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Article

Beneficial Effects of Natural Alkaloids from *Berberis glaucocarpa* as Antidiabetic Agents: An *In Vitro*, *In Silico*, and *In Vivo* Approach

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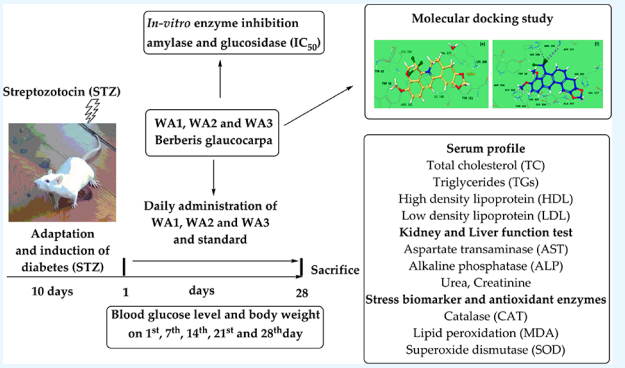
ABSTRACT: Diabetes, also known as diabetes mellitus (DM), is a metabolic disorder characterized by an abnormal rise in blood sugar (glucose) levels brought on by a complete or partial lack of insulin secretion along with corresponding changes in the metabolism of lipids, proteins, and carbohydrates. It has been reported that medicinal plants play a pivotal role in the treatment of various ailments such as diabetes mellitus, dyslipidemia, and hypertension. The current study involved exploring the acute toxicity and *in vivo* antidiabetic activity of berberine (**WA1**), palmatine (**WA2**), and 8-trichloromethyl dihydroberberine (**WA3**) previously isolated from *Berberis glaucocarpa* Stapf using a streptozotocin (STZ)-induced diabetic rat model. Body weight and blood glucose level were assessed on a day interval for 4 weeks. Biochemical parameters, antioxidant enzymes, and oxidative stress markers were also determined. In an acute toxicity profile, the **WA1**, **WA2**, and **WA3** were determined to be nontoxic up to 500 mg/kg (b.w). After the second and third weeks of treatment (14 and 21 days), the blood glucose levels in the **WA1**-, **WA2**-, and **WA3**-treated groups were significantly lower than those in the diabetic control group (476.81 ± 8.65 mg/dL, $n = 8$, $P < 0.001$). On the 21st day, there was a decrease in the blood glucose level and the results obtained were 176.33 ± 4.69, 197.21 ± 4.80, and 161.99 ± 4.75 mg/dL ($n = 8$, $P < 0.001$) for **WA1**, **WA2**, and **WA3** at 12 mg/kg, respectively, as opposed to the diabetic control group (482.87 ± 7.11 mg/dL, $n = 8$, $P < 0.001$). Upon comparison with the diabetic group at the end of the study (28 days), a substantial drop in the glucose level of **WA3** at 12 mg/kg (110.56 ± 4.11 mg/dL, $n = 8$, $P < 0.001$) was observed that was almost near the values of the normal control group. The treated groups (**WA1**, **WA2**, and **WA3**) treated with the samples displayed a significant decline in the levels of HbA1c. Treatment of the samples dramatically lowered the lipid level profile. In groups treated with samples, plasma levels of triglycerides, total cholesterol, and LDL were significantly lowered [F (5, 42) = 100.6, $n = 8$, $P < 0.001$]; these levels were also significantly decreased [F (5, 42) = 129.6 and 91.17, $n = 8$, $P < 0.001$]. In contrast to the diabetes group, all treated groups had significantly higher HDL levels [F (5, 42) = 15.46, $n = 8$, $P < 0.001$]. As a result, hypolipidemic activity was anticipated in the samples. In addition to that, the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) was considerably elevated in the groups treated with the sample compared to the diabetic control group ($n = 8$, $P < 0.001$).



1. INTRODUCTION

Diabetes mellitus (DM) is a serious condition that is spreading quickly.[1] By 2045, there are expected to be 693 million cases worldwide, up from the 425 million patients with DM who had a confirmed diagnosis in 2017.[2] According to the conventional classification, DM is divided into kinds 1 and 2, with type 2 diabetes making up the majority (>85%) of cases of DM overall.[3] It has numerous side effects, including adult blindness, kidney failure, localized soft tissue death, neuropathy, cardiac difficulties, and mortality.[4] Individuals with diabetes and their families, the healthcare system, and the overall economy all experience considerable financial hardships as a result of diabetes and its associated consequences. Diabetes-related direct healthcare expenses were anticipated to be $760 billion USD globally in 2019 and are predicted to increase to 825 and 845 billion USD globally by 2030 and 2045, respectively.[5] To treat diabetes, various synthetic drugs are being utilized, all of

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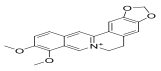
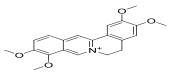
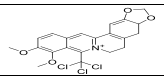
Table 1. α-Amylase and α-Glucosidase Inhibitory Activity with IC_{50}

Sample codes	Chemical Structures	IC_{50} μg mL	
		α-amylase	α-glucosidase
Berberine (**WA1**)		67.31	191.40
Palmatine (**WA2**)		81.22	225.67
8-Trichloromethyldihydroberberine (**WA3**)		53.21	148.78
Acarbose		39.98	123.3

which have adverse effects (such as dipeptidyl peptidase 4 inhibitors, metformin, thiazolidinediones, sulfonylureas, glucagon-like peptide 1, and sodium glucose cotransporter-2). Thiazolidinedione as well as biguanide therapy, for example, can cause weight gain, and both of these drugs are nephrotoxic. Medications based on incretins cause gastrointestinal issues.[6]

