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IN SILICO AND *IN VIVO* ANTI-DIABETIC STUDIES OF *PTEROCARPUS MILBREADII* EXTRACT USING MOLECULAR DOCKING AGAINST DIPEPTIDYL PEPTIDASE-4 AND ALLOXAN-INDUCED HYPERGLYCEMIC RATS

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ABSTRACT

Diabetes mellitus is a disease of both man and animal especially dogs. This present study evaluated the possible anti-diabetic effects of Pterocarpus milbreadii (PM), a culinary vegetable using computer-aided technique and in alloxan-induced hyperglycemia in rats. AutoDock Vina was used for molecular docking of three most abundant compounds (ascorbic acid, phytol and octadecanoic acid) obtained by gas chromatography-mass spectrometry analysis of the methanol leaf extract of PM against Dipeptidyl peptidase-4 (DPP-4). The in vivo anti-diabetic experiment was evaluated using 20 male albino wistar rats assigned into four groups (A–D) of five rats per group. A single intraperitoneal injection of alloxan monohydrate (160 mg/kg) was used to induce diabetes in groups B–D rats while rats in group A served as normal control. Rats in groups A and B received distilled water whereas groups C and D rats were respectively treated with 200 mg/kg and 400 mg/kg of the extract. Fasting blood glucose (FBG) values were evaluated post treatment. Results showed that ascorbic acid showed a binding energy (in Kcal/mol) of –5.3 compared to phytol (–5.2) and octadecanoic acid (–4.8) while Sitagliptin, the standard drug, produced a binding energy of –8.3 when docked against DPP-4 enzyme. The extract, at 400 mg/kg reduced by 51.96 %, the FBG levels of the diabetic rats 24 hours post treatment compared to that of the diabetic untreated (5.16%). It was concluded that the methanol leaf extract of PM has in vivo hypoglycemic potentials and in silico ability to inhibit DPP-4 enzyme.

Keywords: *Alloxan monohydrate, Diabetes, Dipeptidyl peptidase-4, Molecular docking, Pterocarpus milbreadii*

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterised by hyperglycemia (increased blood glucose), polyuria, polydipsia and polyphagia (Attama *et al.* 2021). Lack of insulin or impaired sensitivity to insulin has been fingered as the cause of this condition (Padhi, Nayak and Beher 2020). Types 1 and 2 DM are distinguished. In type 1 DM, an autoimmune disease destroys pancreatic beta cells making them unable to secret insulin while impaired sensitivity of the cells to insulin precipitates type 2 DM (Wong, Al-Salami and Dass 2017).

Dipeptidyl peptidase-4 (DPP-4) also called adenosine deaminase complexing protein 2, is a serine exopeptidase saddled with the role of cleaving X-proline or X-alanine dipeptides from the N-terminal polypeptides (Vanhoof *et al.* 1995). It is known to cleave a lot of substrates ranging from chemokines, neuropeptides, growth factors and vasoactive peptides (Chen 2006). Inhibition of DPP-4 prolongs incretin effects since the enzyme inactivates incretins. The incretins (gastric inhibitory polypeptide and glucagon-like peptide-1) are gut hormones known to stimulate decreases in blood glucose levels after meal by stimulating insulin release and inhibiting glucagon expression (Rosenstock and Zinman 2007). Drugs known as DPP-4 inhibitors such as Sitagliptins which interact with catalytic sites of the enzyme, have been in use for management of type 2 DM for over two decades now (Deacon 2020). The DPP-4 inhibitors do not pose the risk of hypoglycemia development and they have good safety profile and high tolerability (Deacon 2020).

Alloxan monohydrate, a cyclic urea derivative and a diabetogenic agent, is a glucose analogue which is very toxic. Alloxan has been in use for induction of experimental DM owing to its selective toxicity to pancreatic beta cells (Lenzen 2008). Researchers in the time past have reported successful induction of DM using alloxan monohydrate single intraperitoneal injection at the dose of 160 mg/kg (Attama *et al.* 2021; Aba and Edeh, 2019). Alloxan monohydrate induces DM by induction of reactive oxygen species (ROS) that enhances selective necrosis of the pancreatic beta cells and by inhibition of glucose-stimulated insulin secretion through the inhibition of glucokinase, a pancreatic glucose sensor (Ighodaro, Adeosun and Akinloye 2017).

