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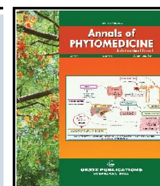

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Exploring antihyperglycemic and histopathological analysis of *Oxalis corniculata* L. hydroethanolic extract in STZ induced diabetic rats with *in silico* α-amylase inhibitor assessment

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Abstract

Diabetes mellitus is an epidemic and multifaceted metabolic disorder defined by hyperglycemia, that reduces insulin secretion sensitivity, leading to damage to vital organs. This study aimed to investigate the antioxidant and antidiabetic effects of the hydroethanolic extract of *Oxalis corniculata* L. (OCHEx) in addition to the hypoglycemic effect of bioactive compounds identified from GC-MS profiling using molecular docking studies to explore possible α-amylase inhibitors. The antioxidant capacity of the OCHEx were determined using *in vitro* DPPH, ABTS, and total antioxidant assays. Antidiabetic, biochemical, and histopathological studies were performed in an *in vivo* STZ induced rat model. The OCHEx showed antioxidant activity against DPPH, ABTS, and total antioxidant expressed in IC_{50} value with 124.56 ± 2.40 µg/ml, 159.17 ± 2.98 µg/ml and 147.83 ± 1.91 µg/ml, respectively. Furthermore, the highest inhibition percentages of α-amylase (71.0 ± 2.8), α-glucosidase (74.6 ± 4.2), and acarbose (92.1 ± 3.7) were obtained at 200 µg/ml. OCHEx treatment significantly reduced blood glucose levels and concurrently improved blood insulin levels, lipid profiles, and liver marker enzymes significantly ($p<0.05$), with improvement in the pancreas and liver revealed through histopathological studies, indicating favour able effects in mitigating diabetic complications. Furthermore, GC-MS screening identified nine compounds, four of which showed potential as α-amylase inhibitors, as investigated through *in silico* and ADME studies. Our study concludes that OCHEx is a valuable source of antioxidants and ameliorates the risk of diabetes owing to the presence of potential bioactive compounds.

1. Introduction

In the progression of many chronic diseases, diabetes mellitus (DM), is an epidemic of widespread endocrinal metabolic disorders characterized by raised blood glucose levels resulting from inadequate insulin secretion or β-cell destruction. DM is also associated with impairment in crucial biomolecule metabolism, caused by abnormal hormonal action (Khavandi *et al.,* 2013; Sanjeev and Divya, 2021). Diabetes is a complex condition influenced by several factors, including genetic, environmental and metabolic. Other factors, like family history, obesity, sociodemographic, sedentary lifestyles, poor-dietary and inactive exercise habits are the key risk factors responsible for diabetes prevalence (Al-Goblan *et al.*, 2014; Kyrou *et al.,* 2020). Diabetes is classified mainly into Insulin-dependent (IDDM), referred to as type 1 diabetes (T1DM) is caused by the autoimmune destruction of pancreatic β cells resulting in inability in insulin production, while non-insulin dependent (NIDDM) often known as type 2 diabetes (T2DM) is defined by insulin resistance and relative insulin deficit status (Choi *et al.,* 2021; Tan *et al.,* 2019). Approximately 90-95% of individuals are diagnosed with T2DM, making it the most widespread form of the disease (Wu *et al.*, 2014). The International Diabetes Federation (IDF) estimated nearly 642 million will experience T2DM by the end of 2040. Individuals with T2DM are vulnerable to various acute conditions like diabetic ketoacidosis and multiple chronic complexities such as macrovascular complications including cardiovascular disease, Cancer, amputation, retinopathy and diabetic nephropathy (Barski *et al.,* 2013; Chawla *et al.* 2016; Martin-Timon *et al.,* 2014).

Reducing the risk of T2DM (Swetha and Velraj, 2023) is a major healthcare concern. Over time, several new classes of oral hypoglycemic drugs have been approved to reduce blood glucose levels including GLP-1R agonists, DPP4 inhibitors, SGLT2 inhibitors and Sulfonylureas. These medications aim to encourage insulin sensitivity and improve glucose absorption, administrated either

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single or in combination. However, despite their efficacy in reducing blood sugar, they may also exhibit various adverse effects and remain relatively expensive, posing, accessibility challenges, particularly for individuals in developing countries (Chen *et al.,* 2011; Zhao *et al.,* 2015). Plants have been employed for centuries in conventional medical practices to alleviate chronic as well as acute illnesses due to esteemed for their less harmful, cost-effective and easy accessibility. Their therapeutic properties are based on the abundance of various classes of phytochemicals including; alkaloids, flavonoids, phenolic acid, terpenes, saponins, cardiac glycosides and phytosterols (Heinrich *et al.,* 2021; Patel *et al.,* 2012; Tahrani *et al.,* 2016; Ullah *et al.,* 2020).

Oxalis corniculata L. belongs to the family Oxalidaceae is a small perennial, weediest herb, vascular plant species distributed globally and most widespread near agriculture fields and wetland regions (Swami and Malpathak, 2018). Ethnomedicinal studies have revealed that *O. corniculata* is utilized for chest pain, headache, stimulant, anaemia, diarrhoea and dysentery (Ibrahim *et al.,* 2013). Phytochemicals investigation has revealed an enormous number of vital compounds notably flavonoids glucoside, phenol and palmitic acid (Badwaik *et al.,* 2011). Previously, the plant was proven to have carbohydrate degradation enzyme inhibitory characteristics through *in vitro* antidiabetic investigations (Agila and Kavitha, 2012). Additionally, the aqueous extract has been reported antioxidant, antihyperglycemic and antihyperlipidemia effects explored through *in vivo* studies (Himaja and Das, 2015). However, irrespective of these findings, there remained a significant insufficiency of details information regarding the specific bioactive substances responsible for lowering blood glucose levels. Consequently, this study endeavours to assess the *in vivo* antidiabetic properties of novel bioactive compounds.

This present study aimed to screen the OCHEx for antioxidant and antidiabetic activity in STZ induced diabetic rats. Additionally, we conducted GC-MS to identify putative phytochemicals and molecular docking study to investigate possible novel α-amylase inhibitors, aiming to address not only postprandial diabetes but also early diabetic symptoms characterized by insulin resistance. Furthermore, our investigation revealed the druglikeness behaviors of identified phytochemicals.

2. Materials and Methods

2.1 Collection and authentication of plant

O. corniculata whole plant was collected from the herbal garden of the Department of Biological Science, SHUATS, Prayagraj, India (25.4137°N 81.8491°E). The authentication was done by Dr. Satya Narayan, Department of Botany, University of Allahabad, Prayagraj-211002, Uttar Pradesh, India, with Accession Number DUTHIE-1-2020.

2.2 Preparation of hydroethanolic extract of *O. corniculata*

The hydroethanolic extract of *O. corniculata* (OCHEx) was prepared as the method reported by Agila and Kavitha (2012) with a few modifications. The whole plant was kept shaded and dried for 10-13 days after being thoroughly washed and pulverized into fine powder. A total weight of 250 g of dried sample was macerated and ground into fine powders. A Soxhlet apparatus was used to perform a hot extraction operation in a hydroethanolic solvent for 48 h at 50-60°C. Using Whatman filter paper (No. 1), the extracts were filtered. The filtrate was then placed into a rotary evaporator for concentration and kept at 4°C until further investigation.

2.3 *In vitro* antioxidant estimation

2.3.1 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant effect of OCHEx ability was assessed by the DPPH method following Rahman *et al.* (2015). Briefly, 0.1 mM of DPPH was prepared in 2.95 ml of methanol. Test sample at different concentrations was mixed in 1 ml solution and allowed to incubate for 25-30 min at room temperature and obtained absorbance at 517 nm. The antioxidant results were represented in IC_{50} values (µg/ml) gallic acid equivalent.

2.3.2 Phosphomolybdate (total antioxidant) assay

The phosphomolybdate assay of OCHEx was performed standard method described by Wan *et al.* (2011) with slight modification. Briefly, 0.5 g of extract was mixed with reagent constituents with sulphuric acid, sodium phosphate, and ammonium molybdate at concentrations of 0.6 M, 28 mM and 4 mM, respectively. The test sample was incubated at 37°C with vigorous shaking for 90 min. and absorbance was assessed at 765 nm opposite methanol as blank. The percentage inhibition result was obtained in IC_{50} values (µg/ml) of standard ascorbic acid equivalents.