Numerous methods, including diets, drugs, and workouts, have been used to regulate blood glucose and reduce the potentially fatal consequences of diabetes[7] based on a variety of variables, including the rate of nutrient uptake, the impact of digestive enzymes on nutrient-rich foods, the activity of insulin, and the tissue's reaction to insulin.[8] Acarbose, voglibose, metformin, gliclazide, glimepiride, glyburide, repaglinide, nateglinide, sitagliptin, saxagliptin, rosiglitazone, pioglitazone, and orlistat have been utilized as oral antihyperglycemic agents for the management of DM.[9] When treating DM, medicinal plants might be utilized as an alternative to synthetic drugs. According to Samarakoon et al., natural herbal remedies for diabetes (more than 400 herbs may be found in the literature) are secure and effective alternatives to Western medicines.[10] Since more than a decade, researchers have been looking for safe and effective antidiabetic medication bases in plants, but the mechanisms by which antidiabetic activity develops and the best ways to focus their search are still unknown.[11] Natural plant-derived molecules and extracts, such as capsaicin, berberine, curcumin, ginsenosides, genistein, gingerols, catechins, resveratrol, stevioside, simple phenolic compounds, anthocyanins, and hesperidin, obtained from various species, are used to treat diabetes and have been found to have various mechanisms of action.[12] Alkaloids are nitrogen-containing, varied, basic chemical compounds with low molecular weight that are present in fungi, bacteria, animals, and plants. However, their movement within each kingdom is relatively constrained.[13] Alkaloids can exist as homo-oligomers or hetero-oligomers as monomers, dimers, trimers, or tetramers. The heterocyclic/nonheterocyclic chemical structure and biological or natural origin (certain sources) are used to categorize them. These secondary metabolites with high bioactivities, which serve as a rich source for drug disclosure, are produced by about 20% of plant species. A total of 12,000 alkaloids from plants with varying medicinal relevance have been evaluated.[14] These chemical entities have the potential to be used for effective antifungal, antimalarial, antioxidant, anticancer, cardioprotective, vaso-relaxing, anxiolytic, anti-inflammatory, analgesic, and antidiabetic activities.[14,15]

2. RESULTS

2.1. *In-Vitro* Assessment of Enzyme Inhibition. Table 1 displays the findings of the *in-vitro* α-amylase and α-glucosidase inhibitory activity of isolated substances in terms of IC_{50} (μg mL).

2.2. Molecular Docking Study. The preferred binding interactions and/or positions of all ligands in the catalytic pocket of α-amylase and α-glucosidase were further analyzed (Figure 1). The binding energies of synthesized ligands indicated moderate to decent fittings in the active sites of target proteins (Table 2). For the amylase enzyme, the crucial amino acids involved were Val49, Ile51, Trp58, Trp59, Tyr62, Gln63, Tyr151, Val157, Arg161, Leu166, Val98, Asp197, and Lys200. Additionally, the orientation of berberine (**WA1**) IC_{50} 67.31 μg/mL (docking score −5.82 kcal/mol) in the amylase enzyme predicted a benzodioxole ring interacting with active-site residue Hid207 through $\pi-\pi$ stacking whereas oxygen of the dioxole ring made it more stable by picking up a hydrogen bond interaction with neighboring Gln63 (2.105 Å) and penetrating inside the pocket of amylase, contributing toward higher inhibition and a stable ligand protein complex. In contrast, in the glucosidase enzyme, the berberine (**WA1**) with IC_{50} 191.40 μg/mL (docking score −6.51 kcal/mol) was predicted to form a hydrogen bond interaction with active-site residue Lys200 (2.657 Å) (Figure 1a,b).

In the case of amylase, the methoxy oxygen of palmatine (**WA2**) IC_{50} 81.22 μg/mL (docking score −4.32 kcal/mol) formed one hydrogen bond (2.535 Å) with active-site amino acid Gln63 and its phenyl rings were further stabilized by π- and cation interactions with Trp58 in the active site. For the glucosidase enzyme, palmatine (**WA2**) IC_{50} 225.67 μg/mL (docking score −5.55 kcal/mol) picked up two salt bridges with side-chain residues Glu233 and Asp300 and stabilized by surrounded hydrophobic and polar interactions (Figure1c,d).

The binding interaction and *in-vitro* validation study revealed that compound 8-trichloromethyldihydroberberine IC_{50} 53.21 μg/mL (docking score −6.07 kcal/mol) is the most active among the present series and binds well in the active binding site of both α-amylase and glucosidase (Table 2 and Figure 1e,f).

The crucial residues involved in inhibition and in forming an enzymatic binding pocket of the target proteins are Trp58, Tyr62, Ile148, Tyr151, Val157, Leu166, Arg195, Asp197, Lys200, His201,205,297, Gly304,306, Tyr158, Gly161, Thr245, Leu246, and Ala307, as reported by Mughal et al.[16] The most active compound 8-trichloromethyldihydroberberine (**WA3**) is predicted to form strong hydrogen bond interactions

