AMELIORATION IN INSULIN RESISTANCE AND β-CELL FUNCTION BY DPP-4 INHIBITION POTENTIAL OF *TRIGONELLA FOENUM* SEED EXTRACT IN TYPE-2 DIABETIC RATS

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ABSTRACT

The current study was aimed to evaluate amelioration in insulin resistance, β-cell function and pancreas protection by DPP-4 inhibition potential of *Trigonella foenum* (TF) seed extract in corticosteroid induced type-2 diabetic rats by *in slilico*, *in vitro* and *in vivo* assays. The corticosteroid induced diabetic status of animal model was considered at the HOMA indices, insulin and glucose levels. The *in vitro* assay of DPP-4 inhibition showed up to 60.4±2.8% and activity of DPP-4 in serum was observed to be 39.12±1.3% in *TF* seed extract treated groups. Furthermore, the FTIR spectra interpreted availability of potent functional groups in possessing bioactive compounds. Additionally, HPLC studies confirmed that gallic acid is the leading compound present in *TF* seed extract and shows significant binding energy obtained from -3.6 to -3.7 with DPP-4 residues LYS-71, ASN-74, GLU-91, THR-94, PHE-95, ILE-102, ASN-103, and ASP-104 via hydrophobic bonds. Significant changes were observed in HOMA indices, histopathology and others supportive parameters in treated groups. The study revealed promising results against insulin resistance, β-cell function and protective alterations in pancreas.

Keywords: β-cell function, pancreas protection, HOMA, HbA1C, Insulin resistance, *TF*(TF).

INTRODUCTION

Dietary combinations are formulated from ancient times for therapeutics of various ailments in the Ayurveda, Unani, Chinese and other medical systems of old civilizations[1]. Moreover, some potent drugs such as aspirin, metformin, anti-cancers drugs, digitalis and other leading drugs have been invented from herbal resources[2]. All together less than 1% of higher plants have been pharmacologically evaluated and some of them are having antidiabetic potential[3]. The reported several plants have been used individually or in formulations for treatment of diabetes and its complications. But the major glitches with herbal formulations are the active constituents: not being well defined with their interactive interpretations[4]. Thus, it is absolutely necessary to recognize the active constituents and their molecular interactions which lead to therapeutic effectiveness of the product and correspondingly to standardize the product and explore to mode of action of plants using model systems[1]. Plants have a peculiar plethora of potent bioactive phytocompounds, with a capacity to resolve different metabolic disorders by following free radical scavenging activities and targeting key enzymes inhibitions[5]. In similar context, several studies concluded that most of the antidiabetic bioactive phytocompounds target in individual or multiple manner to key enzymes i.e. dipeptidyl-peptidase-4 (DPP-4), α-glucosidase, α-amylase, lipase, aldose reductase and protein tyrosine phosphatase 1B (PTP1B) and other key catabolic carbohydrate metabolism enzymes for therapeutics of diabetes[6,7]. Recently, several studies have focussed on the inhibition of DPP-4 enzyme for therapeutics of diabetes, which is one of the serine based proteolytic enzyme cleave to GLP-1 and reduced internal insulin secretion[8]. Therefore, the DPP-4 inhibition considered as the main therapeutic target by synthetic and herbal DPP-4 inhibitors. *Trigonella foenum* (TF) seeds are one of oldest Indian food ingredients used in numerous food recipes used in folklore medicines for therapeutics of various ailments[9]. It is also reported that seed of *TF* possesses potent bioactive compounds such as alkaloids, polyphenols, saponins, flavonoids, steroids and others[10]. The current study was made to evaluate effect of DPP-4 inhibition potential of *TF* seed extract on insulin resistance, β-cell function and pancreas protection in corticosteroid induced type-2 diabetic rats.

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MATERIAL AND METHODS

Chosen extract, drugs and chemicals

The seed extract of *TF* was provided by Amsar, Pvt Ltd., Indore (MP), India. The extract was characterized in detail i.e. solvent used hydro alcohol, off whitish colour, bitter organoleptic test, characteristic odour, 5:1 herb ratio and extractive in water NLT 50. The dose of extract used was 100mg/kg per day for experimentation against 0.41/kg (as per prescription of 25 mg for 60kg human subject once a day) mg sitagliptin for 60kg. Standard drug sitagliptin (Januvia) and dexamethasone injection IP 20 ml (MERIDECA) purchased from local market, Jodhpur (Rajasthan), India.

