reductase or promoting lipolytic enzyme activities[54]. Besides this, the parameters of renal and hepatic functions, i.e., creatinine, urea, uric acid, total protein, alkaline phosphatase, SGOT, and SGPT have remained under normal ranges which shown least toxicity of extract as proven by several studies[55]. Consequently, the *in-silico* analysis of dominating withanolide, i.e., withaferin – A performed efficient binding with DPP-4 enzyme at different binding sites as demonstrated by molecular docking. This kind of binding affinity may be supported through the availability of functional groups, as shown in the IR spectra of extract. The availability of functional groups may promote binding affinity and inhibition potential of possessing phytocompounds[56,57].

Besides this, histopathology revealed that diabetes-induced degenerative changes in both endocrine and exocrine parts of the pancreas, such as an irregular arrangement of islets, vacuolization of islets cells, vascular congestion, and presence of inflammatory cells. The degenerative changes may be caused by oxidative stress resulting from hyperglycemia and decrease the antioxidants levels and increase the free radical's contents as significantly altered levels of antioxidant enzymes (SOD (super-oxide dismutase), catalase, and glutathione peroxidase) [58,59]. Whereas the treatments caused significant changes in antioxidants levels, which may be promoted through free radicals scavenging potential of possessing phytocompounds, and sitagliptin may result to normalcy in histomorphometrically to the pancreas by recovering degenerative changes of vesiculation and cellular arrangements[13].

Therefore, the *Withania somnifera* root extract possessing withaferin-A and withaferin – A like withanolide or phytocompounds having the potential to reduce insulin resistance as well as the protective potential to the pancreas in type – 2 diabetes.

## 5. Conclusions

Based on results, it can be illustrated that the *Withania somnifera* (L.) Dunal root extract exhibiting competent phytocompounds like withaferin -A (steroidal lactone) and other withanolides with the availability of potent functional groups having the capacity to bind with dipeptide peptidase -IV and regulating incretin levels. This kind of phenomenon may directly or indirectly reduce insulin resistance and pancreas protective potential by following the DPP-4 inhibition, antioxidant potential, and other linked mechanism for the amelioration of type 2 diabetes severity by the interaction of possessing phytocompounds. Further, it can be validated by compounds levels evaluation or formulation, which may promote new clue of DPP-4 inhibition based phytomedicine development for therapeutics of type 2 diabetes.

## Funding

This research received no external funding.

## Acknowledgments

We would like to acknowledge the late Dr. R. Jatwa, DAV, Indore for his support by *in-vitro* assessment of DPP-4 and Dr. Asheesh Shanker, Associate Professor & Coordinator, Bioinformatics Programme, Centre for Biological Sciences, Central University of South Bihar, BIT Campus, Patna-800014, Bihar, India for molecular docking analysis.

## Conflicts of Interest

The authors declare no conflict of interest.

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