Gas chromatography-mass spectrometry (GC-MS) hyphenated system has been an excellent method of separating and identifying phytochemical constituents of plants (Razack *et al.* 2018). Molecular docking on the other hand plays important roles in computer-aided drug discovery (CADD) by assisting in evaluating the interactions between a ligand (such as plant phytochemicals and secondary metabolites) and the active site of the protein of interest (Sliwoski *et al*. 2014; Lee and Kim 2019).

Pterocarpus milbreadii (PM) is a plant indigenous to rainforest zones. The leaves of the plant are used to make a local “Oha” soup in the southeastern part of Nigeria (Aba and Udechukwu 2018). In folklore, the leaves are used in the management of headache, fever, convulsions and as antimicrobial agents (Usunomena and Igwe 2016). Studies have shown that the leaves have a lot of nutritional constituents (Usunomena and Igwe 2016). It is believed that the full therapeutic potentials of this important plant are yet to be explored.

This study was therefore tailored to investigate the possible *in vivo* hypoglycemic effects and *in silico* anti-diabetic potentials of the compounds present in the methanol leaf extract of PM through molecular docking of the compounds against DPP-4 enzyme.

METHODS

Animals

Male albino wistar rats weighing between 100 g and 105 g were obtained from the breeder. The rats were acclimatised for two weeks. The environmental temperature where the animals were housed varied between 28°C–32°C. The animals were kept in stainless wire mesh cages and provided with good clean water *ad libitum*. They were fed with standard commercial feed (GuineaR growers, Benin).

This study was approved by the Faculty of Veterinary Medicine, University of Nigeria Nsukka Institutional Animal Care and Use Committee (IACUC) with the approval number: FVM-UNN-IACUC-2023-10/122a

Plant Material

The leaves of the plant material (*Pterocarpus milbreadii*) used in this study were collected from the wild and identified by a plant taxonomist.

Preparation of the Plant Extract

The leaves of PM were air dried at a very low intensity of sunlight to avoid denaturation of the active ingredient. They were pulverised and stored in an air tight container pending its use. About 500 g of the powdered leaves were soaked in 1.5 liters of 100% methanol with intermittent shaking every 2 hours for 48 hours. The mixture was filtered using Whatmann No. 1 filter paper. The filtrate was concentrated using rotary evaporator and the extract was stored in a refrigerator at 4°C.

Induction of Experimental Diabetes Mellitus

The rats were assigned into four groups (A–D) of five rats per group. Diabetes was induced in rats assigned to groups B–D while rats in group A served as normal control. Diabetes was induced in rats using the method described by Venugopal, Prince and Pari (1998). The rats were injected with alloxan monohydrate dissolved in distilled water at a dose of 160 mg/kg body weight intraperitoneally, after overnight fasting (18 hours). Meanwhile before the injection with alloxan monohydrate, the blood glucose levels of the rats were taken using Accu-Check glucometer. This was done by tail snip of the rats and allowing blood to drop on the Glucometer strip. The value was digitally read off on the screen of the glucometer. After induction, the rats were kept in clean stainless-steel cages and fed with commercial feed and were also given clean water for about 2 days. The rats were fasted overnight before the assessment of their blood glucose status on the 2nd day. The fasting blood glucose values above 7 mMol/L (126 mg/dl) were considered diabetic.

Treatment with the Methanol Extract of PM

Upon establishment of diabetes, the rats in groups C and D were administered with 200 mg/kg and 400 mg/kg of the extract while rats in groups A and B were treated with equal volume of the vehicle. Fasting blood glucose levels were checked at zero hour, one hour, three hours and six hours post extract administration.