FTIR examination and gallic acid isolation from *TF* seed extract

The sample of *TF* seed extract was analysed with FTIR spectroscopy with scan range of 400 to 4000cm^{-1} with a resolution of 4 cm 1 and annotated IR spectra have shown presence of bonding or functional groups at relevant spectra[11]. The HPLC isolation of leading compound was also performed in comparison to standard compound by using suitable solvent media[10].The HPLC isolation of gallic acid from extract was performed with standard compound by column C18 Syncronis and detection at -278nm through solvent system of chloroform: ethyl acetate: formic acid (5:4:1). The flow rate was 0.5mL/min at 20.0µL loaded sample.

Experimental Animals

Examined healthy albino rats were chosen with weights ranging 150-200 g and kept in polypropylene cages by following standard photoperiod (14 h light: 10 h dark). The temperature was maintained at 26 ± 3°C. The balanced diet and *add libitum* water were provided to the animals as per CPCSEA guidelines. (Reg. No.1646/GO/a/12/CPCSEA valid up to 27.03.23).

Induction of type 2 diabetes by corticosteroid

The type 2 diabetes induction was made by corticosteroid i.e. dexamethasone (1.0 mg/kg) administration along with high sucrose diet for 21days, as reported by several studies[12]. The monitoring was made by analysing variables from the first day to the end of 21st day, i.e. food intake and daily water consumption, glycemic status (at proper three equal durations i.e. 1st,7th,14th and 21st day), insulin concentration, HOMA-IR, HOMA β % and HOMA sensitivity[12].

DPP-4 inhibition assays

The *in vitro* DPP-4 assays were made by following through measurement of stable chromophore which was generated by the cleavage of Gly-Pro-p-nitroanilide by DPP-4 enzyme. The DPP-4 inhibition through the chosen extract and standard inhibitor (sitagliptin) were determined by measuring the release of 4-nitroaniline assayed by 0.1 M Tris-HCl (pH 8.0) and 2 mM Gly-Pro p-nitroanilide (substrate)[13].

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

Biochemical assessments of serum

Glucose, HbA_{1C}, insulin, lipid profile and renal – hepatic functions assays

Measurements of blood glucose, insulin and $HbA1_C$ were made by using an oxidase-peroxidase, ELISA based method and standard method through commercially available kits, respectively[14–17].

Total cholesterol, triglyceride and HDL-cholesterol were assessed by routine standard methods and other linked parameters i.e. VLDL-cholesterol and LDL-cholesterol were calculated by following Friedewald's formula[18–21]

LDL-C(mg/dl) =TC (mg/dL) - HDL-C (mg/dL) - TG (mg/dL)/5

Analysis of homeostatic model assessment (HOMA) of β – cell function

The HOMA analysis was interpreted by insulin and blood glucose through standard formula of HOMA-IR, HOMA-β and insulin sensitivity by using the **HOMA Calculator,** released in 2004 by Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM)[22].

Antioxidant assays

The homogenates of liver and kidney tissues were made in PBS (0.1M, pH 7.4) through centrifugation at 15,000 rpm for 30 min at 4^{0}C and the supernatant was used for subsequent analysis. The antioxidant assays i.e. SOD (super oxide dismutase), catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPx) and lipid peroxidation were made by following standard methods[23,24]

Histopathologic studies

The histopathology of the pancreatic tissues was made by following routine laboratory protocol by hematoxylin and eosin staining. The pancreatic tissues were collected form autopsied animals and fixed in 10% formalin. The dehydration of fixed tissues was followed by ascending series of ethanol, clarified by xylene and embedded in paraffin wax[25].

In silico analysis

Molecular docking of dipeptidyl peptidase IV (DPP-4) with gallic acid was performed to analyze binding affinity and interaction between the molecules using Auto Dock tool with default parameter[26]. Human dipeptidyl peptidase IV (DPP-4) crystallized structure with inhibitor was procured from protein data bank (PDB 3F8S). Three-dimensional structure of gallic acid was downloaded from PubChem Database.

Statistical analysis

All data are expressed as mean ± SEM. The statistical evaluations were made by single one-way ANOVA. A *p* value of 0.05 or less is considered under calculations for interpretation of significances of values.