2.3.3 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

The antioxidant effect of OCHEx against ABTS was accessed as per the standard method described by Wan *et al.* (2011) with slight alternation. Briefly, a mixture of 7 mM of ABTS solution and 2.45 mM of potassium persulfate dissolved in distilled water to prepare ABTS cation. The mixture was incubated for 12-16 h in the dark. The mixture was diluted to accomplish an initial absorbance of 0.70 ± 0.02 at 734 nm. An aliquot of 0.3 ml of extracts was mixed with 3 ml of mixture and incubated for 6 min. The reduction of absorbance was measured in a spectrophotometer at 734 nm. The results were represented in IC_{50} level (µg/ml) of trolox equivalent.

2.4 Determination of α-amylase and α-glucosidase inhibition

2.4.1 Inhibition of α-amylase enzyme

The α-amylase inhibitory activity was followed by Ahmed *et al.* (2014). Briefly, samples ranging from 50 µg/ml - 200 µg/ml were mixed in freshly prepared enzyme (U/ml) in 20 mM Na-phosphate buffer at pH 6.8 and incubated at 37°C for 15 min. Starch with 1 ml of 0.2% (w/v) dissolved in the Na-phosphate buffer to initiate reactions. The reaction mixtures were incubated for 15 min, followed by the addition of 1 ml of di-nitro salicylic acid (DNSA) as a color reagent. Subsequently, test samples were kept in a hot water bath for 5 min and absorbance was measured at 540 nm. Percentage inhibition was determined as follows:

α-amylase inhibition % =

$$\frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

2.4.2 Inhibition of α-glucosidase enzyme

The α-glucosidase inhibition action was carried out following the method reported by Himja and Das (2015). Briefly, OCHEx at varying ranges from 50 µg/ml - 200 µg/ml were mixed with 1.5 ml of 0.5 mM of PNPG (substrate) in freshly prepared 0.02 M phosphate buffer with pH of 6.5. Subsequently, 1.5 ml of α-glucosidase (0.8 U/ml) enzyme prepared in 0.01 M of phosphate buffer (pH 6.5) was added to the reaction mixture and further incubated at 37°C for 30 min. Added 5 ml of 0.2 M Na_2CO_3 to terminate the reaction. The α-glucosidase inhibitory activity was observed at 405 nm and percentage inhibition was determined as follows:

α-glucosidase inhibition % =

$$\frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

2.5 *In vivo* Antidiabetic study

2.5.1 Experimental Design

The experiment was designed after the approval of the Institutional Animal Ethical Committee (IAEC) conducted by the Department of Pharmaceutical Science, SHUATS, under IAEC No. 1813/GO/Re/S/15/CPCSEA/20210728/01. The antidiabetic activity was carried out in adult Albino Wistar rats of either sex, 150-200 g of weight. The animals were habituated for a week in a Laboratory Animal Facility (LAF), SHUATS, and kept in sterile husk-bedded polypropylene rat cages. The ambient temperature was maintained at 24 ± 2°C with a relative humidity of 50 ± 5%. The animals were subjected to a 12 h light-dark phase to facilitate rat thermoregulation. The rats were given unrestricted access to intake standard pellet diet and water consumption *ad libitum*. The entire experiment was carried out for a duration of 28 days. A total of 30 rats, comprising of either sex were used throughout the study. For experimental analysis, these rats were divided into 5 groups, with each group consisting of 6 rats. The distribution of rats into groups was based on their weight (Ahmed *et al.,* 2014). The details of the group included:

Group 1: Normal rats (Control) administrated vehicle saline (0.9% NaCl)

Group 2: STZ induced diabetic rat (50 mg/kg b.w.)

Group 3: STZ induced diabetic rat dose with OCHEx (100 mg/kg b.w.)

Group 4: STZ induced diabetic rat dose with OCHEx (300 mg/kg b.w.)

Group 5: STZ induced diabetic rat dose with Glibenclamide (5 mg/kg b.w.)

OCHEx= hydroethanolic extract of *O. corniculata*, STZ = Streptozotocin, b.w.= body weight

2.5.2 Acute oral toxicity

The acute toxicity of Albino Wistar rats was carried out following OECD 423 guidelines provided rat based acute toxicity studies for toxic chemicals. The Albino Wistar rats of either sex was kept overnight fasting with the support of water. The tween 80 mixed *O. corniculata* hydroethanolic extract (OCHEx) with increasing doses of 100-2000 mg/kg of body weight were orally administrated to rats groups. The animals were observed for 24 h for any toxic symptoms (Ahmed *et al.,* 2014).

2.5.3 Induction of diabetes

The rats underwent an overnight fasting period before induction of diabetes. Streptozotocin (STZ) (98% extra pure) from SRL (Product code 14653) was dissolved in ice cold 0.1 M citrate buffer at pH 4.5. The solution was then induced to each group of rats excluding group 1 via intraperitoneal (i.p.) in a single dose of 50 mg/kg according to body weight. After 72 h of STZ induction, the plasma blood glucose level was observed and animals with fasted blood glucose level greater than 230 mg/dl was considered in the antidiabetic study (Kumar *et al.,* 2013).

2.5.4 Blood glucose level estimation, collection of blood serum and organs

After the completion of the dosing period, all experimental groups of animals fasted overnight. Blood samples were then drawn from the retro-orbital plexus under mild anaesthesia in a desiccator using very thin capillary tubes and measured blood glucose level and insulin level with the help of OneTouch glucometer (India) and standard kit, respectively. For biochemical assays, serum was separated from blood by centrifuging at 10,000 rpm for 5 min. The animals were humanely sacrificed with mild anesthesia and organs such as the liver and pancreas were immediately dissected with extreme precaution. The tissues were washed with DNS and preserved in a neutral formalin solution (10%) for 48 h at –20°C for histopathology studies (Ahmed *et al.,* 2014).

2.5.5 Biochemical analysis and histopathological studies

Animal blood serum was utilized for determining biochemical parameters such as insulin level, lipid profile (Total cholesterol, HDL, VLDL and triglycerides] and liver function markers [aspartate transaminase (AST) and alanine transaminase (ALT)] with the help of analytical standard kit (Kumar *et al.,* 2013). The histopathological studies were analyzed on the liver and pancreas of diabetic and non diabetic rats. First preserved tissues in 10% formalin were washed in alcohol and held for dehydration before being implanted in paraffin blocks. The block was cut into a 5 µm section using a semi automated microtome. The sections were stained with hematoxylin-eosin (H/E) staining for photomicrography observation using a light microscope at 40X magnification. The stained slices were observed with an Olympus (Tokyo, Japan) microscope (Ononamadu *et al.,* 2018).

2.6 Identification of putative phytochemical by GC-MS

The Shimadzu GC-MS QP2010 Ultra was used to analyze volatile phytochemicals in *O. corniculata* extract. The column oven temperature ranged from 80°C to 280°C with specific holding times and ramp rates. Helium gas served as the carrier gas at a flow rate of 1.21 ml/min. and 81.9 kPa pressure. Split sample injection was employed with a linear velocity of 40.5 cm/sec, maintaining a purge flow of 3.0 ml/min and a split ratio of 10.0. High-pressure injection features were disabled. Ion source temperature was 230°C, and interface temperature was 270°C, crucial for GC-MS efficiency. Detector gain mode was relative, with a solvent cut time of 2.50 min. ACQ mode scan with a speed of 33.33 was utilized, covering the mass range of 0.0-799.80 m/z. Phytochemical analysis relied on peak data, retention time, and total area %, compared against the NIST base spectrum for identification (Durgawale *et al.,* 2015; Bhawana *et al.,* 2021).

2.7 Molecular Docking

Molecular Docking is used to analyze the potent phytochemicals as α-amylase inhibitors, accessed through GC-MS profiling. All 9 ligands along with acarbose were selected for molecular docking and druglikeness attributes (Shabnam *et al.,* 2022).

2.7.1 Protein preparation

The high resolution protein structure for the docking study was obtained from Protein Data Bank (http://www.rcsb.org.pdb) in pdb format. The human pancreatic α-amylase intricate with acarbose (PDB ID-1B2Y) belongs to the hydrolase family of enzymes used in the study. To prepare these structures, non-polar group, hydrogen, elimination of water molecules and energy minimization were obtained. The docking function was employed for every ligand using an extra Glide application. Furthermore, a grid was generated in the receptor for the binding site of ligands (Akshatha *et al.,* 2021).

2.7.2 Ligand preparation

The selection process for ligands from the *O. corniculata* extract involved a comprehensive approach all 9 phytochemicals identified through GC-MS studies were chosen based on distinct pharmacological studies reported previously. Subsequently, ligands were retrieved from the PubChem database in 3D SDF format. In ligand preparation, Schrodinger based Glide application was used for energy minimization utilizing the OPLS3e force field. To strike a balance between flexibility and accuracy, particular addressed standard precision (SP). The ADME and biological behaviour of selected ligands were analyzed using Qikprop (Schrodinger) (Akshatha *et al.,* 2021).