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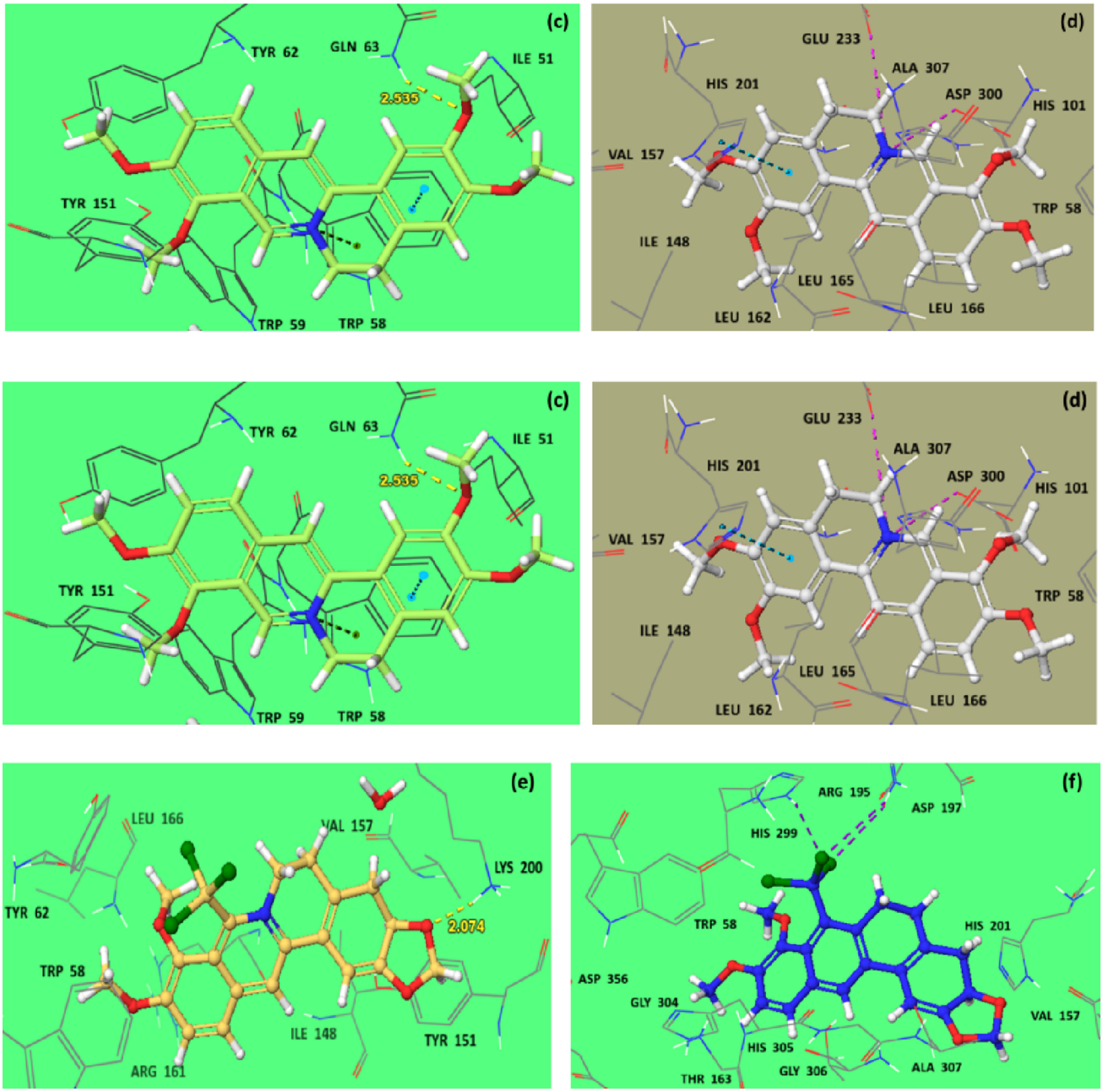

Figure 1. Ligand−protein interaction diagram for berberine (**WA1**) in the catalytic pocket of (a) amylase and (b) glucosidase enzymes. Ligand−protein interaction diagram for palmatine (**WA2**) in the catalytic pocket of (c) amylase and (d) glucosidase enzymes. Ligand−protein interaction diagram for 8-trichloromethyldihydroberberine in the (**WA3**) catalytic pocket of (e) amylase and (f) glucosidase enzymes.

Table 2. Enzyme Inhibitory Assay and Molecular Docking Scores of WA1, WA2, and WA3

	docking score (kcal/mol)	
sample codes	human α-amylase (PDB ID 3BAJ)	human α-amylase (PDB ID 3BAJ)
berberine (**WA1**)	−5.82	−6.51
palmatine (**WA2**)	−4.32	−5.55
8-trichloromethyl dihydroberberine (**WA3**)	−6.07	−7.05
acarbose	−8.93	−8.61

with important active side residue Lys200 (2.074 Å) in amylase, and the trichloromethyl moiety (**WA3**) is predicted to lie in a shallow area closer to Leu166, whereas the trichloromethyl (**WA3**) of this compound IC_{50} 148.78 μg/mL (docking score −7.05 kcal/mol) is forming three halogen bond interactions with amino acid residues Asp197, Arg195, and His299 in the active site of glucosidase to form a stable complex (Figure 1e,f).

2.3. Acute Toxicity. **WA1**, **WA2**, and **WA3** were found to be nontoxic up to 500 mg/kg (b.w.) in the acute toxicity profile and exhibited a normal behavior.

2.4. Assessment of Blood Glucose Levels. Upon comparison to the normal group, the STZ animals' blood sugar levels were markedly increased ($n = 8$, $P < 0.001$). All rat groups had their blood glucose levels periodically (every week) during the course of the 28-day treatment. When compared to the normal group of rats, the untreated (diabetic) group's

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Table 3. Blood Glucose Level of WA1, WA2, and WA3[a]

groups/dose (mg/kg)		blood glucose level in mg/dL				
		day 1st	day 7th	day 14th	day 21st	day 28th
normal control		107.11 ± 4.98	105.61 ± 5.19	104.88 ± 5.87	104.96 ± 4.87	101.81 ± 5.05
diabetic control		457.91 ± 7.43***	461.88 ± 7.71***	476.81 ± 8.65***	482.87 ± 7.11***	470.50 ± 8.93***
WA1	12	460.11 ± 5.17	376.70 ± 4.98*	268.21 ± 5.79**	176.33 ± 4.69***	133.01 ± 5.18***
WA2	12	462.85 ± 5.01	372.61 ± 5.19*	274.19 ± 4.91**	197.21 ± 4.80**	140.04 ± 4.81***
WA3	12	459.88 ± 4.98	367.11 ± 5.01**	253.23 ± 4.81***	161.99 ± 4.75***	110.56 ± 4.11***
glibenclamide	0.5	458.12 ± 6.15	349.36 ± 4.98**	248.70 ± 6.96***	138.49 ± 4.86***	102.19 ± 5.11***

[a]Mean ± SEM (n = 8). Following Dunnett's comparison test, significance was evaluated statistically using one-way ANOVA, with a P value of <0.05 versus the normal control and (*P < 0.05, **P < 0.01, ***P < 0.001, n = 8) vs the diabetic control. **WA1** (berberine), **WA2** (palmatine), and **WA3** (8-trichloromethyl dihydroberberine).

