Evaluation of Novel Phytocompounds and Therapeutic potential of the methanolic extract of Celtis occidentalis against Alloxan-Intoxicated diabetes in rats; In-vitro and In-vivo Antidiabetic and Antioxidant Assessment

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Research Article

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Evaluation of Novel Phytocompounds and Therapeutic potential of the methanolic extract of *Celtis occidentalis* against Alloxan-Intoxicated diabetes in rats; In-vitro and In-vivo Antidiabetic and Antioxidant Assessment

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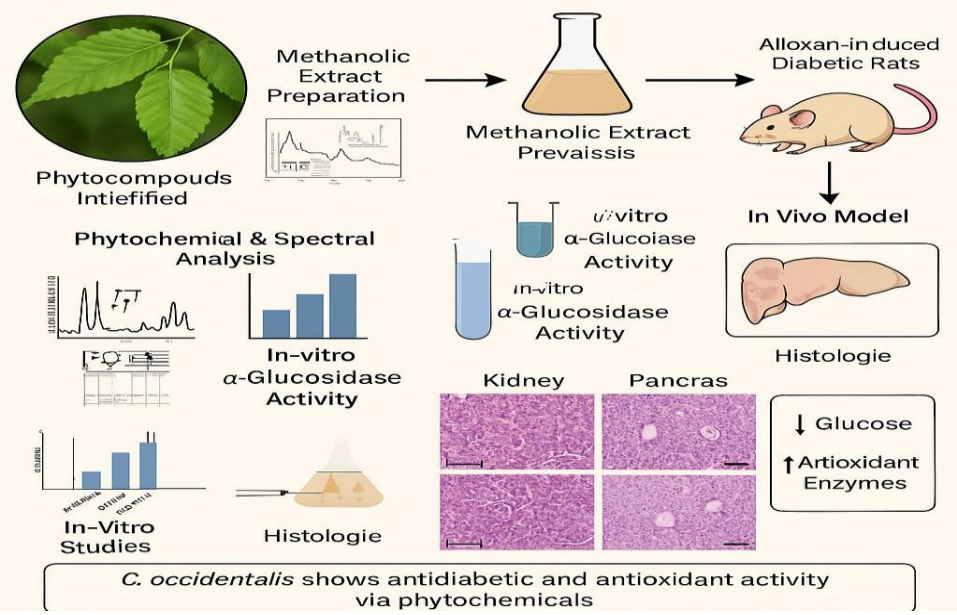
Abstract

Using a variety of chemical compounds and biomolecules, researchers have been working on new antidiabetic drugs for many years. Anti-diabetic research is increasingly using medicinal plants because of their unique qualities, biologically potent phytoconstituents and their fewer side effects. Using the *Celtis occidentalis* leaf extract as a therapeutic agent for diabetes mellitus and oxidative stress was one of the goals of this scientific work. The crude extract of *C. occidentalis* leaves was obtained through a rotary evaporator and was analyzed for a polyphenolic phytochemicals constituents and purity of the novel phytoconstituents using high-performance liquid chromatography-DAD (HPLC-DAD) with a diode-array detector and Fourier transform infrared spectroscopy (FT-IR). The extract has been investigated carefully in-vitro and in-vivo antidiabetic and antioxidant study. Forty albino rats (*Rattus norvegicus*) were used and divided in to five experimental groups each one comprising eight individuals (*n=8*). Control group, diabetic group, standard (glibenclamide) group, extract treated group-1 (**Ex_1D-G**) and extract treated group-2 (**Ex_2D-G**)). In the current research work, renal function tests, blood lipid profile, body weight, liver function tests, blood glucose level, cytokines (TNF-α and INF-γ), antioxidant markers (SODs, CAT, GPx, and GSH), glycated hemoglobin, and oxidative stress indicators (MDA and TBARS) were recorded in animal models. The extract was assayed in vitro for its inhibitory activities against α-glucosidase and α-amylase. 23 polyphenolic compounds were identified by the high-performance liquid chromatogram with a diode-array detector. Experimental animals had noticeable weight changes. The findings indicate that the extract was rich in important phenolic compounds. Induction of diabetes caused a significant increase in plasma glucose levels (>290). Blood glucose and glycated hemoglobin levels were markedly decreased in rats given the extract and glibenclamide. The rats treated with the extract normalized renal/liver function tests, lipid profile, alkaline phosphatase, alanine transaminase, and blood cholesterol, triglycerides, LDL, HDL, cytokines, antioxidant markers, and oxidative stress markers. α-glucosidase and α-amylase scavenging potential at 62.5 µg/mL and 1,000µg/mL were observed to be 69.5% and 86.1%, and 67.4% and 84.3% respectively, both nearing to standard. The plant extract actively restored and

protected the organ tissues histology (liver, kidney, and pancreas) in extract treated groups-1 & 2 in comparison to the diabetic group and work as organs protecting mediator. These findings reveal that the identified novel polyphenolic phytochemicals have strong antidiabetic and antioxidant properties.

Keywords: *C. occidentalis*, Diabetes mellitus, Antioxidant markers, Oxidative stress markers, Cytokines, Histology

Graphical Abstract



Research highlights

- Alloxan induces diabetes, oxidative stress and histological damage.
- *Celtis occidentalis* has potential to recover diabetes and oxidative stress induced by alloxan.
- Polyphenolic compounds have strong antioxidant, antidiabetic, and histo-protective properties.

Introduction

Diabetes mellitus (DM), a common global issue characterized by hyperglycemia, dyslipidemia, and impaired glucose tolerance, can lead to many chronic complications, such as kidney failure, cardiovascular disorders, neurological impairments, and hypertension (Liu et al., 2013; Yulianti et al., 2022). This is occurs due to degeneration of the pancreatic β-cells and can leads to fatty liver and nephropathy because of glucose and lipids overstraining due to instabilities in the metabolism of carbohydrates, lipids, and proteins (Ghosh & Suryawanshi, 2001). The two most ubiquitous types of diabetes are Type I and Type II DM, while gestational diabetes is specific to pregnant women. Since it affects a large portion of the global population, the type 2 diabetes (T2D) is the condition that receives the most attention. This is because it causes insulin resistance in adipose tissue, muscle, and liver due to decreased insulin secretion from pancreatic β-cells (Karthick et al., 2014).

Different factors are involved in the progression of diabetes, but the most common among them is oxidative stress produced by reactive oxygen species (ROS) due to glucose and fatty acids overloading (Wright Jr et al., 2006). According to Rhodes, apoptosis of the beta-cells in pancreas is the motif cause of insulin decline in diabetic patients (Rhodes, 2005). According to the previous literature reported by Volpe et al., the pancreatic β-cells severely damaged by oxidative stress and one of the leading cause of diabetes. Diabetes cause by elevation of blood glucose level is due to the imbalances in ROS production and clearance, which leads to the unusual ROS binding with unsaturated fatty acids, a positive sign of oxidative stress and the primary cause of apoptotic cell death, according to them. The occurrence of diabetes is probably managed by reducing ROS production (Newsholme et al., 2016; Volpe et al., 2018).

Due to the progressive nature and prevalence rate of the disease, it requires better and permanent treatment strategy, including the development and synthesis of new drugs (Modi, 2007). Different effective prophylactic and treatment measures are needed, because only drugs therapy and insulin injections have not been effective role to completely reduce the occurrence (Baynes & Thorpe, 1996). For that purpose variety of herbal medicines were used by humans to cured diabetes as alternative to synthetic medications, with no or less side effects and high recovery potential, which is why research regarding herbal medicines open new door to natural life (Alarcon-Aguilara et al., 1998; Rhodes, 2005).

According to the previous literature, natural drugs are widely used in medical practices because of their ameliorative potential against different ailments and lesser side effects in comparison with synthetic drugs (Marles & Farnsworth, 1995; Tuhin et al., 2017). More than 1200 plants are confirmed that they have medicinal potential against different physiological changes in the body and some are ethno-pharmacologically used to treat diabetes (Wong & Liu, 2010). According to world health organization (WHO), phyto-derived compounds are more warranted than synthetic compounds because of no side effects and production of unwanted by-products in metabolism (Satyavani et al., 2011). To prevent the prevalence of diabetes, there is worldwide demand for phytomedicines and different pharmaceutical companies in Asia and Australia are now conducting different experiment on extracting of various phyto-compounds from plants to recover diabetes and other ailments and to explore its medicinal properties (Kalimuthu et al., 2008).

Celtis occidentalis L. (*C. occidentalis*), commonly known as nettle tree or hackberry native to North America, Australia, Europe and Africa. Plants in Celtis genus have strong therapeutic potential widely used to cure sore throat and menstrual illnesses using its leaves, bark and fruits (Moerman, 1998). Cannabeceae family included 60-70 species of Celtis plant mostly found in temperate zone of Northern Hemisphere and in some parts of Central Africa and South America (Martins et al., 2015). *C. occidentalis* contains different phytochemicals used as antioxidant, hepatoprotective and nephroprotective agents (El-Alfy et al., 2011; A. Muhsin et al., 2024; Muhsin, Zahoor, et al., 2025; A. M. Muhsin et al., 2024). From the above literature it is clear that the plant may also be antidiabetic potential and act as hypoglycemic agent. The present research was aimed to explore the novel bioactive phytochemicals, antidiabetic and organs protective potential of the *C. occidentalis* methanolic extract in Wister albino rat (*Rattus norvedicus*) and to support traditional medicine and their uses in several disorders.

Materials and Methods

Plant collection and Chemicals required

The fresh leaves of *C. occidentalis* were collected from Maidan Lal Qila; district Dir Lower, KPK, and Pakistan from May-July 2021. The plant was identified by Dr. Gul Rahim Department of Botany, from the Herbarium of the University of Malakand with voucher specimen number (UOM.Bot24/Garden 720). Chemicals glibenclamide was purchased from Luqman Chemist and Druggist Chakdara, Pakistan. Alloxan monohydrate, chemical was used for induction of diabetes, was purchased from Sigma Aldrich Chemicals Faisalabad, Pakistan and all other chemicals (analytical grade) were purchased from Lahore, Pakistan.