RESULTS AND DISCUSSION

The DPP-4 inhibitors serve as key antidiabetic agents by pancreatic stimulation by enhancing longevity of incretin hormone and can obtained from natural products or plants can be more cost effective and reliable[27,28]. In the present study, the *TF* seed extract has shown significant results as DPP-4 inhibitors through following investigations.

Phytochemistry by FTIR and HPLC isolation of gallic acid

The phytochemistry of chosen extract (*TF* seed extract) shows annotations of significant interactive functional groups with different picks of FTIR spectra. In TF seed extract, IR spectra (KBr, □, cm^{-1}) showed the presence of potent functional groups i.e. OH, C=O, CH_2 (SP^2); C=C at 3275.97 peak for OH group, 2927.23 of C-H, C=O at 1840.00, C=C at 2308.87 and C=C at 1642.11 (bending) (Fig.1). These observations were also seen by previous researchers, who reported that phenolic compounds are leads in *TF* seeds[29]. The HPLC chromatograms of standard gallic acid confirmed the presence of gallic acid equivalent in TF seed extract through similar peaks at retention time of 3.60 min. which were previously reported by study on varieties of fenugreek[30] (Fig. 2A and Figure 2B).

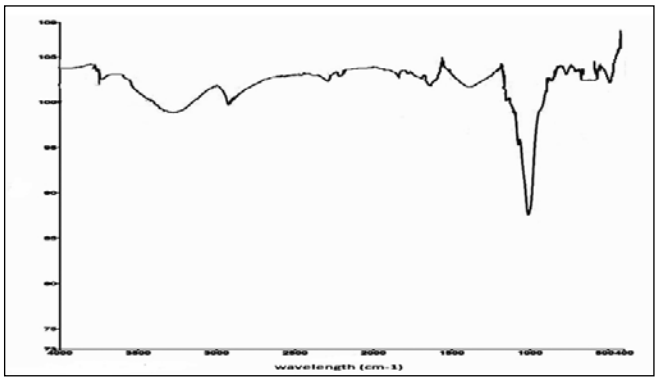

Fig. 1: FT-IR spectra of *T. foenum* seed extract

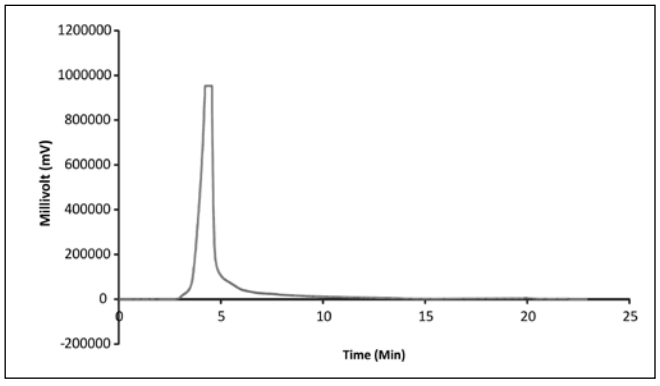

Fig. 2 A: Isolation of standard compound of gallic acid by HPLC

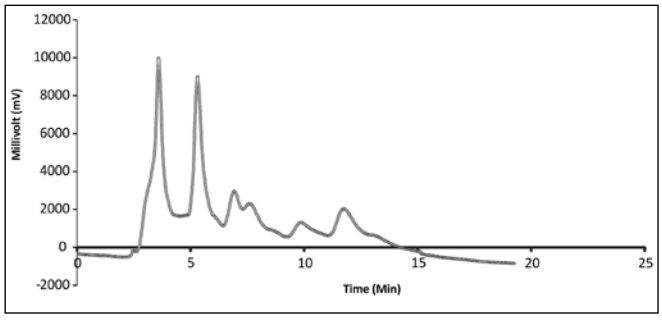

Fig. 2B. Isolation of gallic acid equivalentby HPLC from *T. foenum* seed

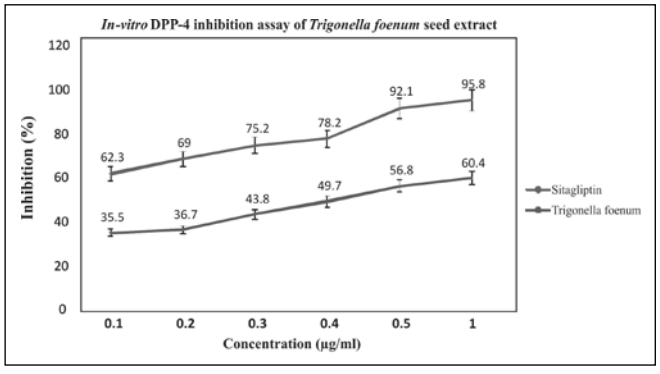

Fig. 3: *In vitro* DPP-4 inhibition assay against graduated concentrations of Sitagliptin and T. foenum seed extract