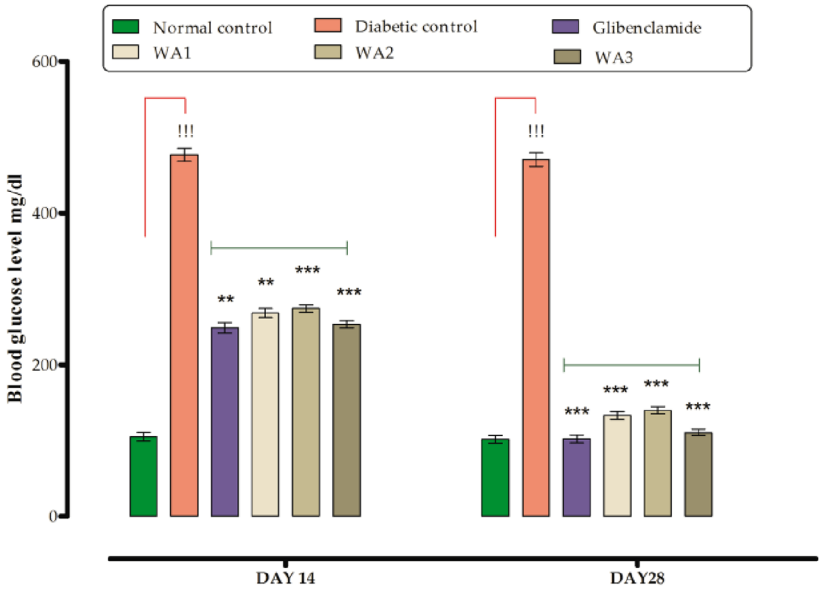

Figure 2. Blood glucose levels of **WA1**, **WA2**, and **WA3** on days 14 and 28. Mean ± SEM (n = 8). Following Dunnett's comparison test, significance was evaluated statistically using one-way ANOVA, with a P value of <0.05 versus the normal control and (*P < 0.05, **P < 0.01, ***P < 0.001, n = 8) vs the diabetic control. **WA1** (berberine), **WA2** (palmatine), and **WA3** (8-trichloromethyl dihydroberberine).

blood glucose levels were noticeably boosted by STZ injection. The glucose levels of the groups did not significantly decrease over the first week of therapy. As compared to the diabetic control group (476.81 ± 8.65 mg/dL, n = 8, P < 0.001), the sample-treated group blood glucose levels were found to 268.21 ± 5.79, 274.19 ± 4.91, and 253.23 ± 4.81 mg/dL (n = 8, P < 0.001) for **WA1**, **WA2**, and **WA3** at 12 mg/kg, respectively, on day 14. When compared to the diabetic control group (482.87 ± 7.11 mg/dL, n = 8, P < 0.001), the blood glucose levels for **WA1**, **WA2**, and **WA3** at 12 mg/kg on day 21 were found to 176.33 ± 4.69, 197.21 ± 4.80, and 161.99 ± 4.75 mg/dL, respectively (n = 8, P < 0.001).

When comparing the treated groups' glucose levels to those of the diabetic group at the end of the study's 28 days, a substantial decrease in **WA3**'s glucose level was seen at 12 mg/kg (110.56 ± 4.11 mg/dL, n = 8, P < 0.001) and was almost next to the normal control group of animals (Table 3 and Figure 2). At the end of the study (28 days), there was a substantial drop in the glucose level of WA3 at 12 mg/kg (110.56 ± 4.11 mg/dL, n = 8, P < 0.001) in comparison to the diabetic group and it was virtually at the levels of the normal control group (Table 3 and Figure 2).

The HbA1c level in the control group of diabetic rats significantly increased (n = 8, P < 0.001) and was reported to be 10.12%. In contrast to the control group's HbA1c of 10.12, the levels of the sample-treated diabetes groups were lower and dropped down to 7.55, 7.88, and 7.39% for **WA1**, **WA2**, and **WA3** at 12 mg/kg, respectively (Table 4). The standard drug significantly reduced the blood level of HbA1c to 6.91%.

The impact of exposure to treated samples on rat body weight was monitored for 28 days. The diabetic group's body weight increased considerably since they did not get any therapy, despite the fact that no noticeable difference in body

Table 4. Effect on Serum Insulin and the HbA1c Level[a]

groups/dose (mg/kg)		HbA1c (%)
normal control		6.04*
WA1	6	7.55***
WA2	6	7.88***
WA3	6	7.39
diabetic control		10.12**
glibenclamide	0.5	6.91***

[a]Mean ± SEM (n = 8). Following Dunnett's comparison test, significance was evaluated statistically using one-way ANOVA, with a P value of <0.05 versus the normal control and (*P < 0.05, **P < 0.01, ***P < 0.001, n = 8) vs the diabetic control. **WA1** (berberine), **WA2** (palmatine), and **WA3** (8-trichloromethyl dihydroberberine).