Extraction of plant material

The leaves were washed and cleaned with distilled water thoroughly and shade dried for 15 days and grinded into fine powder with electric blender. About 300 g of fine powder was soaked with 2 L methanol (95%) for 20 days and was placed in electric shaker for complete extraction. This was followed by filtration and the methanol was evaporated using rotary evaporator at 50 °C. Later, it was subjected in a water bath for one day to evaporate the remaining solvent thoroughly. After drying completely, the crude methanolic extract of *C. occidentalis* was weighed (50.7 g) and stored in a glass container in a vertical freezer to protect from humidity.

Evaluation of the Phenolic Phytochemicals

The phenolic compounds from the freeze *C. occidentalis* crude extract were extracted using HPLC-DAD system (water/methanol-1:5). The Agilent polytetrafluoroethylene syringe filter was used to filter the solution into high-performance liquid chromatography (HPLC) vials. The Agilent Infinity Better 1260 HPLC system was used to separate and identify the phenolic constituents. The system included a C18 reversed-phase column of 100mm in length, 4.6mm in breadth, and 3.5µm in particle size, as well as a diode array detector. The separation was carried out using a solvent system composed of solvent A (methanol-acetic acid-deionized water, 10:2:88 v/v) and solvent B (methanol-acetic acid-deionized water, 90:2:8 v/v). The separation was completed in 25 minutes of elution using the specified procedure (Zeb, 2015). The phenolic components have been identified and measured with reference standards. The quantity calculated was expressed in micrograms per gram of extract.

Fourier transforms infrared spectroscopy (FT-IR Spectroscopy)

The FT-IR examination was done at the Centralized Resources Laboratory (CRL) University of Peshawar (Peshawar KPK) (Model: Cary 630; Made by: Agilent technologies, USA), to identify the functional groups responsible for the bio-reduction Ag-NPs. The functional groups of the prepared sample (Ag-NPs) were scanned and compared with functional group of the potassium bromide bits employing spectrophotometer. The instrument (spectrophotometer) was used to employ KBr pellets with a wavelength ranging from 400 to 4000 cm−1 and biosynthesized Ag-NPs were placed on the glass surface of the sample stage instantly (Kamaraj et al., 2022).

Alpha (α)-Glucosidase Scavenging Assay

The α-glucosidase scavenging potential of the crude methanolic extract of *C. occidentalis* was performed with slight modification (Shai et al., 2011). Glibenclamide was used as a standard parallel with methanolic extract in inhibition assay. The α-glucosidase solution (0.5units/mL) was prepared in phosphate buffer (0.1 M, 6.90pH) and mix with P-Nitrophenyl-α-D-glucopyranoside (5 mM) as substrate solution. Different concentrations of the plant extract i.e., 1000, 500, 250, 125 and 62.5 µg/ml were prepared and the enzyme solutions were thoroughly mixed and incubated for 15 min at 37 ◦C in dark condition. Following the incubation, sodium carbonate solution (0.2 M, 80 µL) was added. The combined solution excluding the α-glucosidase was used as a blank solution. The absorbance of the solutions was recorded at 405 nm. The % inhibition assay of α-glucosidase was calculated by the following formula;

$$\%\ \boldsymbol{\alpha\text{-}glucosidase\ inhibation} = \frac{Abs(control) - Abs(sample)}{Abs\ (control)} \times 100 \qquad (i)$$

- Abs-absorbance, control- blank solution, sample- *C. occidentalis* extract and glibenclamide, standard drug

Alpha (α)-Amylase Inhibition potential

For the assessment of α-amylase inhibition by plant extract previously reported procedure was followed with little modification (Jung et al., 2006). The mixture was prepared containing sodium phosphate buffer 200 µl of 0.02M, 20 µl of enzyme and the plant extracts in concentration range 62.5, 125, 150, 500 and 1000 µg/ml followed by the addition of 200 µl of starch and incubated for 15 minutes at room temperature. 400 µl of 3, 5-Dinitrosalicylic acid (DNS) reagent was added to the solution for termination of reaction and placed in water bath at boiling temperature for 6 minutes and the cooled. Finally the mixture was diluted with 20 ml of distilled water and the absorbance was recorded with UV spectrophotometer at 540 nm. The % α-amylase inhibition assay was calculated by the following formula;

$$\%\ \boldsymbol{\alpha\text{-}amylase\ inhibation} = \frac{Abs(control) - Abs(sample)}{Abs\ (control)} \times 100 \qquad (ii)$$

- Abs-absorbance, control- blank solution, sample- *C. occidentalis* extract and glibenclamide, standard drug

Animal Model and Experimental design

Forty Swiss albino rat male sex weighing between 150 to 200 g (Age: 4 and half months) has been used for the current activity. Animals were kept in the transparent glass cages each cage contained with eight individuals (***n=8***) and the cages were marked with respective experimental group. The experimental detail is given in Figure 1. They were acclimatized for one week in the animal house, at the University of Malakand at 12 h light/dark cycle and room temperature was set at 25 ± 2 °C. The animals have free access to feed and drunken water and the experiment were run for forty days. After completion of experimental duration all the animals were euthanized via anesthesia using isoflurane (5% in 100% oxygen). The protocols for the current research as well as for animals handling were observed according to the guidelines established by the American Physiological Society for Human and Animal

Research (Association, 2001), and also approved by the Department of Zoology, University of Malakand with (Ref. no. E-SA-11-2009).

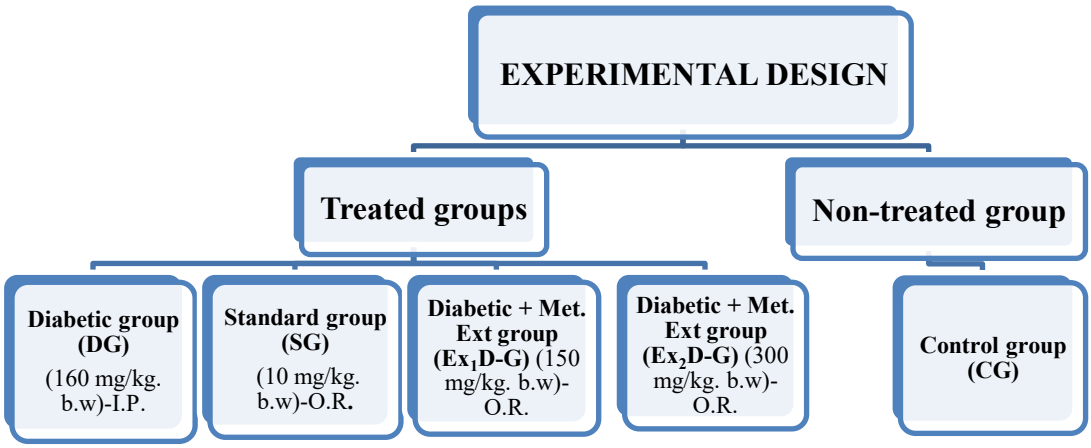


Figure 1. Experimental design of different treated groups

Induction of Diabetes

Before induction of diabetes the body weight and fasting blood glucose level of all experimental animals were recoded. Diabetes was induced with alloxan monohydrate a single-dose of 160 mg/kg b.w. (body weight) via intraperitoneal (I.P.) route in each animal. For the confirmation of diabetes induction, Accu-check glucometer (Model GU) was used to measures fasting blood glucose after 3 days of consecutive administration of alloxan monohydrate. Animals with blood glucose level > 290 mg/dL has been considered diabetic animals and selected for further studies. After successful induction of diabetes in all group animals except control, the rats were randomly grouped. Treatment to all animals were started after 7 days of alloxan injection and the experiment was continued for 40 days following standard protocols (Ul Haq et al., 2022). The methanolic extract of *C. occidentalis* was administered via oral route (O.R.) for 37 days (total dose 5.5 g/kg. b.w.) and glibenclamide was given to the standard group of dose 10 mg/kg (b.w.).

Blood glucose test

The blood glucose levels of experimental rats were measured with a glucometer (Accu-Chek) and suitable strips. After a 12-hour fast, blood was tail puncture and blood sugar was measured: before prior to diabetes, and then on the 1st, 8th, 16th, 24th, and 32nd days are following diabetes induction.

Body and selected organs weight

Weight of each individual was measured five times: once before alloxan induction and again on the 1st, 11th, 21st, and 31st days next to diabetes induction, using an ordinal weighing scale. The rats' anterior abdominal walls were cut

midline, and the animals were put to sleep at the end of the experiment using ketamine/xylazine (80/10 mg/kg, i.p.). Following their removal, the heart, kidney, pancreas, and liver were drained, washed with saline, and weighed using a sensitive balance (Mettler Toledo, Germany). The organs were subsequently preserved in a 10% formalin solution for histopathological examination. The organ-to-body weight ratio was used to calculate the relative weight of each organ (%).

$$\%\ Organ\ weight = \frac{Organ\ weight}{Total\ body\ weight} x\ 100$$

Blood samples collection

The blood samples from all experimental groups were collected via 5 mL syringe. 2 mL blood of each group animals was stored in EDTA tubes and the remaining 3 mL blood of each group animals was centrifuged at 1000x for 15 minutes. After centrifugation the supernatant yellowish color serum was separated in eppendorf tubes for further analysis.

Analysis of Glycated hemoglobin (HbAc1)

1 mL of blood stored in EDTA tube was used and centrifuged for ten minutes at 15,000 rpm at 25 °C after being cleaned three times with 8 mL of saline solution. The hemolysate was made by combining 1.5 mL of water with 500 µL of sediment cells. Fresh or stored hemolyzed samples at -80 °C were used for glycated hemoglobin analyses. Routine hemoglobin assays were performed using an HPLC ion exchange chromatography system (Hi-AUTO A1c A. Menarini Diagnostics—Arkray KDK) with a photometric detection unit operating at 415-500 nm, with a healthy subject's HbA1C ranging from 4.30 to 5.90% (Lapolla et al., 1999).