GC-MS Analysis

The clear methanol leaf extract of PM was subjected to GC-MS analysis using a Trace 1310 gas chromatograph coupled to an ISQTM 7000 single quadrupole mass spectrometer equipped with an auto-sampler operated through a computer running the Chromeleon Dionex version 7.2.10.23925 software (Thermo ScientificTM, Italy). A DB-5 fused silica capillary column (27 cm × 27 cm × 17.7 cm; 12.9 L) coated with 5% phenylmethyl polysiloxane was used to analyse target compounds. Helium was used as the carrier gas at a constant linear velocity of 1 mL min^{-1}. A 1 µL sample volume was injected in a split mode of 10:1 ratio. The gas chromatography (GC) oven ramp temperature program was, 40°C (held for 5 minutes), 80°C at 5°C $mins^{-1}$ (held for 5 minutes) and finally 250°C at 10°C min^{-1} (held for 10 minutes). The injector, transfer line and ion source temperatures were set at 280°C , 250°C and 200°C, respectively, while the run time for the analysis was 48 minutes. The inlet temperature was 150°C with a front inlet purge flow of 3 mL/min. The mass spectrometer was operated in the selected ion mode (SIM) with an electron ionisation voltage of 70 eV using the electron impact (EI) mode. The MS scan range was set at 45 to 500 atomic mass unit (amu) with a dwell time of 0.2 seconds. The MS start time was set at 2.46 minutes, and the front inlet pressure to 100 kPa. The resultant spectra were matched with the database of the NIST (National Institute of Standards and Technology) version 2.3 (2017). Prior to sample injection, the syringe was first prewashed with the matrix (methanol) and sample so as to eliminate interferences from contaminants.

Molecular Docking

The bioactive compounds from PM (that were abundant and less toxic) were selected for molecular docking analysis. The ligands were docked into the active site of DPP-4 for best fit orientation using BIOVIA, Discovery Studio (version 2021) and AutoDock Vina software. Thereafter, the binding energies were calculated. The ligands and the target protein (DPP-4) were prepared following the standard procedure of protein and ligand preparation and the files were submitted to AutoDock Vina. The obtained binding energy and binding contacts of each ligand, and the docked data were analysed using Discovery Studio Visualiser.

Preparation of Ligands

The chemical structures of the bioactive compounds obtained from PM methanol leaf extract were retrieved from the PubChem compound database at NCBI (http:// pubchem. ncbi. nlm. nih. gov/).

Target protein retrieval: DPP-4 with identification number, 1NU6, was selected for its interactions with the bioactive compounds from PM. The 3D X-ray crystal structure of the protein was retrieved from Protein Data Bank (PDB).

DPP-4 Preparation

The Discovery Studio software was used to process and prepare the protein (DPP-4) and convert raw PDB structure into prepared protein models. An X-ray crystal structure of the protein (DPP-4) was prepared by removing the water molecules present in the structures. Then, Discovery Studio software was used to analyse protein structure, hydrogen bond

interactions and non-bond interactions of ligands with the active site residues and generations of high-quality images.

Docking

The prepared ligands and target protein (DPP-4) were analysed using AutoDock Vina to perform the docking. The various conformations for ligand in the docking procedure were generated and the final energy refinement of the ligand pose was performed. The docking score of the best pose into the target proteins for all the tested bioactive compounds was calculated.

Data Analysis

Data generated during the anti-diabetic studies were analysed with One-way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Variant means were separated with Duncans Multiple Range post hoc test. Results were expressed as Means SEM in table and graph. *P*-values were accepted at $p < 0.05$.

RESULTS

The results indicate that the FBG levels of all the rats were statistically the same ($p > 0.05$) before induction (pre-induction) of diabetes with alloxan monohydrate. However, post diabetes induction, the mean FBG values of the rats in groups B–D were significantly higher than that of the rats in group A (normal control). Following treatment with extract, the FBG values of all the rats decreased steadily but marginally until 24 hours duration of the experiment (see table 1).