2.7.3 Docking protocol

Molecular Docking studies were conducted using Schrodinger Maestro (11.5) Glide Suite tools to analyze ligand interaction with the α-amylase receptor. Standard precision (SP) and extra precision (XP) modes were employed to balance flexibility and accuracy. Ligands and receptor proteins were selected and prepared, with a grid generated around the receptor's active site based on inhibitor binding. Docking was performed to obtain Glide scores and docking scores (kcal/mol). Binding interactions between ligands and receptor amino acid residues were examined to validate the results. Clusters were sorted based on the lowest energy representative of each binding mode. Ligand efficacy was determined by negative binding energy, total hydrogen bonds, and other intermolecular interactions (Sajal *et al.,* 2022).

2.8 Statistical analysis

Prism graphpad 9.0v statistical tools was used to analysis the data. The provided data were expressed as mean ± SEM. Groups compare were assessed through One-way ANOVA, with statistical significant value were considered to be set p<0.05.

3. Results

3.1 GC-MS profiling

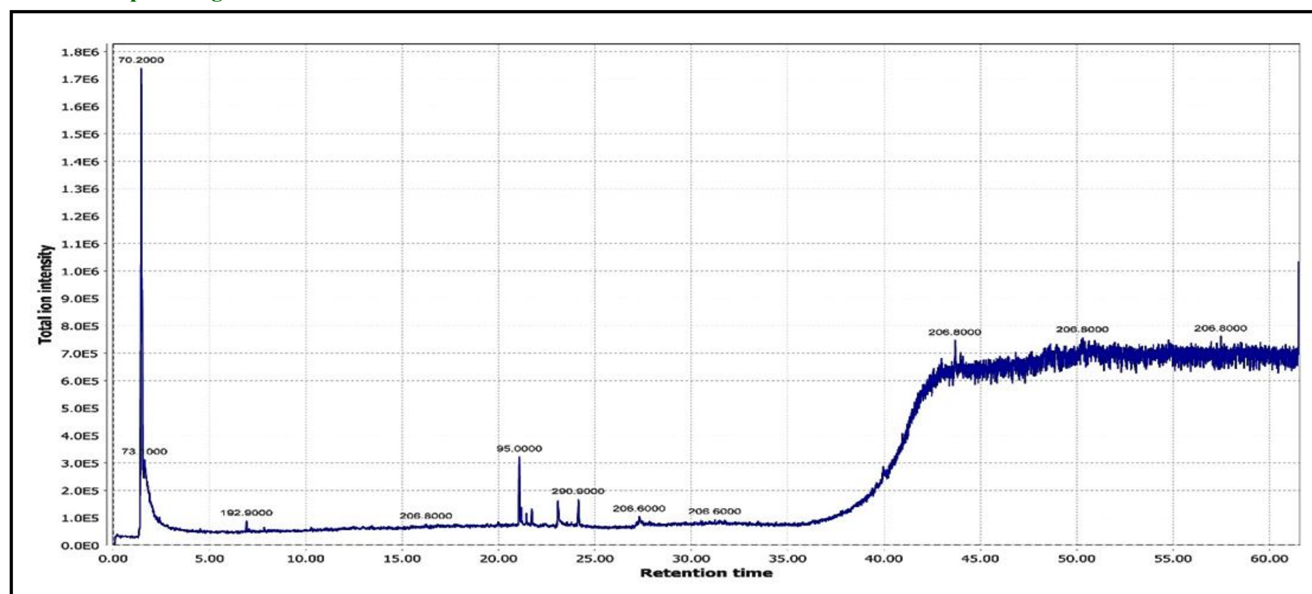


Figure 1: GC-MS chromatogram of *O. corniculata* extract.

GC-MS phytochemical investigation of *O. corniculata* identified total 9 compounds and chromatogram shown in Figure 1. All identified compound based on molecular structure, retention time and area covered by component presented in Table 1.

3.2 Antioxidant activity

The results of antioxidant activity demonstrated that OCHEx exhibits significant potential against DPPH, ABTS and phosphomolybdate in a dose dependent manner. The IC_{50} values of OCHEx were determined to be 124.56 ± 2.40 µg/ml, 159.17 ± 2.98 µg/ml and 147.83 ± 1.91 µg/ml for DPPH, ABTS and posphomolybdanum, respectively. Notably, these were significantly higher than the standard, with no statistically significant difference.

3.3 Effect on α-amylase and α-glucosidase inhibition activity

The inhibition (%) effect of hydroethanol extract of *O. corniculata* was accessed using α-amylase and α-glucosidase inhibition assay.

As shown in Table 2, the lowest inhibition of α-amylase (36.2 ± 0.76%) and α-glucosidase (41.9 ± 1.61%) was observed at 50 µg/ml compared to acarbose (49.7 ± 2.17%), whereas highest inhibition of α-amylase (71.0 ± 1.27%) and α-glucosidase (74.6 ± 1.82%) was observed at concentration at 200 µg/ml, which was lower than standard acarbose with 92.1 ± 1.68%.

Table 1: Phytochemical identified in *O. corniculata* extract using GC-MS

Peak	Retentiontime (min)	Component area	Compound name	Molecularformula	m/z value
1.	1.4540	7464598.9	1,6-Dideoxy-l-mannitol-	$C_6H_{14}O_4$	70.2
2.	1.6258	1087875.4	beta-D-Galactopyranoside, methyl	$C_{14}H_{31}BO_6Si_2$	73.1
3.	21.0761	547847.1	2-Hexadecen-1-ol, 3,7,11,15-tetra-methyl, acetate (phytate acetate)	$C_{22}H_{42}O_2$	95.0
4.	21.1719	173470.1	(5, 9,12)z-octadecatrienoic acid	$C_{18}H_{30}O_2$	70.9
5.	21.7232	143439.3	1,4-Eicosadiene	$C_{20}H_{38}$	81.0
6.	23.0895	395130.3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	72.9
7.	24.1402	317534.6	Benzoic acid	$C_9H_{10}O_2$	290.9
8.	46.1177	218568.1	2,5-Dihydroxyacetophenone, 2TMS derivative	$C_{14}H_{24}O_3Si_2$	281.7
9.	47.9598	59040.7	Stigmasterol (stigmast-5-en-3-ol)	$C_{29}H_{50}O$	281.7

Table 2: Effect of *O. corniculata* hydroethanolic extracts (OCHEx) on *in vitro* α-amylase and α-glucosidase inhibition activity

Concentration (µg/ml)	Inhibition (%)		
	Acarbose	α-amylase	α-glucosidase
50	49.7 ± 2.17	36.2 ± 0.75	41.9 ± 1.61
100	72.4 ± 0.84	54.4 ± 1.10*	51.1 ± 1.59
200	92.1 ± 1.68	71.0 ± 1.27*	74.6 ± 1.82

The data are expressed as mean ± SEM, where, *(p<0.05) significant compared to acarbose.

3.4 Acute toxicity

The experiment on acute toxicity was carried out on Albino Wistar rats following the guidelines outlined in OECD Guideline 423. The finding revealed that the administration of OCHEx exhibited no sign of mortality at a dose of 2000 mg/kg of body weight over 24 h. Throughout the course of experiment, no abnormalities in the appearance of animals were observed. Furthermore, dosage of 2000 mg/kg did not cause the rat's death during the study. The finding revealed that a single dosage of (OCHEx) did not exhibit any adverse effects or toxicity, indicating that the lethal dose (LD_{50}) could be higher than 2000 mg/kg/body weight in rats.

3.5 Effect on blood glucose level

The effect of orally administering OCHEx on blood glucose level is detailed in Table 3. After receiving STZ, all groups were observed for diabetes development, with blood glucose level of ≥230 mg/dl considered to be diabetic. Blood glucose level was observed at 0, 14 and 28 days. There was significant reduction in blood glucose level at the end of the 28th day parodic was observed in the group administered with 300 mg/kg (48.53%) with significant (p<0.001) compared to the 100 mg/kg (35.29%). Glibenclamide (5 mg/kg) administrated group showed highest reduction of blood glucose level significantly (p<0.001) as compared to normal group. The glibenclamide showed highest significant reduction with 51.95% compared to OCHEx doses (Figure 2).