Matrix and sample preparation for MALDI-TOF MS

The sample was prepared using a sandwich layer method. To create a matrix layer, a droplet (0.5 µL) of 30 g/L SA matrix solution dissolved in 100% ethanol was applied to a smooth stainless steel sample plate and dried. In just a few seconds, a fine crystalline layer formed. A droplet (0.5 µL) of 30 g/L sinapinic acid matrix, dissolved in acetonitrile and 0.1% trifluoroacetic acid (1:1), and analyte solution (1:100 dilution), mixed with equal volumes (5 µL), was placed on the surface of the crystalline seed layer and dried in the air at room temperature and then inserted into the Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) instrument at Chughtai Lab Lahore (Lapolla et al., 1996).

The Glycated β-hemoglobin measured by MALDI-TOF (GHb_M correspondingly) was calculated as percentages of the modified form, glycated, with respect to the free form of β-chain hemoglobin, according to the subsequent equations:

$$\boldsymbol{GHbA} = \frac{Intensity\ \beta\_Glycated\ Hbm/z16031}{Intensity\ \beta\_free\ Hbm/z15868} \qquad (iii)$$

Biochemical Analysis

The stored serum was analyzed for the estimation of different biochemicals, such as cholesterol, triglycerides, LDL, HDL, ALP, ALT, bilirubin, serum amylase, serum urea and creatinine, different antioxidant markers (SODs, CAT, GPx, & GSH), lipid preoxidation markers (TBARS & MDA)and insulin.

The xanthine-xanthine oxidase method, which produces superoxide, can be used to measure the activity of superoxide dismutase. Cu, Zn, and Mn were used to measure the SOD enzyme's activity. The appropriate volume of serum was first mixed with 1 mL of an ethanol/chloroform combination (5/3, v/v). This was followed by centrifugation at 4,0009g and an evaluation of the enzyme activity in the ethanol phase of the supernatant (Kaya et al., 2007). The SOD unit was the quantity of enzyme that decreased the nitro-blue tetrazolium reduction rate by 50%, and the activity was reported as U/g of hemoglobin.

The activity of the GSH and GPx enzymes were then assessed. In order to monitor the activity of GPx and GSH (EC 1.6.4.2), hydrogen peroxide was used to start the enzyme reaction in the tube, which also contained reduced glutathione, sodium azide, glutathione reductase, and nicotinamide adenine dinucleotide phosphate. A spectrophotometer was then used to measure any variation in absorbance at 340 nm. Units per gram of hemoglobin were used to quantify erythrocyte activity (Stuart et al., 2021). The rate constant of H_2O_2 breakdown at 240 nm was used to compute the CAT activity. Ask (s-1)/g of hemoglobin was used to obtain the data. According to Alvarez et al. (2009), the tests were carried out at 25°C (Alvarez et al., 2009).

Serum samples have been identified to contain malondialdehyde (MDA) and thiobarbituric acid reactive compounds (TBARS). The levels of MDA in blood samples were ascertained using the thiobarbituric acid reaction technique. Two milliliters of anticoagulant-free blood samples were centrifuged at 8000g for approximately ten minutes at 4°C in order to extract the serum. At 532 nm, the reference curve line for MDA equivalents generated by acid-catalyzed hydrolysis of 1, 1, 3, 3-tetramethoxypropane was compared to the absorption of thiobarbituric acid reactive compounds. Proteins with TBARS and MDA levels of nmol/mg were measured (Alvarez et al., 2009).

Assessment of serum cytokines

To identify inflammation in pancreatic tissues, cytokines including IFN-γ and TNF-α were measured by means of the ELIZA kit (Roche, Schaffhausen, Switzerland) following previous literature (Al-Amarat et al., 2021; Muhsin, Dilnaz, et al., 2025).

Histopathological analysis of organ tissues

Liver, kidney, and pancreas were collected from all the treated and non-treated groups. The organ tissues were immediately cleaned with saline water and stored in 10% formalin. After a while, small pieces of each organ were processed and dehydrated in grading ethanol (70%, 80%, 90%, & 100%) followed by xylene clearing and were

placed in paraffin wax for proper impregnation. The blocks were prepared and sectioned with rotary microtome of 2 um thickness and then stained with hematoxylin and eosin stains and were observed under compound microscope.

Statistical analysis

The collected data is shown as Mean ± SD. In order to explain significant variation among various experimental groups, one-way ANOVA and student t-test, subsequent to which post hoc Tuckey's multiple tests with a significance level of $p<0.05$ were employed in SPSS (version: 27).

Results

Phenolic Contents of the *C. occidentalis* leaves Extract

Twenty-three phenolic constituents were identified in extract via HPLC-DAD (**Figure 2**), with compound information as shown in **Table 1**. The identified novel phytoconstituents were Quercetin-3,4-diglucosidase-3-(6-feruloy-glucosidase), Quercetin-3-(p-coumaroyl-di-glucosidase)-7-glucosidase, Quercetin-3-(p-coumaroyl-di-glucosidase)-7-glucosidase, Phytol, Triferuloyl-dihexose, 6-Octadecenoic acid/ Oleic Acid, Octadecanoic acid, 9, 12-Octadecadienoic acid, and Kaempferol-3-(p-coumaroyl-di-glucosidase)-7-glucoside at peaks 1-7, below 100 µg/g respectively. Compounds at peaks 8-13 were Octadecanoic acid, 9, 12-Octadecadienoic acid, Kaempferol-3-(p-coumaroyl-di-glucosidase)-7-glucoside, Kaempferol-3, 7-glucoside, cis-13,16-Docasadienoic acid, 9-Octadecenal/2-Methyl-Z,Z-3,13-octadecadienol respectively. 9-Octadecenal/2-Methyl-Z, Z-3, 13-octadecadienol (3260.8µg/g) was found in maximum quantity, followed by 9, 12-Octadecadienoic acid (2019.1µg/g). 9, 17-Octadecadienal/cis-9-Hexadecenal was 1720.8µg/g (peak 14), Oxacyclododecan-2-one/trans-13-Octadecenoic acid was 513.5 (peak 15), Kaempferol-3-(p-coumaroyl-di-glucosidase)-7-glucoside (760.3µg/g) was shown at peak 16, and Oleic acid, 3-hydroxypropyl ester (1632.2µg/g) at peak 17. 4-Caffeoyl-5-coumaroylquinic acid (402.1µg/g), Quinic acid/3-o-Coumaroylquinic acid (602.0µg/g), Catechin/Gallic acid/Salicylic acid (350.2µg/g), gamma-Sitosterol/beta-Sitosterol (210µg/g), alpha.-Amyrin/Naringenin/Luteolin-7-glucosidase (427.3µg/g), and apigenin-7-glucosidase/Apigenin-6, 8-glucosidase (430.1µg/g) corresponding at peaks 18, 19, 20, 21, 22, and 23.

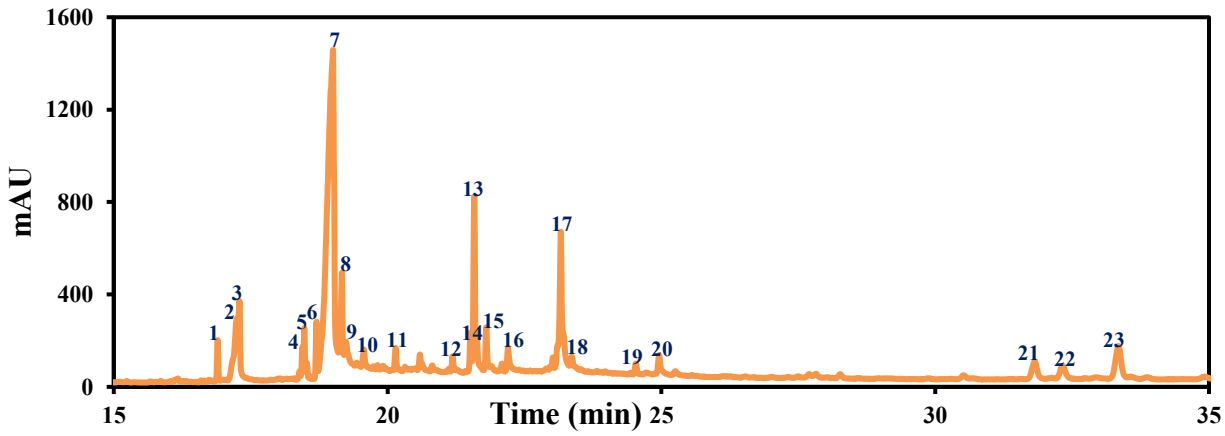


Figure 2. *Celtis occidentalis* leaf extract was chromatographed at 320nm using a C18 reversed-phase column. Particular summit in the HPLC graph reflects a different chemical with specific characteristics given in **Table 1.**

Table 1. Estimation and documentation of essential polyphenolic constituent in *Celtis occidentalis* leaves.