In vitro DPP-4 assay

The *in vitro* analysis of *TF* seed extract with graduated concentrations performed 60.4 % of DPP-4 inhibition in comparison to sitagliptin inhibition of i.e. 95.8%, this may follow by the interaction of bioactive phytocompounds and sitagliptin with DPP-4[31] (Fig. 3).

SERUM BIOCHEMISTRY ANALYSIS

Homeostatic model assessment (HOMA) indices, insulin, glucose and HbA_{1c}

In diabetic animal model, corticosteroid and high sucrose diet administration caused significant abnormalities in HOMA indices i.e. HOMA-IR =2.3, HOMA β % =36.1 and HOMA % S = 44.1. The treatment of *TF* seed extract improved significantly ($P \leq 0.001$) to HOMA-IR=1.5, HOMA β % =78.7 and HOMA % IS = 67.7 in comparison to sitagliptin. These kinds of alterations may be following interactions with internal milieu of β-cells and peculiar stimulatory activities of possesses phytocompounds of *TF* seeds[32]. Similarly, insulin, glucose and HbA1C were altered significantly by following HOMA indices alterations

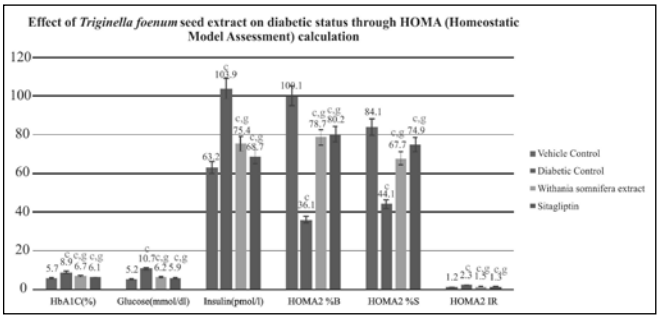

Fig. 4: HOMA (Homeostasis model assessment) analysis of treated groups

Data are presented as means ± S.E.M. (n = 7); c $P \leq 0.001$; d non-significant as compared to the respective control values; g $P \leq 0.001$; and h non-significant as compared to the respective values of the diabetic control group

and may following antidiabetic mechanism of DPP-4 inhibition[33] (Fig. 4).

Histopathology of pancreas

The diabetic pancreas tissues revealed detrimental histopathological changes with different degrees of degeneration in cellular as well as nuclear portions in comparison to normal histoarchitecture as considering the diabetic status (Fig. 5A and 5B)[34]. The treatments of *TF* seed extract and sitagliptin were endorsed retrievals in histoarchitecture in pancreatic tissues with dense nuclei and clear cytological contents, as reported by previous studies that DPP-4 inhibitors stimulate the pancreatic mass[7] (Fig. 5C &5D).

Antioxidant assessment of liver and kidney

Anomalous levels of lipid peroxidation (LPO), SOD (superoxide dismutase), catalase (CAT), GSH and GPxin liver and kidney tissue were observed in diabetic rats. Significant alterations were seen with decreased levels of LOP and GPx whereas the increased levels of SOD, CAT and GSH by treatments of *TF* seed extract and

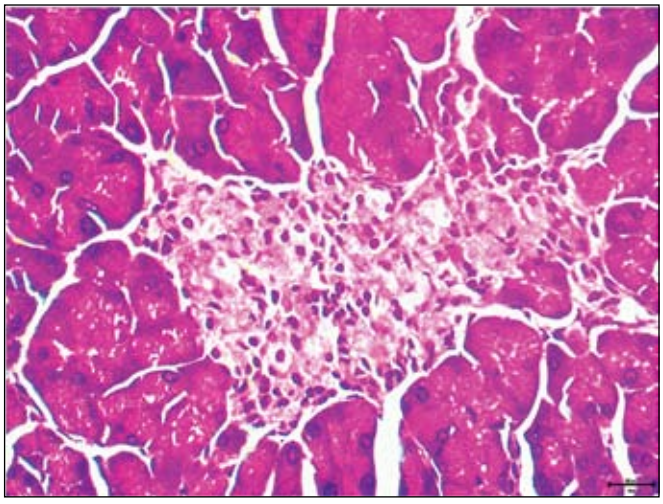

Fig. 5 A: Histo-architecture of intact control pancreas (400x HE)

The arrows indica proper cellular mass of islet β-cells distribution and morphology along with arranged vascularization.