Table 3: Effect of hydroethanolic extract of *O. corniculata* (OCHEx) on blood glucose level

Groups	Treatments	Blood glucose (mg/dl)		
		0 day	14th day	28th days
Group 1	NC	75.97 ± 2.24	80.83 ± 1.19	82.16 ± 1.28
Group 2	DC	236.16 ± 6.87	317.5 ± 8.73**	329.0 ± 6.83 ***
Group 3	OCHEx 100	238.83 ± 4.37	199.83 ± 3.87**	154.16 ± 4.36**
Group 4	OCHEx 300	247.0 ± 4.79	174.50 ± 4.84**	127.67 ± 4.67***
Group 5	GLSC	229.0 ± 1.95	169.67 ± 4.23***	110.83 ± 2.59***

The presented values are expressed as mean ± SEM (n= 6), followed by One way ANOVA, (dunnet's test). *p<0.05 is significant compared to control group (0 day); **p<0.005 is highly significant compared to control group (0 day); ***p<0.001 is most significant compared to control group (0 day); NC: normal (non-diabetic) control, DC; diabetic control (STZ, 50 mg/kg), OCHEx; *O. corniculata* hydroethanolic extract (mg/kg), GLSC: Glibenclamide (5 mg/kg).

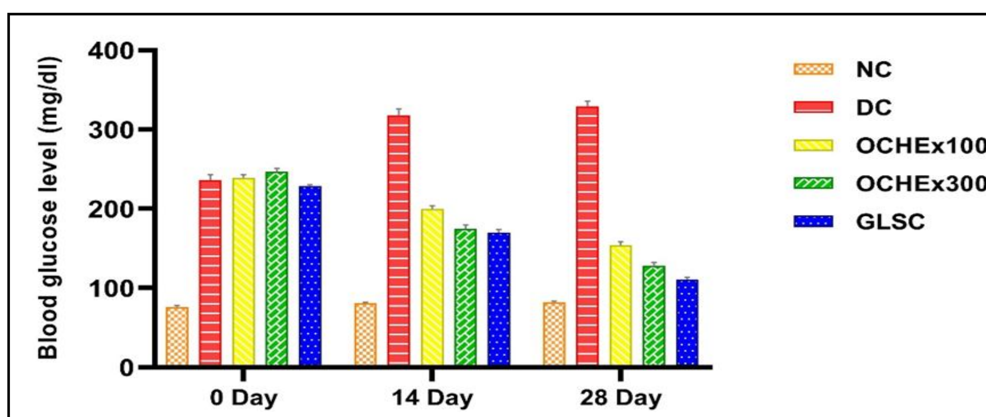


Figure 2: Effect *O. corniculata* hydroethanolic extract (OCHEx) on blood glucose level at 0, 14 and 28 days' period.

NC: normal (non-diabetic) control, DC; diabetic control (STZ, 50mg/kg), OCHEx; *O. corniculata* hydroethanolic extract (mg/kg), GLSC: Glibenclamide (5 mg/kg).

3.6 Effect on Insulin level

The effect of OCHEx on insulin level was observed that extract of 300 mg/kg and standard glibenclamide dose induce release of insulin level as compared to normal group significantly (p<0.05). The STZ induced rat shows lowest insulin production due to destruction of pancreatic β-cell as compared to normal and diabetic group which received extracts. Therefore, extracts help in regenerating pancreas cells and potentially improve insulin level (Table 4).

Table 4: Effect of *O. corniculata* hydroethanolic extracts (OCHEx) on insulin level

Groups	Treatments	Insulin level (µIU/ml)
Group 1	NC	31.2 ± 3.65
Group 2	DC	11.2 ± 2.22
Group 3	OCHEx 100	21.6 ± 3.09
Group 4	OCHEx 300	25.6 ± 2.25*
Group 5	GLSC	34.6 ± 2.69*

The presented values are expressed as mean ± SEM (n= 6), followed by a one-way ANOVA, (dunnet's test). *p<0.05 is significant compared to control group (0 day); **p<0.005 is highly significant compared to control group (0 day); ***p<0.001 is most significant compared to control group (0 day); NC: normal (non-diabetic) control, DC: diabetic control (STZ, 50 mg/kg), OCHEx: *O. corniculata* hydroethanolic extract (mg/kg), GLSC: Glibenclamide (5 mg/kg).

3.7 Effect on total lipid profile

The assessment of the impact of orally administered OCHEx on total lipid profile, including total cholesterol (TC), triglycerides (TG), HDL and LDL revealed significantly**.** The extract demonstrated a noteworthy reduction in cholesterol levels, triglycerides and LDL on compared to diabetic control group as indicated in Table 5. Conversely, the HDL level shown significantly increase against diabetic control group (p<0.005). Prominently, the efficacy of the OCHEx was significantly higher at dose of 300 mg/kg compared to 100 mg/kg in reduction of TC, triglycerides and LDL (Figure 3).

Table 5: Effect of *O. corniculata* hydroethanolic extracts (OCHEx) on total lipid profile

Groups	Treatments	Total lipid profile			
		Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL(mg/dl)	LDL (mg/dl)
Group 1	NC	85.43 ± 2.71	72.8 ± 2.81	36.9 ± 0.35	33.97 ± 1.99
Group 2	DC	218.02 ± 6.4**	194.2 ±2.56***	15.4 ± 1.43***	163.96 ± 2.64**
Group 3	OCHEx 100	151.3 ± 5.65***	185.4 ± 4.49**	25.3 ± 0.67**	88.65 ± 2.38***
Group 4	OCHEx 300	141.5 ± 5.23**	177.0 ± 5.69***	27.3 ± 0.63***	78.8 ± 2.20**
Group 5	GLSC	132.56 ± 3.7***	152.0 ± 5.78**	26.5 ± 0.55***	75.66 ± 1.77***

The presented values are expressed as mean ± SEM (n= 6), followed by a one-way ANOVA, (dunnet's test). *p<0.05 is significant compared to control group (0 day); **p<0.005 is highly significant compared to control group (0 day); ***p<0.001 is most significant compared to control group (0 day); NC: normal (non-diabetic) control, DC: diabetic control (STZ, 50 mg/kg), OCHEx: *O. corniculata* hydroethanolic extract (mg/kg), GLSC: Glibenclamide (5 mg/kg).