Table 1: The effects of PM extract on the FBG values (mg/dl) of alloxan-induced hyperglycemic rats.

Group	Pre-induction	Post-induction	1 hour post treatment	3 hour post treatment	6 hour post treatment	24 hour post treatment
A	77.33 ± 3.8^a	77.66 ± 1.76^a	79.00 ± 1.52^a	79.33 ± 1.20^a	76.00 ± 3.00^a	74.33 ± 2.96^a
B	78.33 ± 1.45^a	410.33 ± 94.73^b	409.33 ± 90.99^b	453.00 ± 86.38^b	447.00 ± 93.05^b	387.33 ± 94.64^b
C	80.66 ± 5.81^a	419.66 ± 99.32^b	363.00 ± 106.92^b	403.00 ± 99.62^b	434.66 ± 86.69^b	300.00 ± 62.11^b
D	81.33 ± 4.17^a	461.33 ± 124.00^b	450.00 ± 120.70^b	365.66 ± 133.37^b	275.33 ± 87.47^b	220.00 ± 59.80^b

Note: Different letter superscripts a, b, across the groups (down the column) indicate significant differences at $p < 0.05$; A (Normal rats + Distilled water 10 mL/kg); B (Diabetic rats + Distilled water 10 mL/kg); C (Diabetic rats + 200 mg/kg PM extract); D (Diabetic rats + 400 mg/kg PM extract).

Twenty-four hours post treatment of the hyperglycemic rats with methanol leaf extract of PM, the group administered with 400 mg/kg recorded 51.96% FBG reduction while the group treated with 200 mg/kg achieved 27% reductions. The mean FBG values of the normal control rats and that of the diabetic untreated showed 4.33% and 5.16% reductions respectively (see Figure 1).

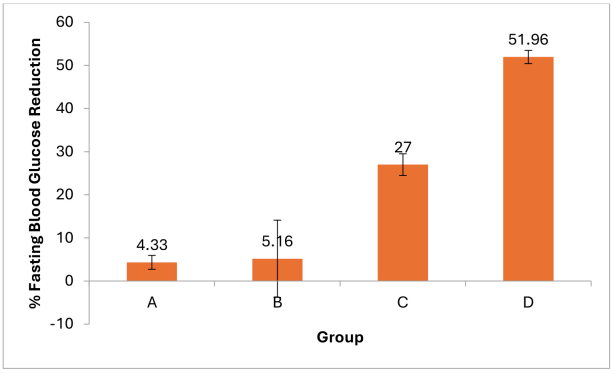

Figure 1: Percentage FBG reductions 24 hours following treatment of alloxan induced hyperglycemic rats with methanol extract of PM; A (Normal rats + Distilled water 10 mL/kg); B (Diabetic rats + Distilled water 10 mL/kg); C (Diabetic rats + 200 mg/kg PM extract); D (Diabetic rats + 400 mg/kg PM extract).

Following GC-MS analysis, ascorbic acid, phytol and octadecanoic acid were found to be the most abundant compounds found in the methanol leaf extract of PM. These compounds therefore were docked (served as ligands) against DPP-4 enzyme while Sitagliptin, a known DPP-4 inhibitor served as a standard control.

Table 2 shows the various binding energies between DPP-4 enzyme and the various ligands (Sitagliptin, ascorbic acid, phytol and octadecanoic acid). The best configurations showed the binding energies (Kcal/mol) of –8.3, –5.3, –5.2 and –4.8 for Sitagliptin, ascorbic acid, phytol and octadecanoic acid respectively.

Table 2: Molecular docking of various ligands and DPP-4 with their resulting affinities in Kcal/mol.