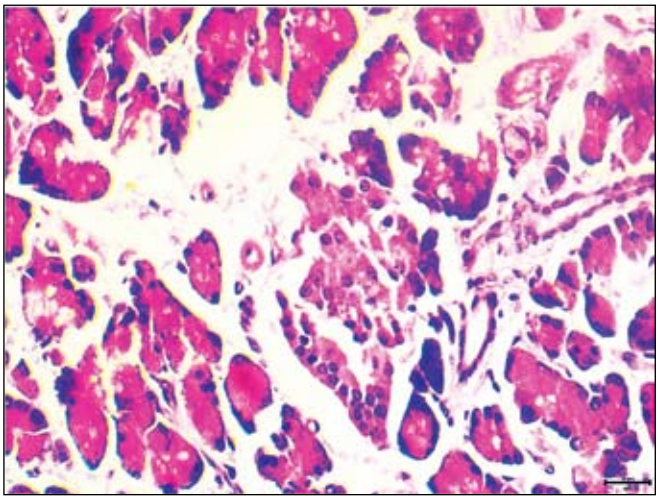

Fig. 5 B. Histo-architecture of diabetic control Pancreas (400 x HE)

Arrows indicate degeneration in pancreatic tissues up to abnormal morphology of nucleus and congestions in vascularization

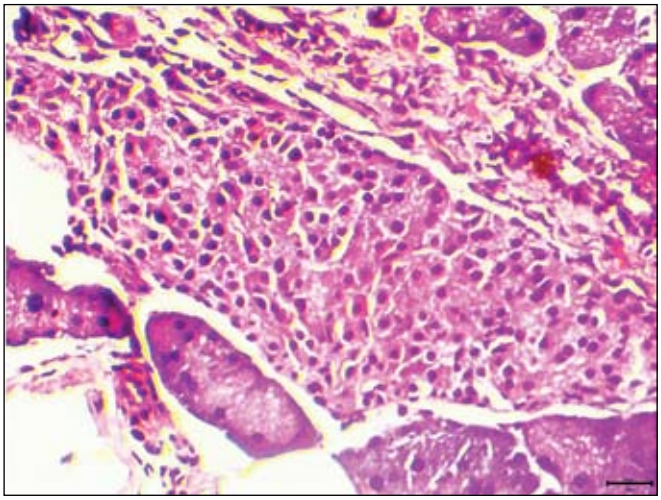

Fig. 5C: Histo-architecture of T. foenum extract treated Pancreas (400x HE)

The arrows demonstre recovery of the islet pancreatic cells and increased peripheral cell mass which is locality of β – cells.

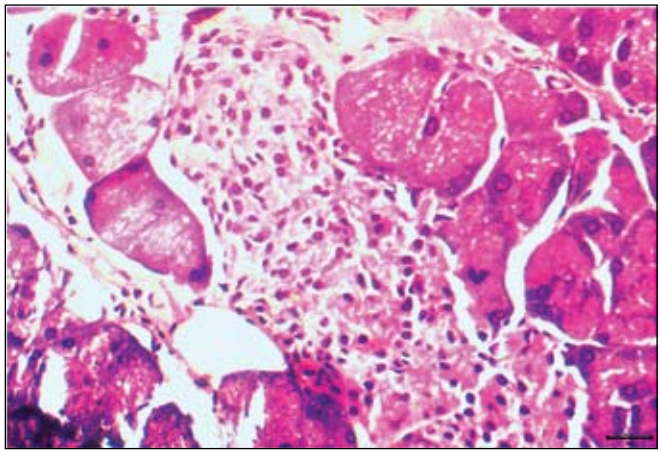

Fig. 5 D: Histo-architecture of sitagliptin treated pancreas (200x HE)

The sign of arrows is pointing out the recovering part of damaged and degenerated area of pancreatic tissues.

sitagliptin in liver and kidney tissues of type 2 diabetic rats. The results suggest following the scavenging potential of free radicals through possesses phytocompounds (Fig. 6)[34].