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Table 5. Effect of WA1, WA2, and WA3 on Body Weight[a]

groups/dose (mg/kg)		1st day	7th day	14th day	21st day	28th day
normal control		184.09 ± 4.91	181.20 ± 5.11	186.05 ± 5.02	189.78 ± 4.94	191.69 ± 4.79
WA1	12	183.77 ± 5.01	180.33 ± 4.91*	178.16 ± 4.96**	174.80 ± 4.77*	170.88 ± 4.84**
WA2	12	182.11 ± 4.98	182.70 ± 4.87*	180.01 ± 4.73**	175.01 ± 4.80*	169.65 ± 4.90**
WA3	12	184.27 ± 4.83	184.20 ± 5.11*	179.11 ± 4.79**	172.20 ± 4.73*	173.39 ± 4.88**
diabetic control		186.69 ± 5.06	178.50 ± 4.97**	166.94 ± 4.95***	160.32 ± 4.77***	153.61 ± 4.79***
glibenclamide		184.11 ± 5.15	183.91 ± 4.87*	189.16 ± 5.13**	187.63 ± 4.86***	194.37 ± 4.90***

[a]Mean ± SEM (n = 8). Following Dunnett's comparison test, significance was evaluated statistically using one-way ANOVA, with a P value of <0.05 versus the normal control and (*P < 0.05, **P < 0.01, ***P < 0.001, n = 8) vs the diabetic control. **WA1** (berberine), **WA2** (palmatine), and **WA3** (8-trichloromethyl dihydroberberine).

Table 6. Effect on Serum Profile of WA1, WA2, and WA3[a]

groups/dose (mg/kg)	normal control	WA1	WA2	WA3	diabetic control	glibenclamide
TC (mg/dL)	81.11 ± 4.20*	92.67 ± 4.81***	101.21 ± 4.32**	85.14 ± 4.01**	201.09 ± 5.31***	84.31 ± 4.95**
TG (mg/dL)	88.90 ± 4.16	112.17 ± 3.10**	114.89 ± 3.81**	104.90 ± 4.01***	196.31 ± 5.12***	102.97 ± 3.69***
LDL (mg/dL)	36.12 ± 1.71	56.33 ± 2.79***	58.79 ± 2.41**	43.88 ± 1.87**	121.77 ± 4.85***	40.17 ± 1.80**
HDL (mg/dL)	42.81 ± 2.16	35.11 ± 1.66**	35.41 ± 1.71**	37.81 ± 1.77***	23.10 ± 1.44***	39.64 ± 1.51***

[a]Mean ± SEM (n = 8). Following Dunnett's comparison test, significance was evaluated statistically using one-way ANOVA, with a P value of <0.05 versus the normal control and (*P < 0.05, **P < 0.01, ***P < 0.001, n = 8) vs the diabetic control. **WA1** (berberine), **WA2** (palmatine), and **WA3** (8-trichloromethyl dihydroberberine).

weight between the treated groups and the control group was observed (Table 5).

2.5. Estimation of the Serum Profile. According to this study, STZ medication increased plasma total cholesterol, LDL, and triglycerides while lowering HDL levels, in contrast to the normal group (Table 6). The level of the lipid profile is significantly reduced after treatment of the samples.

In diabetic groups treated with samples, plasma levels of total cholesterol, LDL cholesterol, and triglycerides were all significantly decreased ($F_{(5, 42)}$ = 100.6, n = 8, P < 0.001; Figure 3), while HDL levels were significantly higher in all treatment groups in comparison to the diabetic group [$F_{(5, 42)}$ = 15.46, n = 8, P < 0.001] (Table 6). Therefore, hypolipidemic activity in the samples is predicted.

2.6. Kidney and Liver Function Tests. Changes in blood levels of glutamate pyruvate transaminase (SGPT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) have been linked to the abnormalities in the liver brought on by STZ. The isolated **WA1**, **WA2**, and **WA3** were subjected to liver and kidney function tests, which resulted in a decline in the serum ALP, AST, and SGPT, indicating the stabilization of the plasma membrane and the reversal of hepatic and kidney tissue damage. The results showed that the administration of the samples considerably (P < 0.05, 0.01, and 0.001, n = 8) decreased the high serum levels of SGPT, AST, and ALP (Table 7).

2.7. *Ex-Vivo* Analysis of Stress Indicators and Antioxidant Enzymes. The MDA level was found to be greater in the STZ-induced diabetic group as a result of lipid peroxidation brought on by an excessive amount of free radical production that would eventually cause tissue deterioration. As revealed in Table 8, diabetic populations, which were given samples, dramatically reduced their MDA levels while in the groups that received the sample treatment, the activity of superoxide dismutase (SOD) was considerably enhanced [$F_{(4, 35)}$ = 37.06, n = 8, P < 0.001]. The catalase (CAT) activity revealed the protecting impact that may be related to antioxidant capabilities (Table 8), and similar types of findings were also seen there.

3. DISCUSSION

Blood glucose levels rise as a result of digestive enzymes hydrolyzing dietary polysaccharides. α-Glucosidase and α-amylase, enzymes belonging to class hydrolases, catalyze the hydrolytic breakdown of starch, glycogen, and various oligosaccharides that cause hyperglycemia.[17] Inhibition of these digestive enzymes with secondary metabolites from plants is a common technique for lowering postprandial blood glucose levels.[18] In order to prevent the formation of an enzyme−substrate complex, which in turn lowers the enzyme activity, alkaloids can attach to the enzymes involved in digestion at either their competitive or noncompetitive sites. The carbazole alkaloids isolated from *Murraya koenigii*, including bisgerayafoline D, bispyrayafoline, bismahanimbinol, *O*-methyl mukonal, *O*-methyl mahanine, and mahanine, have been shown to inhibit α-glucosidase.[19] In addition, mahanimbine inhibited α-glucosidase and α-amylase with IC_{50} value of 99 and 83 μg/mL, respectively.[20] Moreover, the quinazoline alkaloids vasicinol and vasicine from *Adhatoda vasica* effectively block the hydrolysis of sucrose by intestinal α-glucosidase in rats, with K_i (183 and 82 M) and IC_{50} (250 and 125 M), respectively.[21] Additionally, *Piper umbellatum piperumbellactams* A, B, and C had α-glucosidase inhibitory activities of 98, 43, and 29 M, respectively.[31] Vindogentianine from *Catharanthus roseus* inhibits the enzymes α-amylase and α-glucosidase with *p*-nitrophenyl-D-glucopyranoside and dinitrosalicylic acid, respectively.[22] Insulin and glucagon, which have opposing effects on peripheral organs, are secreted by the α-cells and β-cells of pancreatic islets of Langerhans (IoL). Insulin reduces blood sugar levels by promoting skeletal muscle glucose absorption while suppressing hepatic glucose synthesis and slowing lipolysis. By increasing gluconeogenesis and lipolysis, in contrast, glucagon raises the blood glucose level.[23] The quinolizidine alkaloids from *Lupinus* species, lupanine, 13-hydoxy-lupanine, and 17-oxo-lupanine, are able to stimulate insulin secretion in a glucose-dependent way.[24] Oxidative stress brought on by hyperglycemia results in micro- and macrovascular problems.[25] Diabetes antioxidant therapy reduces the problems associated with diabetes.[25,26] In response