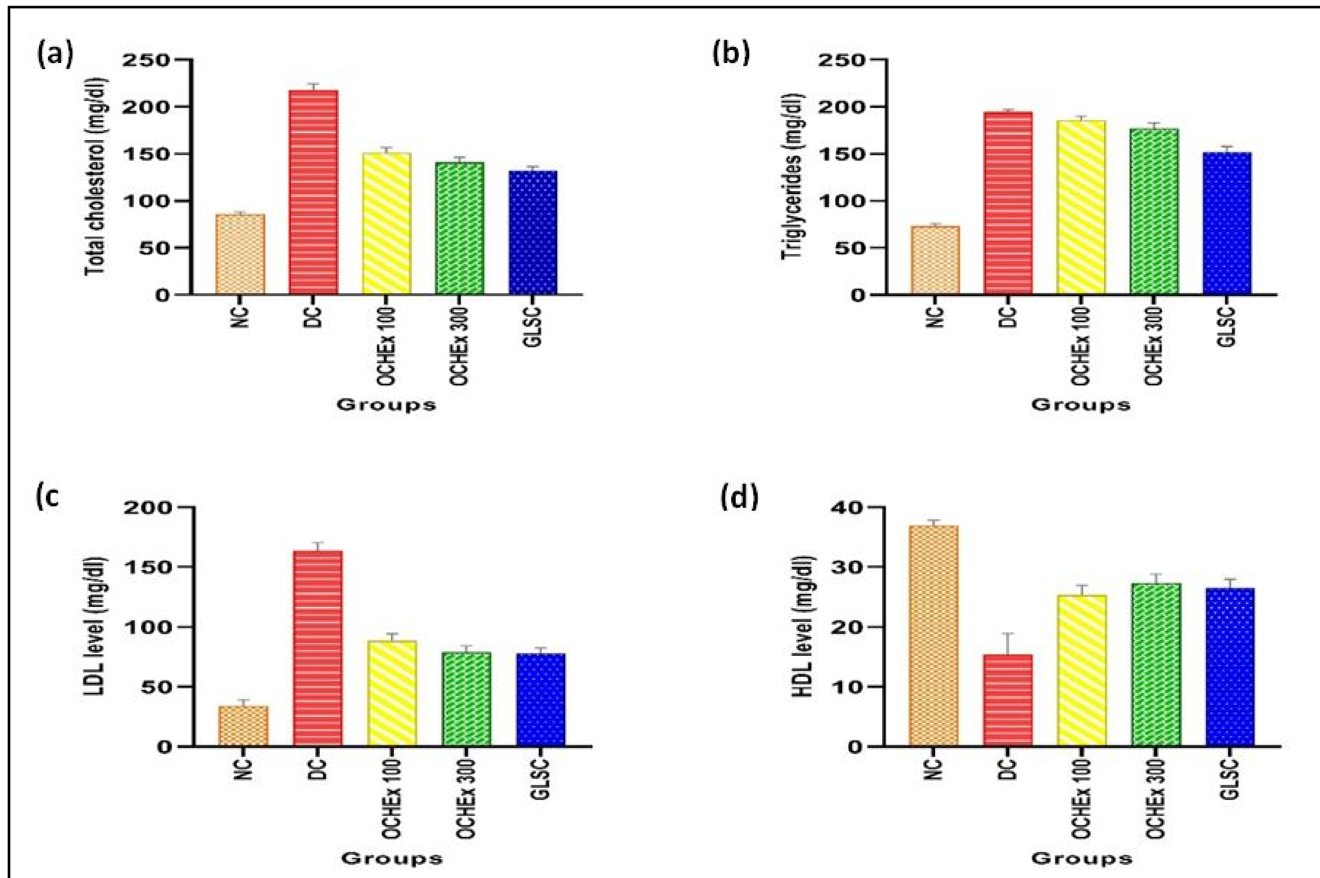


Figure 3: Effect *O. corniculata* hydroethanolic extract (OCHEx) on lipid profile in STZ induced diabetic and normal rats, (a) Total cholesterol, (b) Triglycerides, (c) LDL level and (d) HDL level.

NC: normal (non-diabetic) control, DC: diabetic control (STZ, 50 mg/kg), OCHEx: *O. corniculata* hydroethanolic extract (mg/kg), GLSC: Glibenclamide (5 mg/kg).

3.8 Effect on liver markers

The effectiveness of OCHEx on liver marker enzymes include AST and ALT was evaluated through oral administration of varying doses in STZ induced diabetic rats group. In this experiment, the administration of OCHEx to diabetic rats significantly improved their AST and ALT level compared with diabetic group (Table 6). Notably, OCHEx with 300 mg/kg and glibenclamide standard drug at dose of 5 mg/kg demonstrated the most significant (p<0.005) (p<0.001) reduction compared to diabetic control groups, respectively (Figure 4).

Table 6: Effect of *O. corniculata* hydroethanolic (OCHEx) on Liver marker enzyme

Groups	Treatments	Liver marker enzyme	
		AST (mg/dl)	ALT (mg/dl)
Group 1	NC	35.43 ± 1.33	44.55 ± 1.21
Group 2	DC	151.6 ± 3.81**	79.47 ± 1.46**
Group 3	OCHEx 100	138.1 ± 2.13*	73.70 ± 1.05**
Group 4	OCHEx 300	125.3 ± 1.04**	71.57 ± 2.13***
Group 5	GLSC	90.39 ± 2.25***	65.71 ±2.57**

The presented values are expressed as mean ± SEM (n= 6), followed by a one-way ANOVA, (dunnet's test). *p<0.05 is significant compared to control group (0 day); **p<0.005 is highly significant compared to control group (0 day); ***p<0.001 is most significant compared to control group (0 day); NC: normal (non-diabetic) control, DC: diabetic control (STZ, 50 mg/kg), OCHEx: *O. corniculata* hydroethanolic extract (mg/kg), GLSC: Glibenclamide (5 mg/kg).

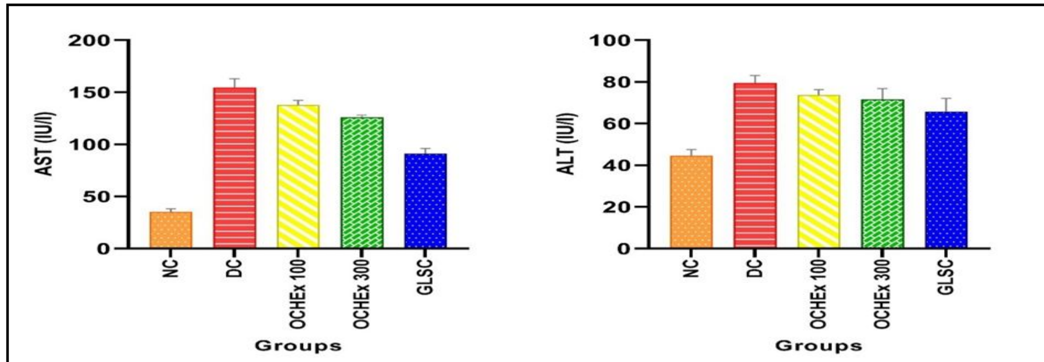


Figure 4: Effect *O. corniculata* hydroethanolic extract (OCHEx) on liver marker enzyme (ALT and AST).

NC: normal (non-diabetic) control, DC: diabetic control (STZ, 50 mg/kg), OCHEx: *O. corniculata* hydroethanolic extract (mg/kg), GLSC: Glibenclamide (5 mg/kg).

3.9 Histology of liver treated with *O. corniculata* hydroethanolic extract (OCHEx)

The histological examinations of the liver observed that liver cell in normal control groups displayed prominent hepatocytes with an intact central vein. The diabetic group (received STZ) liver found damaged characterized by the degeneration of hepatocytes, destruction of central vein and congestion of portal vein. The administration of OCHEx extract as a treatment enhanced histological conditions of liver in STZ induced diabetic groups. The administration of OCHEx extract at a dose of 100 mg/kg body weight, revealed partially affected hepatocytes along with portal vascular ulceration when compared to diabetic control group. However, the dose of 300 mg/kg exhibited improvement compared to both Diabetic control and OCHEx 100 mg/kg dose. Glibenclamide drugs at 5 mg/kg exhibited minor improvement of hepatocytes as compared to diabetic groups (Figure 5a-e).

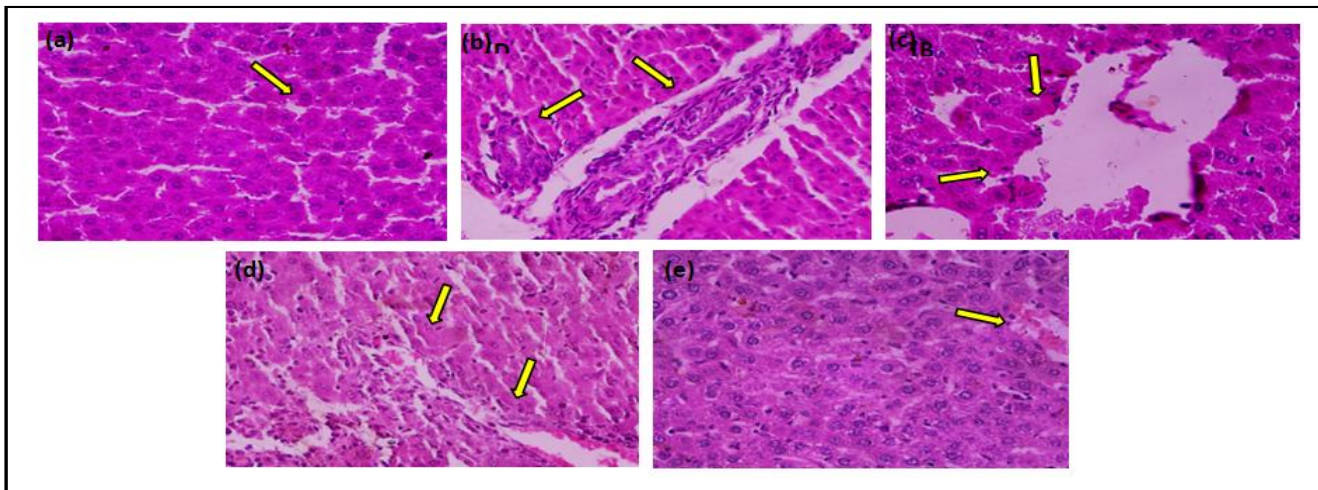


Figure 5: Effect of *O. corniculata* hydroethanolic extract (OCHEx) on histological profile of liver in normal, STZ induced diabetic wistar rats (hematoxylin-eosin (H/E) and magnificence 40X Olympus, Tokyo, Japan). (a) Control, (b) STZ induced diabetic, (c) OCHEx 100 mg/kg, (d) OCHEx 300 mg/kg, and (e) Glibenclamide standard drug. For each group 6 rats were examined and 50 pictures were taken.