Mode/configuration	Sitagliptin	Ascorbic acid	Phytol	Octadecanoic acid
1	–8.3	–5.3	–5.2	–4.8
2	–7.9	–5.3	–5.2	–4.6
3	–7.8	–5.3	–5.0	–4.5
4	–7.7	–5.2	–5.0	–4.5
5	–7.6	–5.2	–5.0	–4.5
6	–7.6	–5.2	–5.0	–4.5
7	–7.6	–5.2	–4.9	–4.3
8	–7.5	–5.0	–4.8	–4.3
9	–7.5	–5.0	–4.8	–4.3

Tables 3–6 indicated also that at various configurations, the active site amino acids of the DPP-4 had different bonding types with the ligands (Sitagliptin, ascorbic acid, phytol and octadecanoic acid). In the same vein, the bonds included H-bonds, Van der Waals forces interactions (VFI), pi-alkyl (PA) and Pi-sigma bonds (Tables 3–6).

Table 3: The active site amino acid residues of DPP-4 and the various bonds and interactions formed with the ligand, Sitagliptin.

Configuration	Conventional Hydrogen bonds	Other bonds and interactions
1	SER:630, TYR:547, TYR:631, SER:309	9VFI, 1PA, 1CH
2	LEU:214, TRP:216	8VFI, 2PA, 4FL
3	TRP:157, VAL:155, ARG:61, ASP:104	7VFI, 1PA, 2FL
4	GLU:361, ARG358, CYS:301	12VFI, 1PA
5	ARG:61, SER:106, TRP216	3PA, 1CH, 1FL
6	TRP:157, TYR:105	6VFI, 1Pi-donor H, 4FL
7	ARG:61	8VFI, 1CH, 5FL
8	ARG:358, ARG:356, VAL:303, ASP:302, GLU:361	8VFI, 1PA, 1FL
9	TYR:361, TYR:666, TYR:547, SER:630	5VFI, 1PA, 1CH

Note: SER = Serine; TYR = Tyrosine; LEU = Leucine; TRP = Tryptophan; VAL = Valine; ARG = Arginine; ASP = Aspartic acid; GLU = Glutamic acid; CYS = Cysteine; CH = Carbon-Hydrogen bond; FL = Floride.

Table 4: The active site amino acid residues of DPP-4 and the various bonds and interactions formed with the ligand, ascorbic acid.

Configuration	Conventional Hydrogen bonds	Other bonds and interactions
1	GLU:205, SER:209, GLU:206, ARG:358, ARG:669	5VFI
2	ASN:710, ARG:125	8VFI, 1CH
3	SER:630, GLU:205, TYR:662, TYR:547	7VFI
4	ARG:125, TYR:662, GLU:206, HIS:740, SER:630	5VFI, 1UAA, 1CH
5	ASP:739, GLY:741, ARG:125	7VFI, 1UDD
6	GLU:206, TYR:662, ASN:710, HIS:740, SER:630, TYR:547	5VFI
7	ARG:125, ASP:709	7VFI, 1UDD, IUAA
8	GLU:117, TYR:132	8VFI, 1UDD
9	TRP:215, SER:212, VAL:303	6VFI

Table 5: The active site amino acid residues of DPP-4 and the various bonds and interactions it formed with the ligand, phytol.

Configuration	Conventional Hydrogen bonds	Other bonds and interactions
1	LYS:122	5VFI, 5PA
2	ASP:739, GLU:738	9VFI, 4PA
3	–	6VFI, 5PA
4	–	6VFI, 6PA, 1CH
5	LYS:250	6VFI, 4PA
6	ARG:253	6VFI, 5PA

(*continued on next page*)

Table 5: (*continued*)

Configuration	Conventional Hydrogen bonds	Other bonds and interactions
7	–	7VFI, 4PA, 1UAA, 1PS
8	–	9VFI, 4PA, 1PS
9	–	9VFI, 4PA, 1PS

Note: LYS = Lysine; UAA = Unfavourable Acceptor Acceptor.

Table 6: The active site amino acid residues of DPP-4 and the various bonds and interactions formed with the ligand, octadecanoic acid.