In silico assessment

The binding energies were obtained ranging from -3.6 to -3.7 (Fig. 7A), showing the accurate presence of binding pocket in DPP-4. The lowest binding energy, -3.7 shows highest binding affinity between DPP-4 receptor protein and best conformation of ligand. The highest binding affinity conformation of gallic acid was used

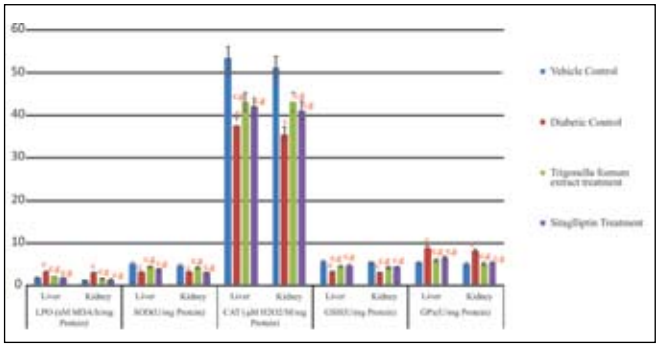

Fig. 6: Effect of DPP-4 inhibition potential of T. foenum seed extract on antioxidant status in liver and kidney

Data are presented as means ± S.E.M. (n = 7); c $P \leq 0.001$; d non-significant as compared to the respective control values; g $P \leq 0.001$; and h non-significant as compared to the respective values of the diabetic control group

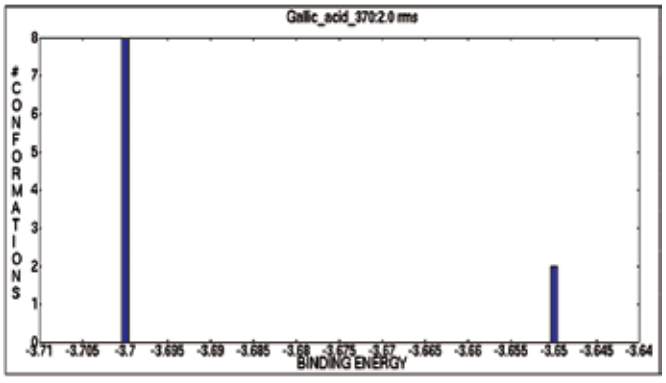

Fig. 7A: Cluster analysis of conformations of gallic acid with their binding energy levels

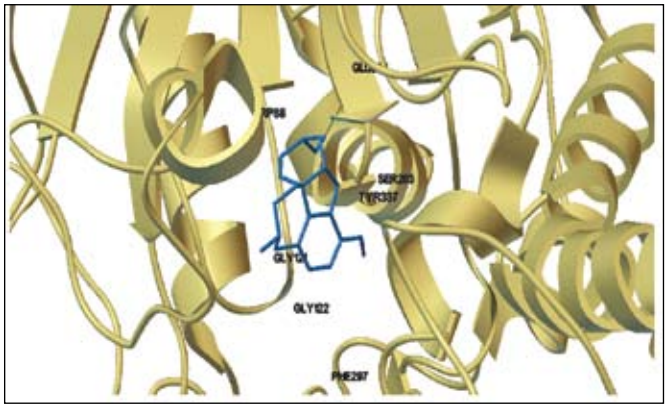

Fig. 7B: Molecular docking interaction of gallic acid with DPP-4 protein

to analyze interaction with DPP-4 protein[35]. Gallic acid interacts with DPP-4 residues LYS-71, ASN-74, GLU-91, THR-94, PHE-95, ILE-102, ASN-103, and ASP-104 via hydrophobic bonds. Gallic acid also forms hydrogen bond with ASN-92[36] (Fig. 7B).

SUMMARY AND CONCLUSION

Based on the above results, it can be concluded that the gallic acid equivalent bioactive phytocompounds of *TF* seed extract has a potential to improve HOMA indices and protective effect on pancreas through DPP-4 inhibition in type 2 diabetes. Further studies are required to validate the individual bioactive phytocompounds through proper formulation levels.

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