Peak #	*R.Time	Compounds Name	λ-max(nm)	Concentration (µg/g)
1	16.899	Quercetin-3,4-diglucosidase-3-(6-feruloy-glucosidase)	26698612	74.2±2.1
2	17.252	Quercetin-3-(p-coumaroyl-di-glucosidase)-7-glucosidase	122703679	72.2±3.9
3	17.297	Quercetin-3-(p-coumaroyl-di-glucosidase)-7-glucosidase	85424147	92.1±2.6
4	18.447	Diosmetin-7-rutionside	17823038	54.1±1.3
5	18.482	Phytol	27593008	34.5±3.5
6	18.704	Triferuloyl-dihexose	42687977	65.0±3.1
7	19.003	6-Octadecenoic acid/ Oleic Acid	1234145869	45.2±3.7
8	19.167	Octadecanoic acid	119081685	472.5±13.5
9	19.245	9,12-Octadecadienoic acid	78217791	2019.1±20.2
10	19.569	Kaempferol-3-(p-coumaroyl-di-glucosidase)-7-glucoside	31159865	901.1±5.6
11	20.149	Kaempferol-3, 7-glucoside	23664506	630.6±6.5
12	21.517	cis-13,16-Docasadienoic acid	34844339	487.6±7.5
13	21.586	2-Methyl-Z,Z-3,13-octadecadienol	154042502	3260.8±14.2
14	21.628	9,17-Octadecadienal/cis-9-Hexadecenal	43973601	1720.8±10.3
15	21.805	Oxacyclododecan-2-one/trans-13-Octadecenoic acid	45101310	513.5±4.5
16	22.203	Kaempferol-3-(p-coumaroyl-di-glucosidase)-7-glucoside	33292891	760.3±8.6
17	23.166	Oleic acid, 3-hydroxypropyl ester	208695995	1632.2±12.2
18	23.961	4-Caffeoyl-5-coumaroylquinic acid	31920338	402.1±4.5
19	24.652	Quinic acid/3-o-Coumaroylquinic acid	48546579	602.0±4.7
20	25.001	Catechin/Gallic acid/Salicylic acid	96293928	350.2±3.2
21	31.816	gamma-Sitosterol/beta-Sitosterol	49644569	210.6±3.1
22	32.149	alpha.-Amyrin/Naringenin/Luteolin-7-glucosidase	97314918	427.3±4.3
23	33.349	Apigenin-7-glucosidase/Apigenin-6, 8-glucosidase	112339219	430.1±5.2

*R.Time: Retention Time

FT-IR Spectroscopic Analysis of Leaf Extract of *C. occidentalis*

The FTIR analysis shows the nature and purity of the manufactured nanoparticles. The FTIR spectra of the Ag-NPs and *Celtis tetrandra* plant were recorded (**Figure 3**). Both spectra have two Infrared (IR) regions: one region of the IR spectroscopy is the functional region and the second one is the fingerprint region. The FTIR spectrum indicates the bands of both the organic compounds and that of the metals. The organic compounds almost found in the functional region showed by absorbance peaks and the second region consists of metals as shown by the peaks in Figure 3. The peaks at the wavelength range from 3688 cm^{-1} to 1028 cm^{-1} shows the functional group region consist of organic constituents present in the crude extract of the plant and the peak below 855 cm^{-1} and at 454.7 cm^{-1} represent the fingerprint region consist of metals. The FTIR spectroscopy was carried out by different scientist to investigate the different functional group in the powder of various plants.

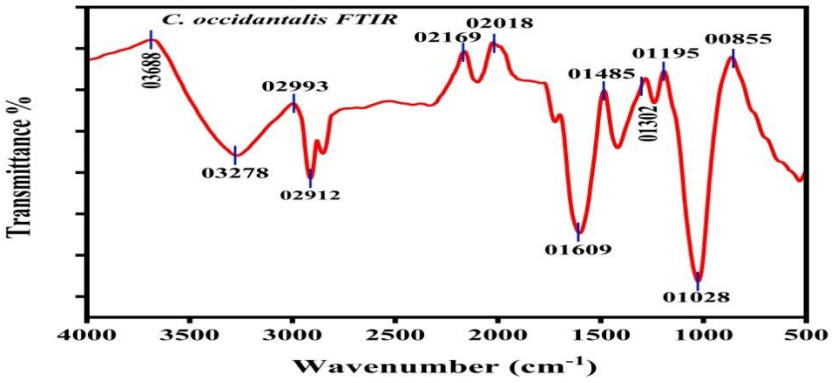


Figure 3. FT-IR Spectrograph of *C. occidentalis* Leaves methanolic extract

In vivo Study

The experimental rats' body weight changed significantly from the control group as shown in **Figure 4**. The plasma glucose levels increased significantly as a result of alloxan induction in comparison with pre-dose values, in all groups'. Rats treated with the extract showed a substantial drop in blood glucose on day 8 ($P < 0.02$), followed by a further decrease on day 16 ($P < 0.002$), and a highly significant decline ($P < 0.001$) on days 24, and 32 when compared to day 1 (**Figure 5**).

The glibenclamide-treated group's plasma glucose levels showed no significant decline ($P < 0.09$) on day 8th, a significant fall ($P < 0.05$) on day 16, a highly significant decline ($P < 0.001$) on days 24, and 32 when compared to day 1, while the untreated (control) and diabetic groups showed a highly significant variation throughout the experiment (Figure 4). Throughout the trial, plasma glucose levels in the diabetic control group stayed considerably higher ($P < 0.001$) than in the extract-treated, control, glibenclamide-treated groups.

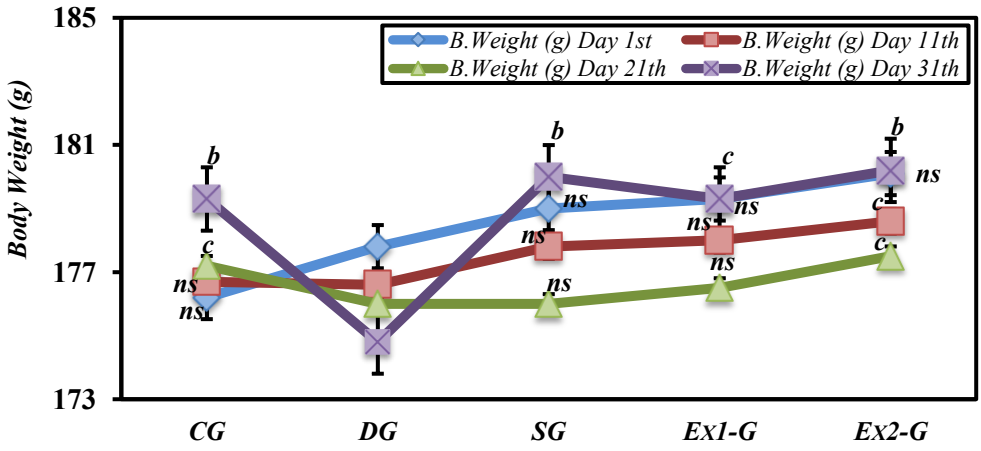


Figure 4. Body weight of different treated groups at specified days. Significance level ***a;*** P<0.001, ***b;*** P<0.01 and ***c;*** P<0.05.

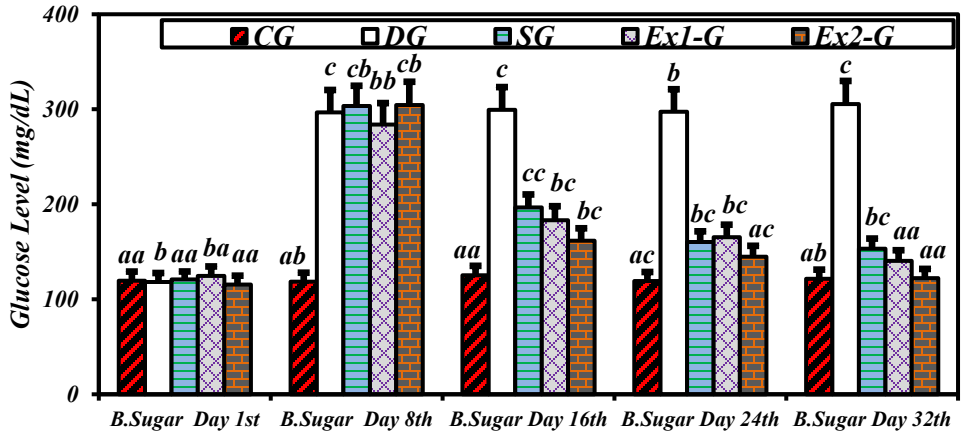


Figure 5. Plasma glucose level of different treated groups at specified days. Significance level ***aa***; P<0.0001, ***ab***; P<0.001, ***ac***; P<0.0005, ***bc***; P<0.005 and ***b***; P<0.01, ***cc***; P<0.03, ***c***; P<0.05.

Effects of *Celtis occidentalis* extract on relative weight organs

Table 2 shows the influence of *Celtis occidentalis* on relative organ weights in all rat groups. The relative weights of all the organs in experimental groups were significantly different. The diabetic group had a larger relative weight than the treated groups. The relative weight of the given organs in the extract treated (**Ex_1D-G & Ex_2D-G)** shows significant (P<0.01 & P<0.001) in comparison with normal, diabetic and glibenclamide groups as shown in **Table 2.**

Table 2. Effect of *C. occidentalis* leaves extract on relative weights of organs in different treated groups of rat

Treatment	Kidney	Heart	Pancreas	Liver
CG	0.70 ± 0.08^{a}	0.40 ± 0.02^{b}	1.39 ± 0.02^{a}	4.25 ± 0.13^{b}
DG	1.12 ± 0.05	0.50 ± 0.01	1.03 ± 0.02	5.66 ± 0.23
SD	0.81 ± 0.03^{c}	0.42 ± 0.06^{ns}	1.21 ± 0.08^{b}	4.70 ± 0.24^{c}
Ex_1D-G	0.76 ± 0.12^{b}	0.40 ± 0.08^{b}	1.34 ± 0.01^{b}	4.30 ± 0.12^{b}
Ex_2D-G	0.64 ± 0.03^{a}	0.38 ± 0.01^{a}	1.41 ± 0.31^{a}	4.10 ± 0.01^{a}

***a*: P<0.001, *b:* P<0.01, *c:* P<0.05, CG;** control group, **DG;** diabetic group, **SG;** standard (glibenclamide) group, **Ex_1D-G;** extract-1 group, **Ex_2D-G;** extract-2 group.

Effects on Glycated Hemoglobin and Insulin

A noteworthy (P< 0.00, P<0.01 & P<0.05) elevation was noted in the serum insulin level in the plant extract-treated group compared to the control, glibenclamide-treated, and diabetic groups (P<abc). In plasma concentration of glycated hemoglobin **(P<*abc*)**. In the plasma level of glycated hemoglobin, a noteworthy reduction was observed in extract treated group compared to the aforementioned groups **(P<*abc*)** as shown in **Table 3** and **Figure 6**.