Configuration	Conventional Hydrogen bonds	Other bonds and interactions
1	ASP:739, GLU:738	6VFI, 5PA
2	LYS:122	6VFI, 4PA
3	TRP:629, VAL:546	10VFI, 2PA, 1PS
4	–	7VFI, 5PA, 1UAA, 1CH
5	ASP739	7VFI, 4PA
6	LYS:122	5VFI, 4PA
7	LYS:554, SER:577, ASP:545	6VFI, 6PA
8	TRP:629, ASP:545	8VFI, 1PA, 1UDD
9	ALA:743, ASP:739, SER:744	7VFI, 5PA

Figure 2 showed that the Sitagliptin, a known standard drug for treatment of type 2 DM had some level of strong binding affinity with some active site amino acid residues of DPP-4; four conventional hydrogen bonding, together with pi-Alkyl bonding occurred (see Figure 2).

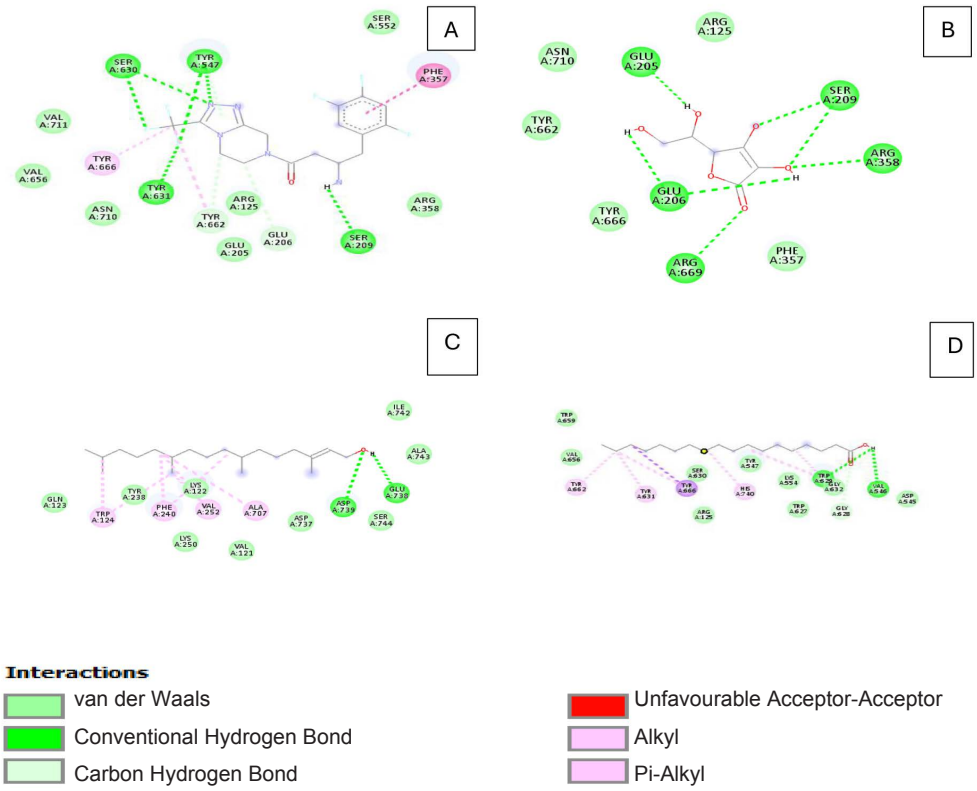

Figure 2: Active site amino acids of DPP-4 showing interactions with: (A) Sitagliptin, (B) ascorbic acid, (C) phytol and (D) octadecanoic acid.

DPP-4 and ascorbic acid interacted via five conventional hydrogen bonding together with Van der Waals forces. The binding interactions of the ligand, phytol and the enzyme DPP-4 were also illustrated in Figure 2. It showed that the ligand had only two conventional hydrogen bonding with the active site amino acid residues of the DPP-4 enzyme with few Van der Waals interactions and Pi-Alkyl bonding. The active site amino acid residue of DPP-4 interacted with octadecanoic acid ligand via multiple bonds notably: hydrogen bond, Van der Waals force interactions and pi-Alkyl bonds.