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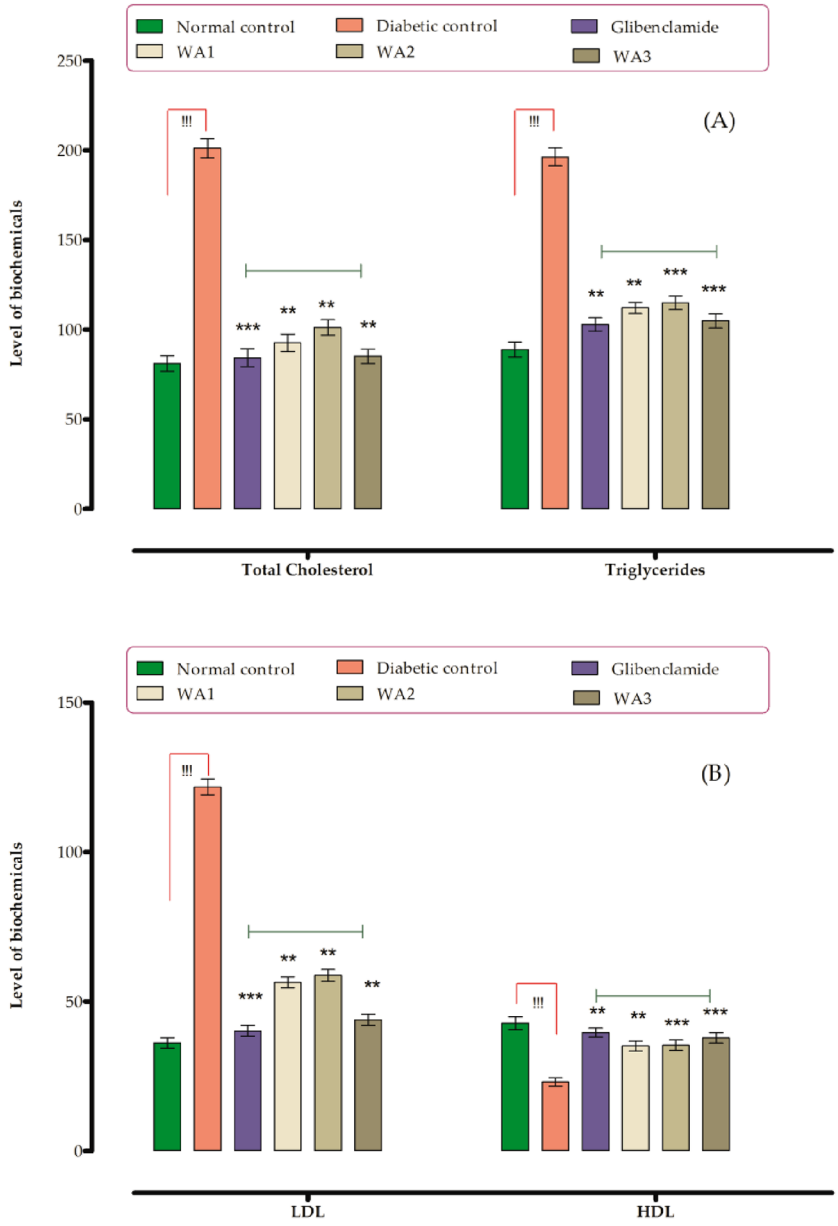

Figure 3. Effect on the serum profile of **WA1**, **WA2**, and **WA3**. Effect on (A) total cholesterol (TC) and triglycerides (TGs) and (B) low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Mean ± SEM (n = 8). Following Dunnett's comparison test, significance was evaluated statistically using one-way ANOVA, with a P value of <0.05 versus the normal control and (*P < 0.05, ***P < 0.01, ***P < 0.001, n = 8) vs the diabetic control. **WA1** (berberine), **WA2** (palmatine), and **WA3** (8-trichloromethyl dihydroberberine).

Table 7. Effect of WA1, WA2, and WA3 on Kidney and Liver Function Test[a]

	parameters	normal control	WA1	WA2	WA3	diabetic control	glibenclamide
kidney	urea (mg/dL)	37.12 ± 1.60*	47.30 ± 1.51**	46.11 ± 1.80***	43.20 ± 1.66***	99.30 ± 3.40***	40.17 ± 1.90
	creatinine (mg/dL)	0.94 ± 0.06	1.07 ± 0.06***	1.09 ± 0.07***	1.04 ± 0.09**	1.88 ± 0.11***	1.01 ± 0.07
liver	ALP (U/L)	116.97 ± 3.87	145.69 ± 3.78***	152.13 ± 4.15**	139.15 ± 3.97***	228.97 ± 4.86***	133.67 ± 3.71
	AST (U/L)	49.91 ± 1.97	71.16 ± 1.79**	70.86 ± 2.11***	62.13 ± 2.01***	98.03 ± 2.97***	55.03 ± 1.94
	ALT (U/L)	68.14 ± 2.04	88.30 ± 2.45**	86.91 ± 2.87**	78.95 ± 2.50***	120.90 ± 2.88***	71.43 ± 2.13