Table 3. Serum insulin and plasma glycated hemoglobin levels in different treated groups

Test	CG	DG	SG	Ex_1D-G	Ex_2D-G	
Insulin (U/L)	17.2±1.0***ac***	5.2±1.2***c***	10.3±0.4***bc***	11.6±1.5***bc***	17.9±0.5***abc***	
HbA1c (%)	6.13±0.3***ac***	9.75±0.2**c**	8.12±0.1***bc***	7.53±0.6**bc**	6.65±0.2***abc***	

***** Significance level **aa**; P<0.0001, **ab;** P<0.001**, bc;** P<0.005 and **b;** P<0.01**, cc;** P<0.03**, c;** P<0.05**, abc/bc/ac;** with respect to **bc** & **c. CG;** control group, **DG;** diabetic group, **SG;** standard (glibenclamide) group, **Ex_1D-G;** extract-1 group, **Ex_2D-G;** extract-2 group.

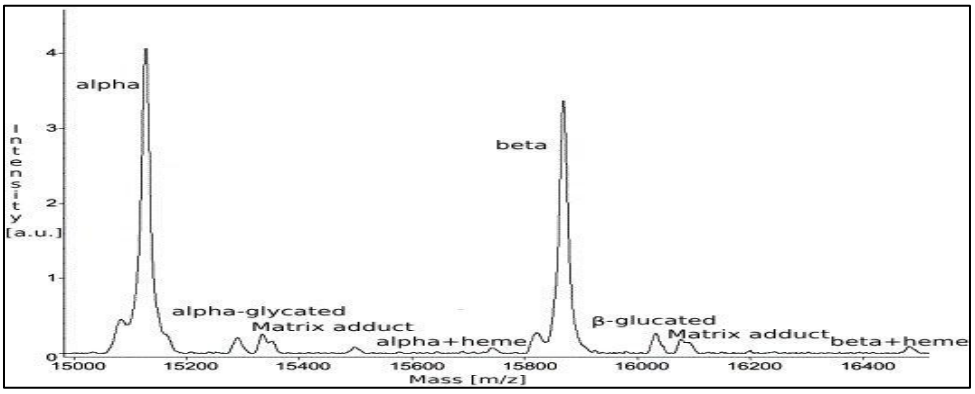


Figure 6. Plasma glycated hemoglobin analysis

Effects on Liver and Renal Function Tests

Serum levels of bilirubin, ALT, ALP, and amylase were significantly (P < 0.001) lower in the extract-treated rats (Ex1D-G & Ex2D-G) at day 32 than in glibenclamide-treated and diabetic groups of rats **(Figure 7)**. At day 32, the extract-treated groups (Ex1D-G & Ex2D-G) showed a significant (P<0.001) decrease in serum urea and creatinine when compared to the previously described treated and non-treated treated groups **(Figure 8)**.

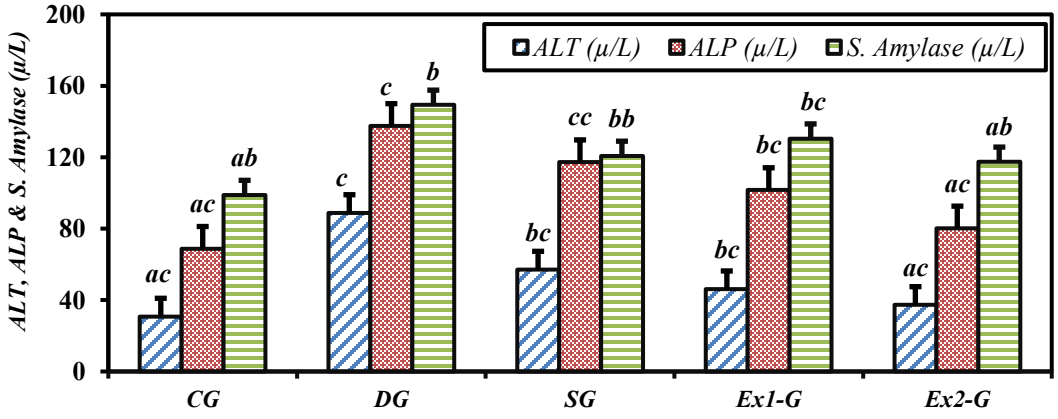


Figure 7. ALT, ALP & Serum Amylase concentration in different treated groups of rat. Significance level ***aa***; P<0.0001, ***ab***; P<0.001, ***ac***; P<0.0005, ***bc***; P<0.005 and ***b***; P<0.01, ***cc***; P<0.03, ***c***; P<0.05, ***ns***; non-significant.

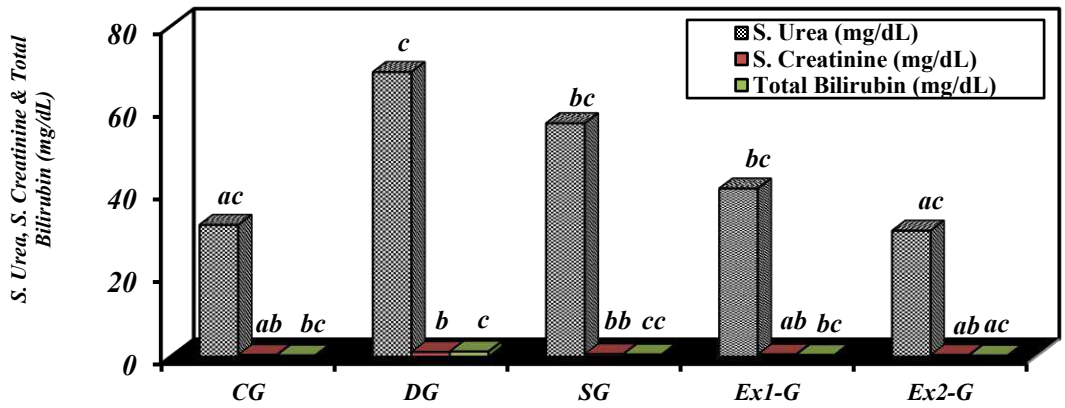


Figure 8. Serum urea & creatinine and total bilirubin level in various treated rat groups. ***aa***; P<0.0001, ***ab***; P<0.001, ***ac***; P<0.0005, ***bc***; P<0.005 and ***b***; P<0.01, ***cc***; P<0.03, ***c***; P<0.05, ***ns***; non-significant.

Effects of *C. occidentalis extract* on Lipid Profile

A significant (P<0.001 & P<0.01) reduction was observed in serum cholesterol and serum triglycerides in the extract-treated (**Ex_1D-G, Ex_2D-G**) groups, compare to DG, and SG groups and same as control group animals, whereas no significant change was perceived in serum HDL and serum LDL level in the diabetic group as compared with the normal control, glibenclamide and extract-treated (**Ex_1D-G, Ex_2D-G**) groups (**Figure 9**).

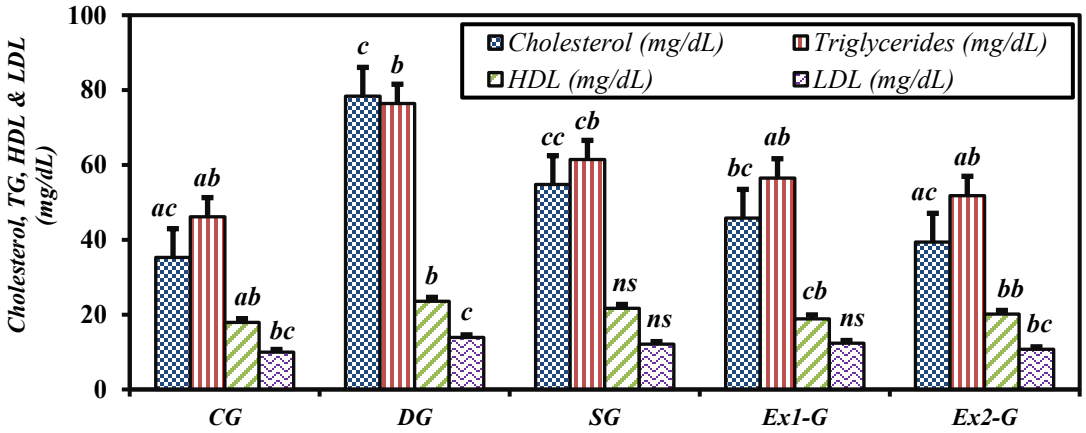


Figure 9. Serum cholesterol, triglycerides, HDL, and LDL levels in different treated groups of rats. ***aa***; P<0.0001, ***ab***; P<0.001, ***ac***; P<0.0005, ***bc***; P<0.005 and ***b***; P<0.01, ***cc***; P<0.03, ***c***; P<0.05, ***ns***; non-significant.

Effects of *C. occidentalis* extract on Serum Cytokines markers

Various alloxan metabolites increase inflammation in various organs, resulting in necrosis, the formation and progression of lesions, intrusion of the inflamed cells because of higher interferon gamma and tumor necrosis factor alpha (INF-γ and TNF-α) levels, and hepatic, renal, and pancreatic dysfunction. INF-γ and TNF-α level of DG group augmented, as seen in Table 4. In the extract-treated (Ex1D-G & Ex2D-G) groups, concentration of the aforementioned inflammatory markers were decline respectively, indicating the anti-inflammatory qualities of the *C. occidentalis* leaf extract that amplify the scarring behavior of alloxan.

Table 4. Fluctuations in cytokine indicators level in various treated groups of rat

Experimental Groups	INF-γ (pg/ml)	TNF-α (pg/ml)
CG	3.12 ± 0.8^{ab}	5.8 ± 0.5^{ac}
DG	6.42 ± 1.1^{b}	24.7 ± 1.01^{c}
SG	4.99 ± 1.2^{ns}	20.1 ± 2.2^{ns}
Ex_1D-G	3.87 ± 1.9^{ab}	9.7 ± 0.9^{c}
Ex_2D-G	2.56 ± 0.3^{ab}	4.7 ± 1.05^{ac}

*** *aa***; P<0.0001, ***ab*; P<0.001, *ac*;** P<0.0005, ***bc*;** P<0.005 and ***b*;** P<0.01, ***cc*;** P<0.03, **c;** P<0.05, ***ns*;** non-significant**, *ab*;** with respect to ***b*. CG;** control group, **DG;** diabetic group, **SG;** standard (glibenclamide) group, **Ex_1D-G;** extract-1 group, **Ex_2D-G;** extract-2 group.