Figure 3 shows the surface views of DPP-4 and the ligands (Sitagliptin, ascorbic acid, phytol and octadecanoic acid) in close proximity. The ligands were seen lying in the front of the active site crypt of the enzyme (DPP-4).

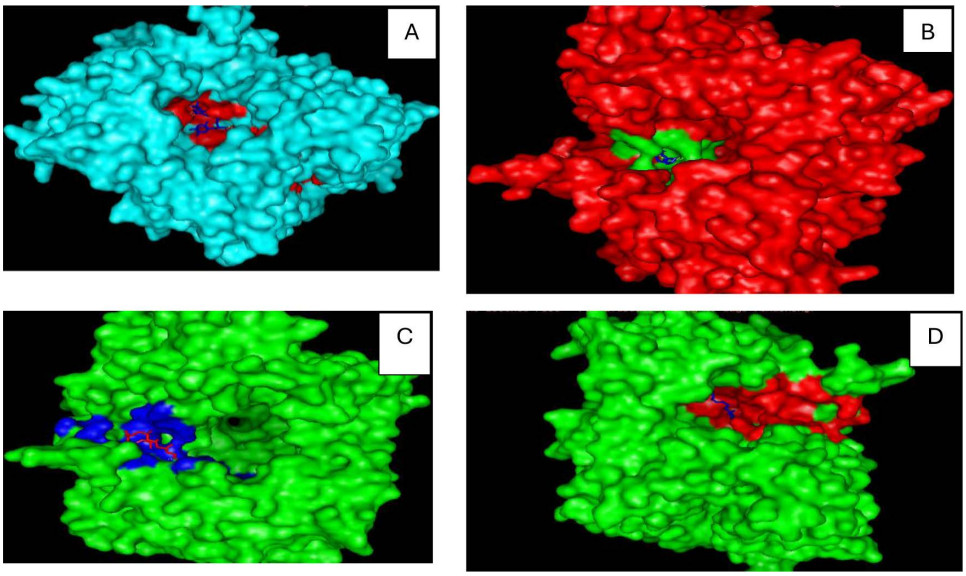

Figure 3: Surface form of DPP-4 showing interactions with: (A) Sitagliptin, (B) ascorbic acid, (C) phytol and (D) octadecanoic acid.

DISCUSSION

The results of the anti-diabetic studies showed that following alloxan monohydrate intraperitoneal injection, the mean FBG values of the rats in groups B–D increased significantly when compared with that of the normal control rats. This implies that alloxan monohydrate elicited the increase in the FBG levels. Alloxan monohydrate with the molecular formular of $C_4H_2N_2O_4$ is both a cytotoxic and carcinogenic organic compound used for induction of experimental diabetes (Lenzen 2008; Ighodaro, Adeosun and Akinloye 2017). Researchers have submitted that the diabetogenic potential of alloxan monohydrate is occasioned by its selective uptake by the beta cells of the pancreas (Szkudelski 2001). Alloxan monohydrate causes multiphase glucose responses ranging from transient intermittent hypoglycemia and hyperglycemia within the first 24 hours following its administration. However, between 24 hours and 48 hours post administration, a permanent hyperglycemia owing to destruction of the beta cells occurs (Ighodaro *et al.* 2017). Between one hour and 24 hours post treatment with PM extract, the FBG of the treated rats reduced appreciably; though the values were still higher than that of the normal control rats (see Table 1).

The mean FBG values of the rats treated with 400 mg/kg recorded 51.96% FBG reduction while the group treated with 200 mg/kg achieved 27% reductions. The mean FBG values of the normal control rats and that of the diabetic untreated showed 4.33% and 5.16% reductions respectively (see Figure 1). This indicates the hypoglycemic potentials of the PM methanol leaf extract. Previously, the aqueous extract of PM was demonstrated to have anti-hyperglycemic effects (Aba and Udechukwu 2018). The anti-diabetic effects of medicinal plants have been attributed to their phytochemical constituents and the secondary metabolites (Aba and Asuzu 2018).