[a]Mean ± SEM (n = 8). Following Dunnett's comparison test, significance was evaluated statistically using one-way ANOVA, with a P value of <0.05 versus the normal control and (*P < 0.05, **P < 0.01, ***P < 0.001, n = 8) vs the diabetic control. **WA1** (berberine), **WA2** (palmatine), and **WA3** (8-trichloromethyl dihydroberberine).

to reactive radicals, antioxidants either accept or donate an electron, prevent the activity or expression of enzymes that produce reactive radicals, or increase the activity and expression of enzymes that are responsible for producing antioxidants.[27] Alkaloids such oriciacridone C, 1,3,5-trihydroxy-4-(c,c-dimethylallyl)-acridone, and oriciacridone F showed antioxidant activity in addition to glucosidase inhibitory action, with IC_{50} values of 60.79, 118.70, and 482

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Table 8. Different Experimental Groups' Markers for Oxidative Stress and Antioxidant Enzymes[a]

groups/dose (mg/kg)	normal control	WA1	WA2	WA3	diabetic control	glibenclamide
SOD (U/mg protein)	10.87 ± 1.40*	7.41 ± 0.59***	7.76 ± 0.63**	8.10 ± 0.77***	3.97 ± 0.62***	8.71 ± 0.57***
CAT (U/mg protein)	41.09 ± 2.34	32.97 ± 1.21**	28.91 ± 1.44**	33.80 ± 1.39***	18.66 ± 1.73***	39.12 ± 2.11***
TBARS (nmol MDA/mg protein)	2.03 ± 0.55	2.89 ± 0.66***	2.97 ± 0.83***	2.90 ± 0.74***	4.61 ± 0.83***	2.56 ± 0.69***

[a]Mean ± SEM (n = 8). Following Dunnett's comparison test, significance was evaluated statistically using one-way ANOVA, with a P value of <0.05 versus the normal control and (*P < 0.05, **P < 0.01, ***P < 0.001, n = 8) vs the diabetic control. **WA1** (berberine), **WA2** (palmatine), and **WA3** (8-trichloromethyl dihydroberberine).

mM.[28] The antioxidant activity of piperumbellactams A, B, and C, respectively, was reported to be 13.1, 67.8, and 86.4 μM. *O*-Methyl mahanine, *O*-methyl mukonal, bispyrayafoline, bismahanimbinol, and mahanine all exhibit antioxidant activity with IC_{50} values ranging from 6.3 to 400 μM.[19] Studies have reported that dysregulation of oxidative stress is the crucial cause for etiology of DM and the development of reactive oxygen species (ROS) associated with high blood glucose level. The oxidative stress plays a prime role in the mechanism of DM and associated complications. The excessive production of free radicals is considered to be due to multiple mechanisms including the polyol pathway, glucose autoxidation, protein glycation, and lipid peroxidation. Antioxidants and antioxidant enzymes present in cells function to prevent the damage done by oxidative stress and DM. In streptozotocin (STZ)-induced diabetic mice, the steroidal alkaloids sarcovagine D and holaphylline from *Sarcococca saligna* likewise demonstrated hypoglycemic effects and managed diabetes-related problems.[29] In the STZ-induced diabetic rat model, *O*-methylmurrayamine A and koenidine lower blood glucose levels by roughly 24.6 and 22.5%, respectively, during the course of 0−300 min, which is equivalent to metformin (25.9%).[30] On *in vivo* experiments, the root of *Combretum dolichopetalum*'s echinulin and arestrictin B demonstrated significant antidiabetic action comparable to glibenclamide.[31]

4. MATERIALS AND METHODS

Berberine (**WA1**), palmatine (**WA2**), and 8-trichloromethyl dihydroberberine (**WA3**) used in this investigation were extracted from *B. glaucocarpa* Stapf, and the spectroscopic results agree with previously published literature.[32] Streptozotocin, Tween-80, α-glucosidase, and substrate (*p*-nitrophenyl-α-D-glucopyranoside) were bought from Sigma-Aldrich in Germany. The local pharmaceutical company provided the glibenclamide. All of the other chemicals were of analytical grade.

4.1. Molecular Docking. A docking study of compounds was performed to investigate their potential to be dual inhibitors and the possible binding mode in the binding sites of α-amylase and α-glucosidase enzymes using a molecular operating environment (MOE, v2015.10). Human pancreatic amylase's X-ray crystal structures were collected from the Protein Database Bank (PDB) (https://www.rcsb.org/pdb/home/home.do). Due to the difficulties of extracting pure and homogeneous human α-glucosidase from endogenous sources, the *Saccharomyces cerevisiae* has functioned as a model structure to test the inhibitory potential of different substances. Although only few homology models have been described,[33] we constructed a 3D homology model for α-glycosidase from the already reported method with little modification.[34] The *S. cerevisiae* isomaltase crystal structure (PDB ID 3AJ7), which has excellent sequence similarity (40%) with the target, was used as the molecular modeling template. Through the use of MOE's search tools, the templates were located and then incorporated into MOE to create its 3D structure. The 3D crystal structure of human pancreatic α-amylase in association with nitrate and acarbose (PDB ID 3BAJ) was retrieved from the RCSB protein data bank. The generated 3D model underwent energy minimization up to 0.05 gradients. Prior to ligand docking, the protonate 3D tool was used to protonate all of the proteins and ligands. In order to lower the energy gap to 0.01 gradient, a database of 3D built ligands was created to be used as the input file for MOE docking with the applied Amber 10:EHT force field. The database was finally docked into the target protein's active site using the Triangular Matcher method and 30 conformations, each of which had a docking score (S). Maestro Schrodinger (v2017-2) was used to create 3D images and investigate each complex's interactions and visual examination.[35]