Antioxidant and Oxidative stress markers

Table 5 showed effects on antioxidant markers in *C. occidentalis* treated groups. A very significant ((P<0.01 P<0.001) reduction was observed in the antioxidant markers (**SODs, CAT, GPx, & GSH**) in diabetic group as compared to the control group. The extract treated groups (**Ex_1D-G & Ex_2D-G**) comparatively shows significant (P<0.01 & P<0.001) elevation in antioxidant markers as in control, DG and SG groups. The oxidative stress (**TBARS &MDA**) markers were significantly raised in diabetic group and in extract treated (**Ex_1D-G & Ex_2D-G**) group's a significant decline was noted as compared to the diabetic and standard groups and same as the control group given in **Table 6**.

Table 5. Effects of *C. occidentalis* extract on antioxidant markers in different treated groups

Experimental groups	GSH (Nm/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
CG	6.42 ± 0.5^{ab}	9.32 ± 1.02^{ac}	115.5 ± 1.12^{ac}	35.45 ± 2.21^{aa}
DG	2.82 ± 0.3^{b}	2.42 ± 1.1^{c}	65.3 ± 1.5^{c}	5.21 ± 1.78^{ns}
SG	3.24 ± 0.22^{bb}	5.45 ± 1.12^{ns}	96 ± 1.10^{bc}	22.53 ± 1.54^{ca}
Ex_1D-G	4.66 ± 0.1^{bc}	7.87 ± 0.9^{ac}	102.6 ± 1.4^{bc}	28.17 ± 2.01^{ba}
Ex_2D-G	6.87 ± 0.3^{ab}	9.47 ± 1.0^{ac}	115.3 ± 1.2^{ac}	35.29 ± 1.89^{aa}

*** *aa***; P<0.0001, ***ab*; P<0.001, *ac*;** P<0.0005, ***bc*;** P<0.005 and ***b*;** P<0.01, ***cc*;** P<0.03, ***c*;** P<0.05, ***ns*;** non-significant**;** with respect to **b & c. CG;** control group, **DG;** diabetic group, **SG;** standard (glibenclamide) group, **Ex_1D-G;** extract-1 group, **Ex_2D-G;** extract-2 group.

Table 6. Effects of *C. occidentalis* extract on oxidative stress markers in different treated groups

Experimental groups	MDA (nmol/mg protein)	TBARS (nmol/mg protein)
CG	2.5 ± 1.2^{ab}	20.2 ± 0.23^{ac}
DG	7.29 ± 1.32^{b}	65.8 ± 1.21^{c}
SG	4.41 ± 0.92^{cb}	42.3 ± 2.11^{ns}
Ex_1-G	3.12 ± 2.1^{ab}	30.9 ± 0.12^{bc}
Ex_2-G	2.1 ± 1.41^{ab}	20.7 ± 0.32^{ac}

*** aa**; P<0.0001, **ab;** P<0.001, **ac;** P<0.0005, **bc;** P<0.005 and **b;** P<0.01, **cc;** P<0.03, **c;** P<0.05, **ns;** non-significant**, ab/ac/bc;** with respect to **b** & **c. CG;** control group, **DG;** diabetic group, **SG;** standard (glibenclamide) group, **Ex_1D-G;** extract-1 group, **Ex_2D-G;** extract-2 group.

In-vitro Study

α-Glucosidase and α-amylase scavenging Assay

In the following **Figure 10**, the *Celtis occidentalis* leaves retain the anticipatory capability in different concentration ranges for α-glucosidase and α-amylase. At 62.5µg/ml, the α-glucosidase and α-amylase showed 69.5±0.52% and 67.4±0.20%, whereas glibenclamide (standard) showed 76.2±0.22% inhibition. In the same way, at 1,000µg/ml, a substantial inhibition of α-glucosidase and α-amylase was 86.1±0.20% and 84.3±54% occurred, almost near to the standard (90.8±0.51%).

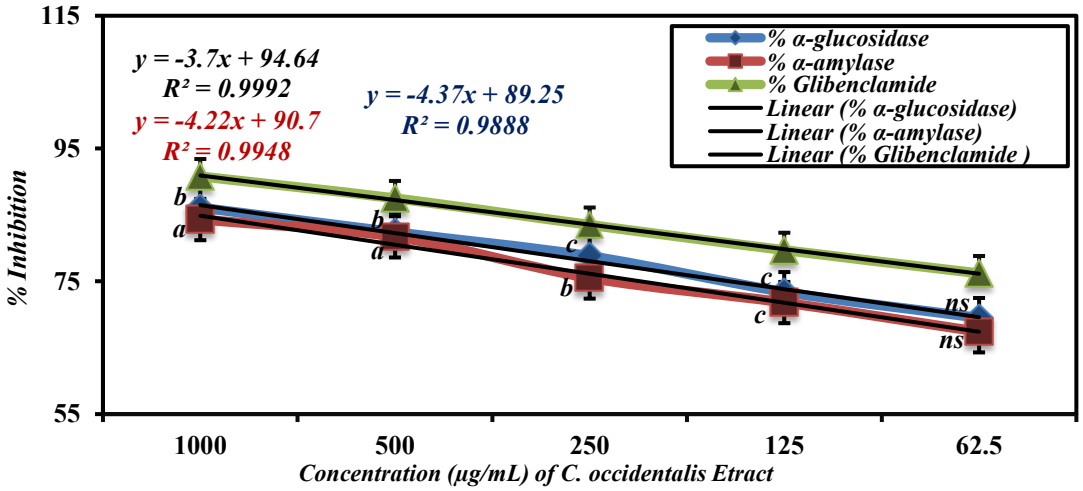


Figure 10. The % inhibition of Celtis occidentalis leaves extract against α-glucosidase and α-amylase in comparison with glibenclamide (standard).

Histopathological assessment

Figure 11 (I, II, & III) represents the effects of alloxan and methanolic extract of *Celtis occidentalis* on the hepatic, renal and pancreatic tissues in rats. The degenerative effects of alloxan were assessed in different treated samples. In the liver tissues of diabetic group, hepatocytes were severely disrupted, dilation of the central vein, inflammatory cells infiltrations, mucous accumulation in cellular spaces and in central vein (**Fig 11 (I)-b**). The anatomical structure of the liver was observed normal in control, standard and extract treated groups, but in comparison with standard group the extract actively regenerated the affected and degenerated regions in extract treated groups (**Ex_1D-G & Ex_2D-G**) as shown in (**Fig 11 (I)-d & e**). The renal tissues anatomy were also rigorously altered by alloxan in diabetic group such as, glomerular congestion, extrusion of the epithelial cell contents into the lumen, tubular necrosis, inflammatory lesions aggregation etc. (**Fig 11 (II)-k**). In treated groups the aforementioned alterations were normalized as compared to the diabetic group, but most potently recovered in the extract groups (**Ex_1D-G & Ex_2D-G**) as in (**Fig 11 (II)-m & n**) as like control group. The pancreatic islets and ovoid β-cells were analyzed in diabetic group were vigorously halted due shrinkage of islets and destruction of the β-cells, zymogen granules, blockage of the major pancreatic duct etc. (**Fig 11 (III)-w**). These architectural distortions were reversed more

actively by the plant extract in (**Ex$_1$D-G & Ex$_2$D-G**) (**Fig 11 (III)-y & z**), and optimized by the standard glibenclamide in standard group (**Fig 11 (III)-x**).

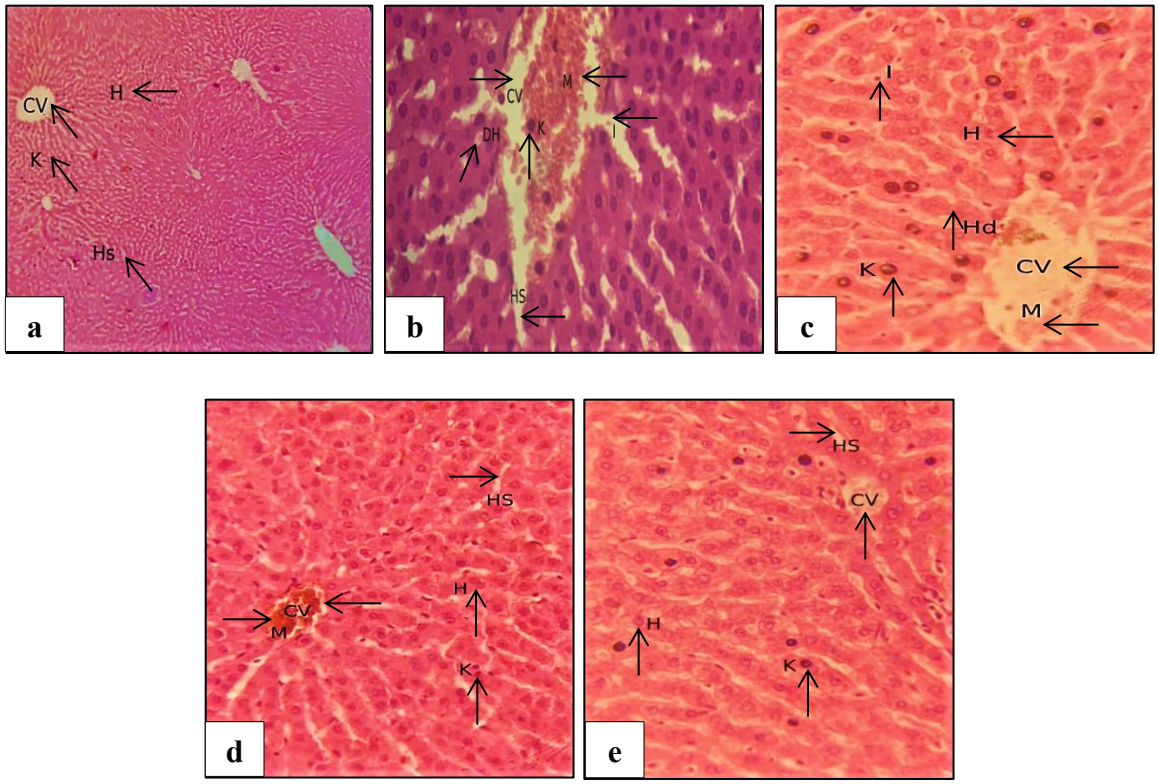
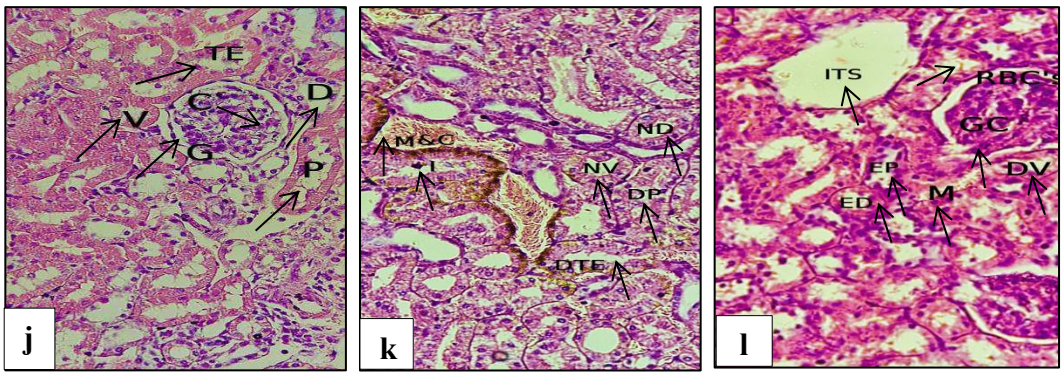