The binding affinities of Sitagliptin (a standard anti-diabetic drug) and DPP-4 were presented in Table 2. The results showed that Sitagliptin associated more with DPP-4 when compared with ascorbic acid, phytol and octadecanoic acid. The binding energies of Sitagliptin, ascorbic acid, phytol and octadecanoic acid were –8.3, –5.3, –5.2 and –4.8 kcal/mol, respectively. Earlier studies by Ralte *et al.* (2022) submitted binding energies of –4.3kcal/mol following molecular docking of the compounds of *P. timoriana* against Cox-2 as being inhibitory. Consequently, the binding energies obtained in the present study suffice to say that the compounds of PM exhibited *in silico* anti-diabetic property by inhibiting DPP-4. The lower the binding free energy, the more the inhibitory activity (Vargas *et al.* 2018).

Tables 3–6 provided the types of bonding and interactions between the ligands and the active site amino acid residues of DPP-4 enzyme. It was observed that various bonds and interactions such as conventional hydrogen bond, Van der Waals force interaction, pi-Alkyl, pi-sigma, carbon-hydrogen bond, unfavourable acceptor-acceptor bond, unfavourable donor-donor bonding were also seen at various occasions as the means of interactions between the ligands and the active site amino acid residues. Compounds (ascorbic acid and Sitagliptin) that showed good binding scores had appreciable conventional hydrogen bonding with the active site amino acid residues of the enzyme. Researchers had reported the importance of hydrogen bonding in the expression of free binding energies and consequently, the inhibitory effects of such association (Ralte *et al*. 2022; Odoemelam *et al*. 2022). Ascorbic acid was the only test compound that made very good conventional hydrogen bond interactions with the active site amino acid of DPP-4 enzyme (see Figure 2). Ascorbic acid, therefore demonstrated superior *in silico* antidiabetic properties compared to phytol and octadecanoic acid.

Findings of various interactions between ligands and their target proteins had earlier been reported (Vargas *et al*. 2018). Sitagliptin, a known DPP-4 inhibitor bonded with the active site amino acid residues of DPP-4 via hydrogen bond mediated by SER610, TYR547, TYR433 and SER 209 (see Figure 2). Pi-Alkyl bonds and Van der Waals interactions were also among the means of association between the ligand and the enzyme. The ascorbic acid ligand interacted with DPP-4 via five hydrogen bonds mediated by GLU 205, SER 209, ARG 358, GLU 206 and ARG 669 amino acid residues of the enzyme. The active site amino acid residue of DPP-4 interacted with octadecanoic acid ligand via multiple bonds notably: hydrogen bond, Van der Waals force interactions and pi-Alkyl bonds. The ASP 739 and GLU 738 were the two active site amino acid residues that connected with the ligand phytol via hydrogen bonding. However, Van der Waals forces, pi-Alkyl and pi-Sigma bonds were observed mediating the interactions with the phytol ligand. Figure 3 shows ascorbic acid, phytol, octadecanoic acid and Sitagliptin in the active site of DPP-4 enzyme. Interactions between active site amino acid residues and the ligands generate the free binding energies which determine the strength of inhibition (Thamaraiselvi *et al*. 2021). Hydrogen bond describes a coulombic interaction between a polar donor (Dn d) and acceptor atom (:Acd). In chemistry, a hydrogen bond is primarily electrostatic force of attraction between a hydrogen atom which is covalently bound to a more electronegative donor atom or group and another electronegative atom bearing a lone pair of electrons-the hydrogen bond acceptor (Arunan *et al*. 2011). Hydrogen bonds can be intermolecular or intramolecular and are usually stronger than Van der Waals forces but weaker than covalent bonds (Gardner and Yajvidi 2016).

CONCLUSION

In considerations of the results of *in vivo* anti-diabetic and molecular docking results, it was concluded that the methanol leaf extract of PM has *in vivo* hypoglycemic potentials and *in silico* ability to inhibit DPP-4 enzyme.

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