4.2. *In-Vitro* Assessment of Enzyme Inhibition. Against α-amylase and α-glucosidase, we measured the inhibitory capacities of **WA1**, **WA2**, and **WA3**. The test samples contained 120 μL of 0.1 M phosphate buffer (pH 6.9), 20 μL of α-glucosidase (0.5 unit/mL), and 10 μL of each of **WA1**, **WA2**, and **WA3** at different concentrations, which were then incubated for 15 min at 37 °C with the combination solutions in 96-well plates. 20 μL of a 5 mM *p*-nitrophenyl-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to the mixture after it had been remixed for a further 15 min to begin the enzymatic reaction. To stop the reaction, 80 L of 0.2 M sodium carbonate was added. Following that, the absorbance at 405 nm was measured using a microplate reader. The positive control was a sample-free reaction system, and a blank without glucosidase was created to account for background absorbance. After 15 min of remixing, 20 μL of 5 mM *p*-nitrophenyl-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to start the enzymatic activity. The positive control was made without the use of α-glucosidase, and the blank was created using a sample-free reaction technique for background absorbance adjustment.[36]

4.3. Animals. Male Wistar rats, 8−10 weeks old, weighing 175−195 g, and Balb/C mice, 23−28 g, were donated by the Veterinary Research Institute (VRI), Lahore. The animals were housed under typical laboratory settings (25°−2 °C, relative humidity of 55°−65%, and a 12 h light/dark cycle). Standard diet and water were also provided. Animals were permitted to adapt to laboratory conditions prior to the studies, and they were handled in accordance with the "Animals Byelaws 2008 of University of Malakand (Scientific Procedures Issue-I)" procedures. The study was conducted out in accordance with the Department of Pharmacy's Ethical Committee's clearance, as evidenced by notification number Pharm/EC-Bg-Np/26-10/22.

4.3.1. Assessment of Acute Toxicity Study. Two phases were used to test the acute toxicity of **WA1**, **WA2**, and **WA3**

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for potential toxicological effects. To groups in the first and second phases, oral dosages of natural items in milligrams per kilogram of b.w. and Tween 80 were administered. In the first phase, one group (the control group) received 2% Tween 80 while the other groups received a variety of oral doses (5, 50, and 100 mg/kg of body weight, respectively). In the first phase, the remaining groups were given varied oral dosages (125, 250, and 500 mg/kg of body weight). Animals were observed for 24 h at first, and then daily for 72 h, followed by 14 days of surveillance to search for evidence of aberrant behavior, allergy manifestations, and death.[37] Based on early pharmacological research and previously published data, the effective dose for pharmacological activity (12 mg/kg b.w.) was chosen.[38]

4.4. Type II Diabetes Induction. Following adaptation, the HFD rats were given an intraperitoneal (ip) injection of 50 mg/kg streptozotocin (STZ) in 0.1 M citrate buffer. To reduce hypoglycemia shock-related mortality, mice were given a 10% glucose solution for 3 days. An SD glucometer (Accu-Chek, Active blood glucose meter, Korea) was used to assess blood glucose levels 72 h after STZ therapy in a tail vein. Diabetic animals had fasting blood glucose levels more than 250 mg/dL.[39]

4.5. Experimental Design for Antidiabetic Activity. A total of 48 rats of similar strain, age, and weight after diabetes induction were divided randomly into six groups each containing eight animals (n = 8), comprising diabetic and normal control test groups given berberine, palmatine, and 8-trichloromethyl dihydroberberine at a dose of 12 mg/kg b.w. (**WA1**, **WA2**, and **WA3**, respectively). The positive control group was given the recommended dose of 0.5 mg/kg. Only the vehicle was given to the control (normal) group and the STZ diabetic groups' animals. For 4 weeks, the compounds were administered orally (12 mg/kg) to the treated animals.

4.5.1. Calculating Body Weight and Blood Sugar Levels. On the first day of each of the weeks (1st, 7th, 14th, 21st, and 28th) for 4 weeks, the blood glucose level and body weight were determined.[40]

4.5.2. Glycated Hemoglobin and Insulin Levels. Insulin and glycated hemoglobin A1c (HbA1c) levels were assessed in each therapy group in accordance with the manufacturer's recommendations.

4.5.3. Assessment of Serum Profile. At the end of the study (28th day), once the antidiabetic assay was finished, all of the animals were humanely euthanized with isoflurane and blood samples were taken to evaluate the biochemical parameters. Lipid profiles including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate transaminase (AST), alkaline phosphatase (ALP), and kidney function parameters including urea and creatinine were all measured.[41] The presence of antioxidant enzymes including catalase (CAT), lipid peroxidation (MDA), and superoxide dismutase (SOD) was tested in a homogenized and treated liver slice for the existence of the oxidative stress signal.

4.6. Statistical Analysis. Data were presented as mean ± SEM (n = 8) and statistically analyzed with statistical software (GraphPad Prism 5.01 version) using one-way ANOVA and Dunnett's *post hoc* test. P value P < 0 05 was considered statistically significant.

5. CONCLUSIONS

In conclusion, DM is a metabolic disorder characterized by an abnormal rise in blood sugar (glucose) levels associated with an imbalance in insulin secretion, along with corresponding changes in the metabolism of lipids, proteins, and carbohydrates. The natural alkaloids berberine, palmatine, and 8-trichloromethyl dihydroberberine from *B. glaucocarpa* Stapf has significantly decreased levels of HbA1c, triglycerides, total cholesterol, and LDL. These alkaloids have an enhanced level of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in animal models. In addition, these compounds may be suitable candidates for curing diabetes after exploring their pharmacological profile.

■ ASSOCIATED CONTENT

Data Availability Statement

All the data contains within this article.

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Notes

The authors declare no competing financial interest.

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