Figure 11 (I): Photomicrographs represent the liver (**a-e**) portions of various treated groups (H & E), at100x & 400 x. (**a**) Liver of control group with normal hepatocytes ‘‘H’’ and endothelial lining of central vein ‘‘CV’’ with no evidences of necrosis and having normal bile duct. (**b**) Liver section of DG showed with necrosis, proliferation of blood and mucus in bile duct, inflammatory cells ‘‘I’’ infiltration and dilation of central vein “CV”. (**c**) Liver histology of SG has slightly recovered “H” with infiltration of inflammatory cells “I” and mucus ‘‘M’’ aggregated in hepatocytes spaces ‘‘HS’’. (**d**) Liver stained section of Ex$_1$D-G shows improvement in recovery of degenerated hepatocytes “Hd” with mild ingested bile duct and slightly accumulated “M” in “CV”. (**e**) Liver histopathology of Ex$_2$D-G shows normal liver parenchyma and no alteration founds in where due to paracetamol over reaction.



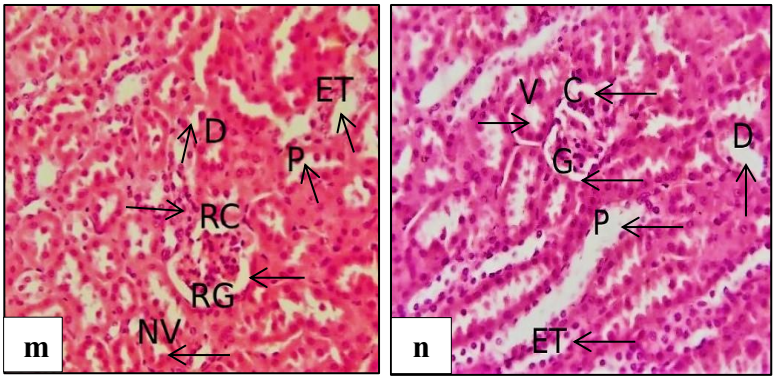


Figure 11 (II): 100x and 400x microscopy images reveal renal (j-n) sections of the treated groups (H & E). (j) Kidney in the control group has normal tubular epithelium (TE), functional glomeruli (G), capillaries of the glomeruli (C), capillaries of the glomeruli (C), capillaries of the glomeruli (C), and proximal and distal convoluted tubules (P & D). (k) DG stained renal segment displaying intense tubular epithelium degeneration (DTE), inflammation cell infiltration (I), and vessel narrowing (NV) as an effect of blood wall degeneration, mucus and cellular debris deposit (M & C), and degeneration of proximal convoluted tubule (DP). (l) SG kidney with glomerular congestion (GC), dilation of blood vessels (DV), mucus (M) agglomeration in the tubules, and (RBCs) in the glomerular capillary space. (m) Ex1D-G kidney with constricted blood vessels (NV), healing of the tubule epithelial wall (ET), young glomeruli (RG), and restoration of the glomerular capillary tuft (RC). (n) Ex2D-G kidney stained photomicrograph without any changes in structural architecture showed similar results to the control group.

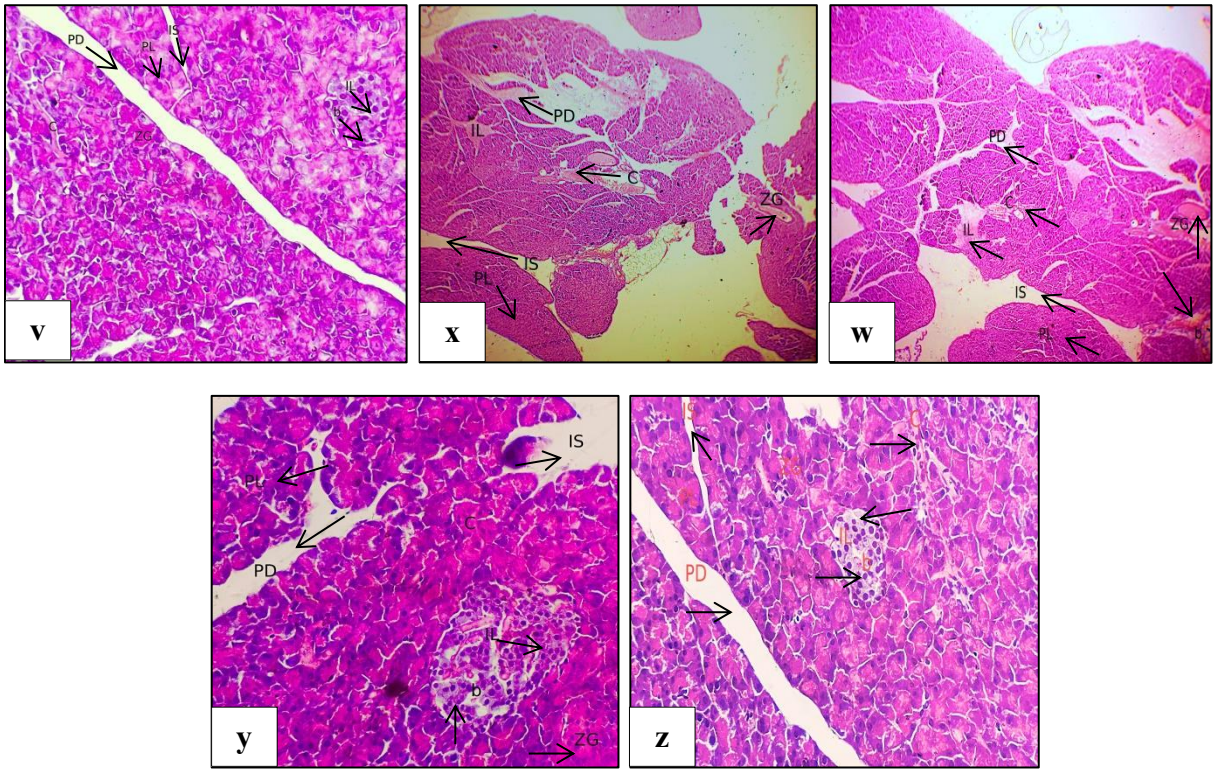


Figure 11 (III): Photomicrographs represent the pancreas (**v-z**) segments of various treated groups (H & E), at100x & 400 x. (**v**) Pancreas section of the control group having normal islets of Langerhans ‘‘IL’’, normal ovoid β-cells (b), zymogen granules ‘‘ZG’’, normal diameter of the pancreatic duct ‘‘PD’’, functional capillaries ‘‘C’’, normal pancreatic lobules ‘‘PL’’ and interlobular septum ‘‘IS’’ . (**w**) Pancreatic section of the DG with altered histology,

shrunk ‘‘IL’’, degenerated β-cells ‘‘Db’’, dilated ‘‘PD’’ disrupted ‘‘C’’ and degenerated ‘‘PL’’. (**x**) Pancreatic section of the SG with rare alterations, rarely shrunk ‘‘IL’’ normal β-cells ‘‘b’’ and slightly dilated ‘‘PD’’. (**y**) Pancreas section of the Ex1D-G with recovered histology. (**z**) Pancreatic section of the Ex2D-G has normal histological architecture, same as the control group.

Discussion

Twenty-three polyphenolic substances were identified using HPLC-DAD. The *Celtis occidentalis* leaves were abundant in quercetin and kaempferol glycosides in addition to phenolic acids. Ertas et al. (2014) measured the total glycosides of kaempferol and quercetin, however they were unable to identify the precise molecules (Ertaş et al., 2014). According to Oh et al. (2004), *S. sarmentosum* was found to contain quercetin and kaempferol glycosides (Oh *et al*., 2004). These findings suggest that several species of the genus Celtis are a great source of flavonoids and other phenolic chemicals. The FTIR analysis shows the nature and purity of the novel compounds. The FTIR spectrum of the *Celtis tetrandra* plant was recorded. Mak et al. (2013) carried out (FTIR) spectral analysis for the powdered flower petals of hibiscus and Cassia and reported polysaccharides, suberin, and lipid and also the OH group of the phenolic compounds (Mak et al., 2013). Similarly, Jamuna et al. (2017) also conducted (FTIR) spectroscopy for essential oil of *Cymbopogon citratus* and concluded CH3, C=O, C-O and functional groups (Jamuna et al., 2017). The finding of these workers supported the present findings.