3.10 Histology of pancreas treated with *O. corniculata* hydroethanolic extract (OCHEx)

Histological investigation the pancreas after administration of OCHEx was observed that cells in pancreas in diabetic group exhibited a reduction of islet of Langerhans due to depletion of cytoplasm of cells within the islets. Additionally, focal necrosis, broad lumen and vacuolation were also observed, resulting in β-cell degeneration compared to normal group. The administration of OCHEx as treatment improved the histological conditions of the pancreas in STZ induced diabetic groups. The administration of OCHEx at a dose of 100 mg/kg revealed partial degeneration of cytoplasm of islets along with damaged blood vessels and as increase size of interlobular duct compared to the diabetes group. However, 300 mg/kg od dose showed improvement in cells physical state compared to both the Diabetic control group and dose with 100 mg/kg. Glibenclamide, administered at 5 mg/kg, exhibited improvement in β-cell regeneration compared to the diabetic groups (Figures 6a-e).

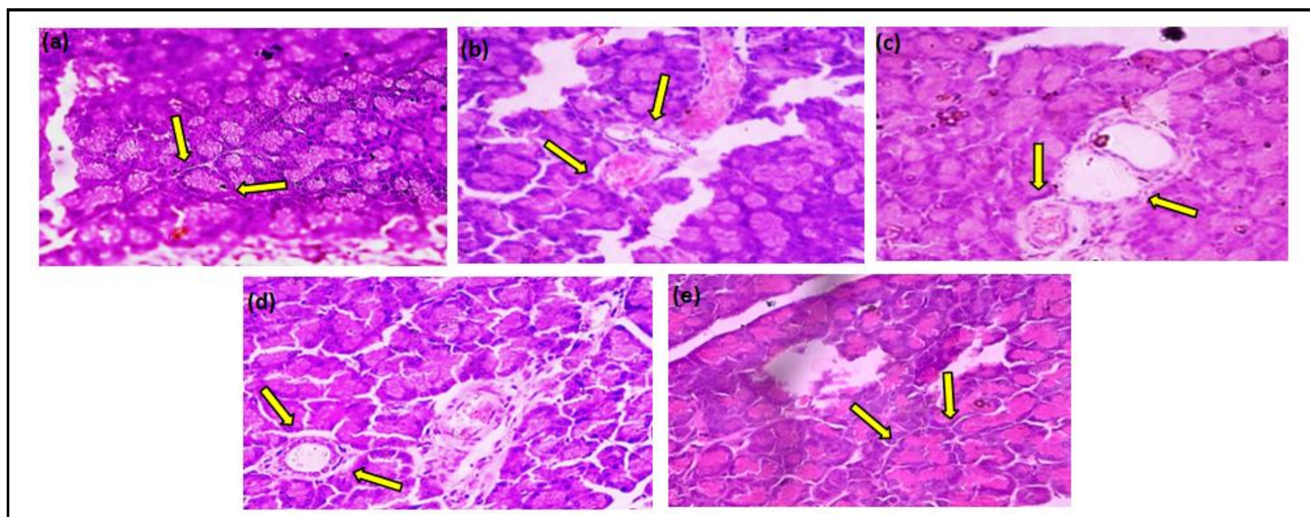


Figure 6: Effect of *O. corniculata* ethanolic extract (OCHEx) on histological profile of Pancreas in normal, STZ induced diabetic wistar rats (hematoxylin-eosin (H/E) and magnificence 40X, Olympus, Tokyo, Japan). (a) Control, (b) STZ induced diabetic, (c) OCHEx 100 mg/kg, (d) OCHEx 300 mg/kg, and (e) Glibenclamide standard drug. For each group 6 rats were examined and 50 pictures were taken.

3.11 Molecular Docking

The molecular docking study used to investigate the optimal ligand-receptor interaction among all 9 compounds identified in *O. corniculata* extract by GC-MS profiling bases on prediction of docking score (kcal/mol) tabulated in Table 7. The docking score prediction indicated out of nine ligands, four ligands act as α-amylase inhibitors of *O. corniculata* extract notably, L2 (β-D-galactopyranoside, methyl), L7 (Benzoic acid), L8 (2,5-dihydroxyacetophenone, 2TMS derivative) and L9 (Stigmasterol, Stigmast-5-en-3-ol) showed good binding interaction with amino acid residue of α-amylase (1B2Y). Furthermore, all four ligands have potential and play a significant role in inhibition of α-amylase. Other ligands include L1 (1,6-Dideoxy-l-mannitol), L3 (2-hexadecen-1-ol, 3,7,11,15), L4 ((5, 9,12) z-octadecatrienoic acid) L5 (1,4-eicosadiene), and L6 (n-hexadecanoic acid), their respective docking scores were comparatively lower. This indicates their potential ineffectiveness against α-amylase when compared to other ligands evaluated. The 2D interaction of four potential ligands with α-amylase are depicted in Figure 7. Acarbose is a known standard α-amylase inhibitor used in the lowering diabetic effect and typically exhibited strong binding affinity. As shown in Table 7, acarbose displayed highest docking score with α-amylase and 2D interaction (Figure 7).

Table 7: Ligand code, ligand's name, docking score (kcal/mol) and amino acid interactions patterns of selected phytochemicals form *O. corniculata* extract with α-amylase (PDB ID-1B2Y)

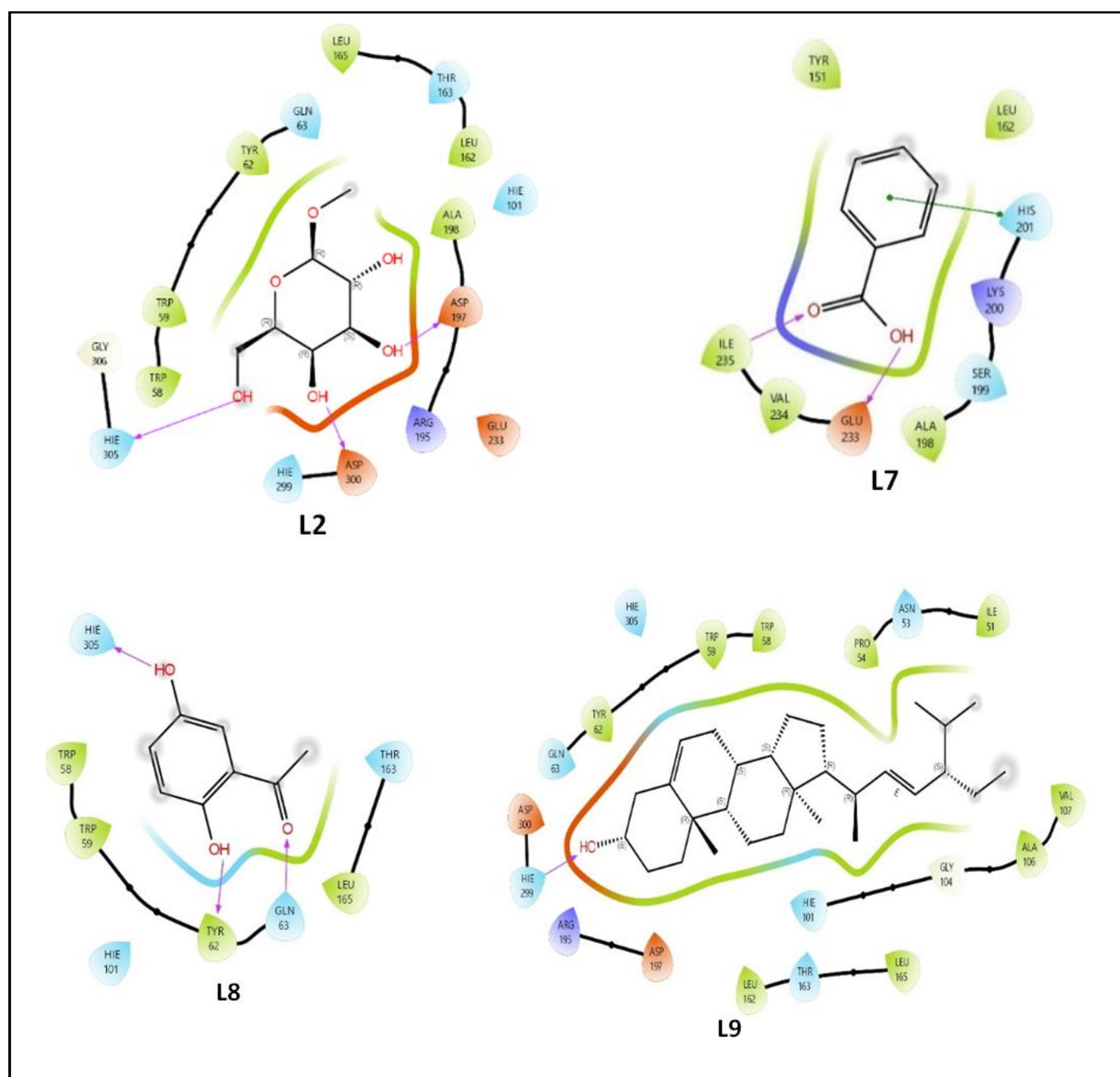
Ligand code	Ligand's name	Docking score (kcal/mol)	Amino acid residue interaction (Hydrogen bond)
L1	1,6-Dideoxy-l-mannitol-	-5.1	*
L2	beta-D-Galactopyranoside, methyl	-6.0	HIS305, ASP300, ASP197
L3	2-Hexadecen-1-ol (phytol)	-5.2	*
L4	(5, 9,12,)z-octadecatrienoic acid	-5.3	*
L5	1,4-Eicosadiene	-.4.9	*
L6	n-hexadecanoic acid	-5.0	*
L7	Benzoic acid	-5.4	HIS201, ILE235, GLU233
L8	2,5-Dihydroxyacetophenone, 2TMS derivative	-5.9	HIS305, TYR62, GLN63
L9	Stigmasterol (Stigmast-5-en-3-ol)	-9.7	HIS299
	Acarbose	-12.07	GLU233, HIS201, HIE101, ASP197, HIS101, ARG195, ASP300, HIS305

*No docking score

L2 showed docking score -6.0 kcal/mol with sharing of three hydrogen bond HIE305, ASP300 and ASP197, respectively. L7 with -5.4 kcal/mol formed two hydrogen bond with ILE235 and GLU233. Whereas L8 displayed docking score of -5.9 kcal/mol, with HIE305, GLN63 and TYR62 against 1B2Y receptor. The highest docking score gained by L8 compound with -9.7 kcal/mol and formed one hydrogen bond with HIE299. All compound includes L2, L7, L8 and L9 had lowest docking score than acarbose. As depicted in Table 7, with docking score of -12.07 kcal/mol, acarbose formed eight hydrogen bond with specific residues GLU233, HIS201, HIE101, ASP197, HIS101, ARG195, ASP300 and HIS305 within α-amylase binding site (PDB id: 1B2Y). This characteristics interaction revealed 't' compared potency of acarbose as an inhibitor when compared to ligands explored in the investigation suggesting greater capacity to inhibit (Figure 7).

3.12 Physiochemical and ADME evaluation

All phytochemical evaluated for molecular docking with 1B2Y were investigated for physiochemical and ADME evaluation performed using QikProp (Schrodinger). The ADME parameters encompass a range of significant standards such as molecular weight, hydrogen bond donor, hydrogen bond acceptor, octanol/water coefficient prediction, water solubility prediction, skin permeability prediction, predicted solvent accessible surface area, percentage oral absorption. These parameters are evaluated according to five rule of Lipinski's, which guides drug development by emphasizing traits associated with improved oral bioavailability. The ADME and physiochemical attributes of selected phytochemicals (L1-L9) have been investigated for molecular docking study as α-amylase inhibitors presented in Table 8.



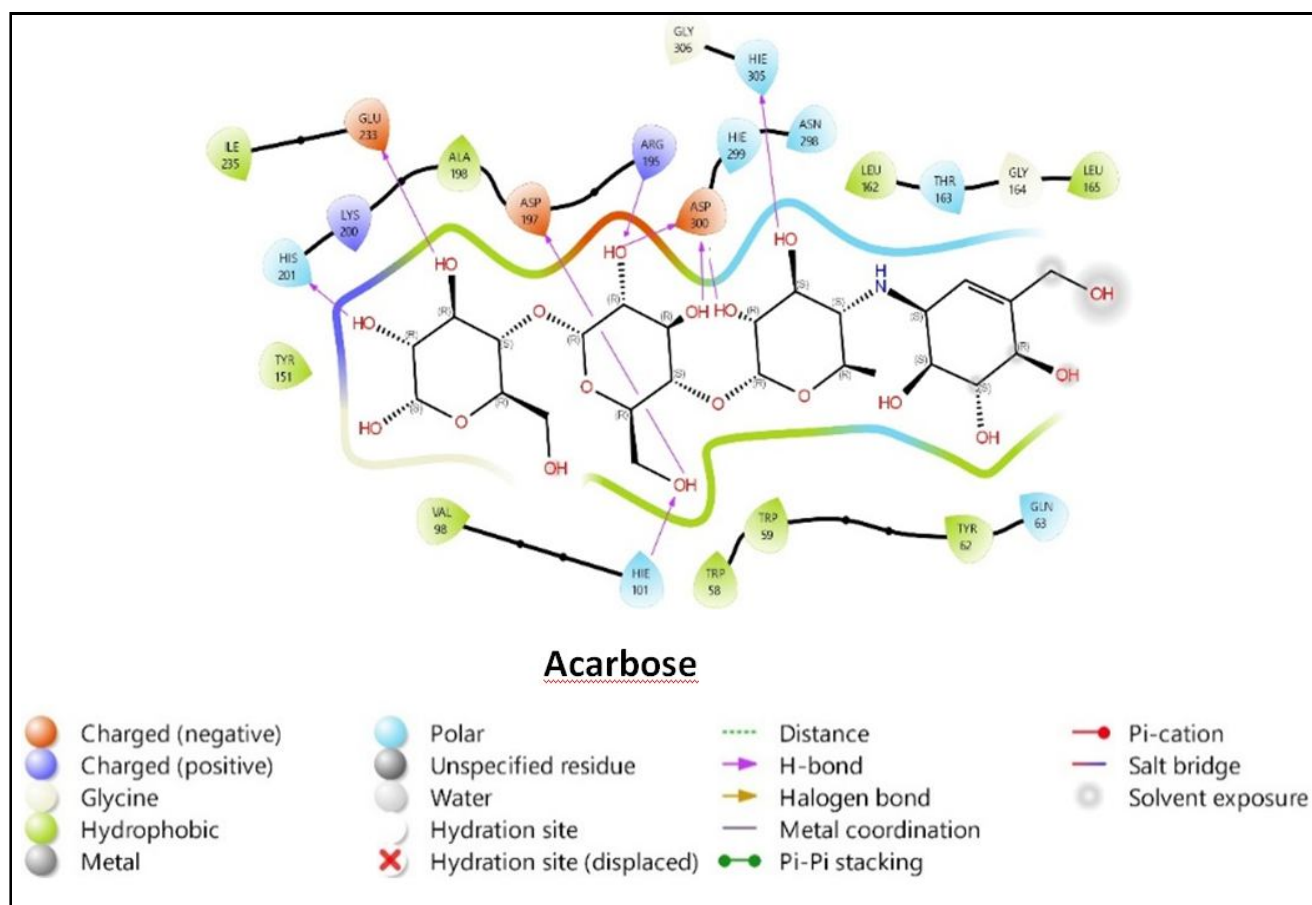


Figure 7: 2D Interaction of L2, L7, L8, L9 and L10 with 1B2Y, violet line represents hydrogen bond (H-bond); L2: Beta-D-Galactopyranoside, methyl, L7: Benzoic acid, L8: 2,5-Dihydroxyacetophenone, 2TMS derivative, L9: Stigmasterol (Stigmast-5-en-3-ol).

Table 8: Physiochemical and ADME evaluation of L1-10 using QikProp (Schrodinger)

Ligand code	MW[a] (g/mol)	HBD[b]	HBA[c]	QlogP[d] (o/w)	QlogS[e] (%)	QlogKp[f]	QlogBB[g]	SASA[h]($Å^2$)	Oralabsorption[i]	RO5[j]
L1	150.17	4	4	-0.76	0.38	-8.29	-0.76	80.92	73.19	0
L2	194.18	4	6	-1.64	0.76	-9.37	-1.44	99.38	61.98	0
L3	296.53	1	1	6.22	-5.98	-2.29	-3.18	20.23	24.72	1
L4	278.43	2	1	5.18	-4.70	-3.50	-3.15	37.30	25.07	1
L5	278.52	0	0	7.34	-6.72	-1.01	-3.36	0.00	25.03	1
L6	256.42	2	1	5.20	-5.02	-2.77	-3.09	37.30	26.98.	1
L7	122.12	2	1	1.44	-2.20	-5.72	-0.44	37.30	85	0
L8	152.15	3	2	1.21	-2.16	-6.06	-0.54	57.53	73.55	0
L9	412.69	1	1	6.98	-7.46	-2.74	-6.10	20.23	24.12	1
Acarbose	645.60	19	14	-6.06	2.13	-16.29	-6.48	321.17	17.01	3

[a]Molecular weight (<500Da g/mol); [b]Hydrogen bond donor (<5); [c]Hydrogen bond donor (<10); [d]octanol/water partition coefficient (range -3.0-1.2); [e] Predicted logarithm of aqueous solubility (range -6.5 -0.5); [f] predicted partition coefficient for skin permeation; [g]predicted blood brain partition coefficient (range -3.0 - 1.2); [h]solvent-accessible surface area; [i]Oral absorption in human (<25% poor; >85% great); [j]Lipinski's rule of 5.