After chronically administering a methanolic crude extract of the selected plant, the current study tested the hypoglycemic characteristic of a plant species, *C. occidentalis*, in rats with diabetes prompted by alloxan. The extract's anti-hyperglycemic action was demonstrated via remarkable decline in plasma glucose concentration. All of these biochemical indicators showed a notable decline. Following a 32-day period of continuous treatment of the plant extract, diabetic rats' plasma glucose levels considerably decreased. The plant has antidiabetic properties, as evidenced by the considerable decline observed on day 8^{th} and the highly significant fall observed on days 16^{th}, 24^{th}, and 32^{nd}. Although it has been used in traditional medicine, sufficient scientific study to evaluate its anti-hyperglycemic qualities has not yet been conducted. Flavonoids and tannins are among the plant's primary constituents. Literature indicates that tannins and flavonoids have anti-hyperglycemic effects (Garg et al., 2010). According to scientific research, flavonol bioside from Hibiscus vitifolius flowers has a significant hypoglycemic effect (Sachdewa & Khemani, 2003).

The flavonoids and tannins existence was verified in the initial phytochemical tests may be the cause of the extract's antidiabetic properties in this study. The plant's potential hypoglycemic mechanism was most likely due to either enhanced peripheral blood glucose transport or increased insulin release from the pancreatic β-cells. In diabetic animals, a number of processes are believed to cooperate to reduce plasma glucose levels. Certain plants have properties similar to those of oral anti-hyperglycemic medications, such as sulfonylureas, which lower blood glucose levels in animals with normal blood sugar levels (Davies et al., 2004). In contrast, some anti-hyperglycemic medications, such as biguanides like metformin, only affect diabetic animals and have no effect on plasma glucose levels in healthy animals (Stumvoll et al., 1995).

Given that the selected plant had not brought any changes in the plasma glucose level in normo-glycemic rats, it is likely that this plant has a mechanism similar to that of metformin. When DM occurs, an excessive increase in plasma glucose levels impacts plasma proteins. Hemoglobin and glucose molecules combine to generate HbA1c, and the level of this upsurge is believed to be associated with the intensification in plasma sugar concentration before meal (Jackson et al., 1979). Therefore, HbA1c works as a marker for estimation of status of diabetes. HbA1c is formed increasingly and permanently for a time and is stable over the life span of red blood cells. It is not affected by diet, exercise, or insulin and even on test day. Since it is slowly formed and dissociates hardly, it shows an actual level of blood glucose (Franklin Bunn, 1981). Hence, HbA1c is thought to be a reliable index and is an imperative parameter in prognosis and management of the disease (Narendhirakannan et al., 2006). Results of the current investigations revealed that, the treatment with selected plant up to 37 days leads to a remarkable reduction in HbA1c concentration in hyperglycemic individuals (Gupta et al., 2009). The extract's effect on the HbA1c level is probably due to increased insulin production, which lowers blood sugar; a decline in plasma glycosylated hemoglobin is directly correlated with lower blood sugar (Ylönen et al., 2003).

In present research, serum urea, serum creatinine, and serum amylase levels significantly increased in diabetic rats with renal and pancreatic failures. In diabetic rats, higher level of urea was mostly due to protein break down or removal of the amino group from amino acids for glucose synthesis from non-carbohydrate sources (Yamaguchi & Weitzmann, 2009). When diabetic rats were given a long-term dose of the extract, their blood creatinine, serum amylase, and serum urea levels significantly decreased, which improved both pancreas and kidney functions. For the extract of additional plant species, comparable findings have been documented (Kedar & Chakrabarti, 1983). The extract used in this study might have enhanced kidney and pancreas functioning because to its antidiabetic and antioxidant properties, which can raise an animal's altered metabolic status, as well as the ability of the islet cells in the pancreas and the kidney tubules to regenerate (Naz et al., 2019).

Liver enzymes are used to assess liver function. The reservoirs where ALP stores are the liver, bones, placenta, and intestine, whereas ALT is found in kidney cells, liver, and muscles (Naz et al., 2019). ALT serves a marker for the identification of hepatic damage when the production of pyruvate and glutamate increase due to alanine break down. Alkaline phosphatases and aminotransferases are found in the cytosol and are drained out when cells are damaged. They serve as a marker for the loss of cell membrane functional integrity (Sivakrishnan & Kottaimuthu, 2014). The hepatocellular function is represented by the serum ALP level. Increased enzyme production may be the cause of the elevation in blood ALP levels (Moss, 1975). These enzymes are released into the bloodstream from injured tissue during liver dysfunctioning and necrosis of the parenchymal cells (BP et al., 2009). In hyperglycemic animals treated with alloxan, the serum ALT and ALP concentrations constantly increases, whereas level of the aforementioned hepatic markers restore to normal in plant treated groups (**Ex_1D-G** & **Ex_2D-G**) and standard group (glibenclamide).

Because of the raised levels of these enzymes in the liver, hepatic impairment is commonly seen. The extract's hepato-protective properties are demonstrated by the early improvement in liver cell secretary phenomenon that results from its successful regulation of ALP. The inclusion of flavonoids, which have the ability to scavenge free

radicals, may have contributed to the extract's hepato-protective effects (Choi et al., 2002). Since these enzymes are believed to be the primary marker of normal liver function, an increased amount of them indicates hepatic damage. Recovery from injury is demonstrated by restoration to normal. Numerous scientific papers state that hyperlipidemia and hyperglycemia coexist in diabetes (Okon et al., 2007). The primary hazard for heart ailment (Greenland et al., 2003), is elevated cholesterol and LDL levels, which are also a major cause of illness in hyperglycemic rats (Sowers et al., 2001).

The diabetic rats had elevated levels of triglycerides and cholesterol, which is linked by means of some notable change in lipids break down and associated metabolites (Sochar et al., 1985). Elevated variations in cholesterol in diabetes are caused by abnormalities in cellular metabolism of cholesterol; the true mechanism underlying these changes is still unknown (Pari & Saravanan, 2002). The diabetic rats' lack of plasma insulin was most likely the cause of the substantial increase in triglycerides. Insulin increases lipoprotein lipase and the subsequent hydrolysis of triglycerides under normal conditions (Morikawa et al., 2007). A higher amount of plasma insulin is most likely the source of the diabetic rats' decreased triglyceride levels. Given that diabetes raises triglycerides, several plant species have shown promise in lowering them (Arkkila et al., 2001).

The current work demonstrated, cholesterol, LDL and triglycerides were markedly increased. Triglycerides, LDL, and cholesterol all significantly decreased following therapy with the plant extract. The extract therefore demonstrated hypoglycemic and hypolipidemic properties. In this study, the amount of insulin was measured and closely examined in relation to the functioning of the pancreas. In diabetic group the level of insulin was decreased severely due to pancreatic inflammation and β-cells degeneration. The level of insulin was reinstated promptly in extract treated (**Ex_1D-G & Ex_1D-G**) as compared to the diabetic and standard groups and same as control group. Higher levels in triglycerides and cholesterol are caused by the absence of insulin in people with diabetes (Morikawa et al., 2007). The findings of this study demonstrate the crude extract's antidiabetic effects in rats with diabetes. A variety of extra-pancreatic and pancreatic processes may work in concert to produce strong antidiabetic effects. This study suggests that the extract and glibenclamide may use an analogous pathway to cause a hypoglycemic impact in rats. According to this study, the extract from C. occidentalis leaves may also be useful in regulating biochemical markers associated with diabetes mellitus, including triglycerides, cholesterol, LDL, HDL, serum creatinine, serum urea, serum amylase, glycosylated hemoglobin, and liver enzyme characteristics like ALT and ALP.

The histopathological alterations in different organ tissues (liver, kidney, and pancreas) were evaluated. The liver tissues in diabetic group show severe alterations such as hepatocytes degenerations, inflammatory cells infiltrations, necrotic lesion, dilation of central vein etc. due to alloxan-induced oxidative stress and declining the serum concentration of different antioxidant markers as shown in (**Fig 11 (I)-b**) (Naz et al., 2019). These alterations were significantly mitigated in extract treated groups (**Ex_1D-G & Ex_2D-G**) as compared to the diabetic and standard groups and same as control group (**Fig 11 (I)-d & e**).

The renal tissues were also assessed and showed severe changes in renal histology such as glomerular congestion, tubular necrosis, dilation of peritubular capillaries, cellular infiltration etc. in diabetic group (**Fig 11 (II)-k**) (Muhsin

et al., 2025). These alterations were significantly normalized in extracted treated groups (**Ex_1D-G & Ex_2D-G**) in comparison with diabetic, standard, and control group (**Fig 11 (II)-m & n**). The histopathological changes in pancreas were analyzed and evaluated as degeneration of the ovoid β-cells, dilation of central pancreatic duct, shrunken islets of Langerhans, aggregation of zymogen granules, blockage of capillaries etc. as shown in (**Fig 11 (III)-w**) (Morikawa et al., 2007; Pari & Savaranan, 2002). The recovery of these alterations was significantly observed in extract treated group (**Ex_1D-G & Ex_2D-G**) as compared to the standard and diabetic group and same as control group (**Fig 11 (III)-y & z**).

Conclusion

This study provides scientific evidence that C. occidentalis, which is high in vital phenolic phytochemicals, can be used as a tonic for diabetes and can be utilized in place of chemically synthesized drugs that have additional negative effects. Long-term clinical trials are encouraged in order to identify the active ingredient responsible for the antidiabetic effect, reveal the actual mechanism underlying the plant's ability to lower blood sugar and cholesterol, and examine the histological recovery of various organ tissues.

Data availability statement

The datasets generated for this study are available on request to the corresponding author.

Ethics statement

All institutional and national guidelines for the care and use of laboratory animals were followed. The animal study was reviewed and approved by the ethical committee of the Department of Zoology, University of Malakand with (Ref. no. E-SA-11-2009).

Author contributions

AM: conceptualization, experimentation, writing-review and editing original draft, statistical analysis. DN: supervision and formal analysis, SN&MW: methodology & experimentation.

Conflicts of Interest

The authors have no conflict of interest.

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Data availability

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