4. Discussion

Diabetes is one of the major endocrinal metabolic disorders, which causes disturbances in the metabolism of fat, protein, and carbohydrates, leading to hyperglycemia and hyperlipidemia and eventually causing malfunction in multiple organs. Over the period, several reports have suggested that phytochemicals have worked ethno-medicinally against several chronic and acute type diseases, including antibacterial, anticancer, antidiabetic, neuroprotective, antiaging, and cardiovascular and CNS related diseases (Leon and Maddox 2015). Nevertheless, it still lacks comprehensive scientific recognition. As a result, studies conducted into more secure and efficient antioxidant and antidiabetic agents are still crucial. The present study investigates the impact of *O. corniculata* hydroethanolic extract (OCHEx) on improving the performance of â cells in STZ induced diabetic rats, followed by biochemical and histopathological studies.

Oxidative stress is the result of a disparity between the body's antioxidant defenses, which counteract the damaging effect of reactive oxygen species and their generation (Hassan *et al*., 2015). OCHEx free radical scavenging properties were explored using DPPH, ABTS and total antioxidant assay, revealing extract contains active antioxidants like flavonoids and phenol which could possibly cause the reduction of ROS species produced during oxidative stress (Ahmed *et al.,* 2014), aligns with the findings reported by Agila and Kavitha (2012).

The present findings revealed that OCHEx displayed a comparatively lower inhibitory effect on α-amylase and α-glucosidase when compared to acarbose, a drug known for its significant role in inhibiting the action of digestive enzymes and decline postprandial hyperglycemia. This finding is also supported by Agila and Kavitha (2012). Moreover, recent studies investigated that STZ transported through glucose and damaged pancreatic β cells through ROS and NO generation The finding of the study revealed that OCHEx improved β cells regeneration in STZ induced rats due to its ROS scavenging properties. Based on the result, OCHEx accomplished hypoglycemic action increased insulin sensitivity and declined blood glucose level, which is consistent with earlier studies reported showing antidiabetic activity and promoting glycogenesis (Agila and Kavitha*,* 2012; Himja and Das, 2015). The OCHEx antidiabetic action was comparable to second generation sulfonylurea class drug, *i.e*., glibenclamide or glyburide, orally administrated, treatment of NIDDM help in the stimulation of insulin secretion from pancreatic β cells through ATP sensitivity potassium channel and increasing potassium and calcium ion (Yashwant *et al.,* 2011; Rambiritch *et al.,* 2014). Therefore, developing a diabetes treatment that simultaneously reduces the risk of cardiovascular disease is still challenging. In light of this., treatment of extract in dose dependent mode resulting a significant reduction in lipid profile including total cholesterol, triglycerides level and LDL levels and a significant rise in HDL levels in STZ induced diabetic rats. The antihypercholesterolemia effect of *O. corniculata* is presumed to be due to the inhibition of the enzyme responsible for cholesterol formation in the cholesterol biosynthetic pathway. The histopathological study suggested that high blood glucose conditions cause biochemical alternations resulting in liver damage. The liver is the most vital organ playing a crucial role in glucose metabolism by converting blood glucose into glycogen through glycogenesis ultimately producing energy for the body. The liver enzymes such as AST and ALT are very crucial indicators or biomarkers for accessing liver injury (Islam *et al.,* 2020). Studies have shown that diabetes is linked to significant elevations in AST and ALT levels (Mohamed *et al.,* 2016; Shibabaw *et al.,* 2016).

In present study, GC-MS analytical technique has been employed for identifying and quantifying compounds within extracts. This technique is an asset for investigating the presence of volatile, essential oil, sterols and non-volatile components (Akshatha *et al.,* 2021). Furthermore, nine components were identified from OCHEx, which have been selected for *in silico* study for α-amylase inhibition (Figure 1). The α-amylase serves as a key enzyme secreted by salivary glands, crucial in breakdown starch and glycogen during digestion. α-amylase plays a crucial role in digestion by breaking down complex carbohydrates into simpler sugars like glucose and maltose. It hydrolyzes the glycosidic bonds within starch molecules, facilitating their fragmentation into smaller units (Akshatha *et al.,* 2021). We present the results of the first docking research of compounds from *O. corniculata* extract as α-amylase inhibitors. Molecular docking and ADME study found that the compound includes β-D-galactopyranoside methyl (L2), Benzoic acid (L7), 2,5-dihydroxyacetophenone 2TMS derivative (L8) and stigmasterol (Stigmast-5-en-3-ol) (L9) exhibited highest docking score and formed hydrogen bonding interaction with α-amylase (1B2Y), suggested their potential as α-amylase inhibitors and elucidating the performance of molecular interaction for targeting ligands (Sruthy and Balasubramanian, 2023).

Earlier study revealed that compound, benzoic acid has shown antidiabetic and antihypercholesterolemic activity (Gayathri and Kannabiran 2009). Dihydroxyacetophenone, a nitrobenzaldehyde derivative has shown antioxidant and antidiabetic activity (Tajammal *et al.,* 2017). Furthermore, stigmasterol, a phytosterol has anti-inflammatory and antioxidant activity Similarly, octadecatrienoic acid and stigmasterol offer various biological activities, including antidiabetic and antioxidant activity (Bakrim *et al.,* 2022; Sujatha *et al.,* 2010). Therefore, the identified phytochemicals in *O. corniculata* have distinctive biological activity and the majority of compounds exhibited potential antioxidant and antidiabetic activity. Hashim *et al.* (2013) investigated *in silico* inhibition activity of α-amylase of compound 9, 12-octadecadienoic acid from plant *Phyllanthus virgatus.* In addition, 2-Hexadecen-1-ol, known to be a fragrance material or essential oil in plants, exhibited antioxidant, antihyperglycemic and cholinesterase inhibition activity (Nazir *et al.,* 2021). Additionally, n-hexadecanoic and 5, 9, 12 z-octadecatrienoic acids have been reported to have free radical scavenging and antiinflammatory properties (Aparna *et al.,* 2012). This *in silico* studies explained that acarbose, a typical α-amylase inhibitor, produced strong hydrogen bonds with residues with highest negative value compared to other studied components. Moreover, docking studies revealed that L2 and L8 phytochemicals interacted with residue HIS305, whereas, L9 was interacted with HIS299 of enzyme residue. Overall the research indicated that natural compounds, specifically, acarbose, bind to α-amylase's catalytic site *via* amino acid residue comparable to those of native ligands, especially significant enzyme residues (Ayoola *et al.,* 2024). The ADME profile of these phytochemicals suggests their potential use as druglikeness, particularly in the management of T2DM. Finally, ADME (absorption, distribution, metabolism, excretion) analysis was performed for all identified compounds as drug candidates using

the Qikprop module in Schrodinger Metro Molecular docking tools (Table 8). ADME parameters evolved druglikeness of possible drug candidates chosen either experimental or computational methods. This comprehensive study contributes to determining the druglikeness and assessing their promising prospects as therapeutic agents. Our study revealed that hydroethanolic extract of *O. corniculata* reducing blood glucose level by inhibiting the action of α-amylase enzyme, particularly in postprandial hyperglycemic condition. It is plausible that OCHEx exhibits antihyperglycemic characters, possible due to the presence of the putative antidiabetic compound.

5. Conclusion

Our study concluded *O. corniculata* hydroethanolic extract exhibited promising antioxidant activity at different doses, likely caused by the presence of diverse phytoconstituents identified through GC-MS profiling. In the antidiabetic investigation, OCHEx mitigates the risk of elevating blood glucose levels by stimulating plasma insulin release from β cells. Additionally, it led to a reduction in total cholesterol, triglycerides, and LDL and significantly increased HDL levels of blood serum. In the same way, it curtails the levels of liver marker enzyme suggesting its potential as an alternative hepatoprotective and antidiabetics. The finding of the present study could potentially be valuable for the design and development of novel drugs with improved inhibiting efficacy against various complication due to hyperglycemia. Therefore, identified compounds need advanced *in vivo* and *in vitro* studies to validates as therapeutic candidate and explore their appropriate function which would advance our understating of their role in diabetes management.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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