Studying the molecular mechanism of *Cleome droserifolia* extract antidiabetic effect in rats

Doaa I. Kabil
Department of Home Economics
Faculty of Specific Education
Tanta University, Egypt
doaa.kabeal@sed.tanta.edu.eg

Mohamed A. Kelany
Food science and Nutrition
Faculty of Agriculture
Sohag University, Egypt
Mohamd.kelany@agr.sohag.edu.eg

Karim S. Elsaid
Department of Chemistry
Faculty of Science,
Tanta University, Egypt
kareem.ali@science.tanta.edu.eg

Amany A. Abd El-Aziz
Department of Home Economics
Faculty of Specific Education
South Valley University, Egypt
amany.mohamed@sed.svu.edu.eg

Abstract:
Diabetes is a global disease that endangers human health. Metformin (Met) drug is used as a first-line treatment for type 2diabetes mellitus (T2-DM). It was noted that Met had several adverse effects on vital organs. Natural products have counted as much safer agents for T2-DM treatment. This study aimed to evaluate the antidiabetic effect of *Cleome droserifolia* extract (CDE). Forty male Sprague Dawley rats were divided into 5 groups (GP) (n = 8) as the follows: The 1st group (Gp1) served as a negative control. From Gp2 to Gp5 were fed on a high-fat diet (HFD) for 12 weeks and were induced with a30 mg/kg streptozotocin (STZ) injection as a single intraperitoneal (i.p) dose. Then, Gp3 was treated with ١٥٠ mg/kg of Met orally for 4 weeks on a daily basis.GP4 was treated with 100 mg/kg CDE. GP5 was treated with Met/CDE as in Gp3 and Gp4. Phytochemical analysis showed CDE has a high level of phenolic and flavonoids. The percentage of body weight % (b.wt.%)changes, biochemical, and histopathological investigations were evaluated. Co-treatment of T2- DM with Met/CDE led to significant improvement in the % b.wt of T2-DM rats and improved serum glucose level, increase in the C-peptide levels, and improvement in the hepatic functions and antioxidants/antioxidant status. CDE could play an important role in regulating T2-DM and can be developed as a promising natural material for diabetes management. In silico study showed an important combination with specific phenolic acids that have antidiabetic activity.

Keywords: Type 2 diabetes mellitus, Metformin, *Cleome droserifolia*, Total antioxidant capacity, Antidiabetic effect.

دراسة الآلية الجزيئية لتأثير مستخلص نبات السموا *Cleome droserifolia* كمضاد لمرض السكر لدى الجرذان

المستخلص

يعتبر مرض السكر من الامراض الخطيرة التي تؤثر على صحة الانسان كما يعتبر ميتفورمين من اهم العقاقير المستخدمة في علاج السكر من النوع الثاني لكنه اثار جانبية على اعضاء جسم الانسان. تم اجراء هذه الدراسة لتقييم تأثير اضافة نبات السموة لعقار الميتفورمين وذلك للحد من اثاره الجانبية. تم اجراء التحليل الفيتوكيميائى لمستخلص السموة. استخدم في هذه الدراسة ٤٠ من الجرزان يتراوح وزنهم (١٢٠-١٣٠) تم تقسيمهم الى ٥ مجموعات: المجموعة الأولى تم تغذيتها على الوجبة القياسية (مجموعة ضابطة سالبة): المجموعات من الثانية الى الخامسة تم تغذيتها على الوجبة عالية الدهن لمدة ١٢ اسبوع ثم حقنها بمادة استريبتوسيتوزين (٣٠ ملجم / كجم). المجموعة الثانية (لمجموعة الضابطة الموجبة المصابة بمرض السكر من النوع الثاني)، المجموعة الثالثة والتي تناولت ميتفورمين (١٥٠ ملجم /كجم) فقط لمدة ٤ اسابيع. المجموعة الرابعة تناولت (١٠٠ملجم /كجم) من مستخلص السموة فقط، المجموعة لخامسة تناولت عقار ميتفورمين مع مستخلص السموة وذلك لمدة ٤ اسابيع. اظهرت النتائج تأثير مستخلص السموة في ضبط وزن الجسم بالمقارنة بالمجموعة الضابطة المصابة بالسكر كما اظهرت التأثير الخافض لسكر الدم لمستخلص السموة لتأثيره المضاد للأكسدة لمحتواه من الفينولات والغلافونيدات وتحسن في وظائف الكبد والفحص الهستوباثولوجى للأنسجة. كما أظهرت الدراسة باستخدام النمذجة الحاسوبية (In silico) وجود تركيبة فعالة من أحماض فينولية معينة تمتلك خصائص مضادة لمرض السكري.

الكلمات الدالة: السكر من النوع الثاني – ميتفورمين– السموة – التأثير المضاد للأكسدة – التأثير المضاد للسكر.

INTRODUCTION

Diabetes mellitus (DM) is a chronic endocrine disease characterized by persistent hyperglycemia, which is often caused by the absolute or relative deficiency of insulin secretion or insulin resistance (IR) **(Shi *et al.,* 2018)**. DM is usually classified into three major categories including type-1 diabetes mellitus (T1-DM), T2-DM, and gestational diabetes **(Nuckols *et al.,* 2018)**. It is estimated that there are currently 537 million adults aged 20 to 79 worldwide who have DM, and this number is expected to rise to 783 million by 2045. In 2021, diabetes resulted in 747,000 deaths in the Southeast Asia region, with the total economic impact reaching 10.1 billion USD **(IDF, 2021)**. In T2 DM counted as the highest endemic type all over the world **(Khan *et al.,* 2020)**.T2-DM symptoms included

blurred vision, fatigue, feeling very hungry or thirsty, increased need to urinate, slow healing of cuts or sores, and unexplained weight loss **(Yang *et al.*,2018)**.The complications of T2-DM classified microvascular or macrovascular types **(Harding *et al.*, 2019)**.For T2-DM treatment different approaches have been approved to decrease IR sensitivity and enhance the uptake of glucose into the cells. Recently, novel approaches have been applied to treat T2-DM, for instance, the use of nanoparticles and stem cells has emerged as potential settings for treatment **(Simos *et al.*, 2020 and Tillman *et al.*, 2022).**

Metformin (Met), is one of the most prescribed medications as a fist- line therapy for T2-DM. Met has many physiological advantages. It can activate adenosine monophosphate-activated protein kinase, upregulate glucose transporter 4 genes, increase glucose uptake and decrease oxidative stress, resulting in reduced hepatic gluconeogenesis and aiding lipid metabolism **(Hundal *et al.*, 2000)**. Met inhibits the mitochondrial respiratory chain complex 1 that responsible for energy metabolism and activation of AMP-activated protein kinase (AMPK) **(Vial *et al.*, 2019)**. The most common side effect of Met concentrated on the kidney and the liver tissues. Previous studies showed an adverse effect of Met upon the treatment of diabetic persons and reported that accumulation of Met led to an impairment in renal function and was associated with lactic acidosis **(Hsu *et al.*, 2017** and **Hao *et al.*, 2018)**.

Due to the severe side effects of the current medication for T2-DM, including Met, researchers have been directed to screen natural products (NPs) for complementary alternative therapies **(El-Said *et al.*, 2022)**. The interest in exploring NPs as a new source of different drugs, specifically anti-diabetic drugs, has increased in recent decades **(Yadav,2024)**. According to **Aboraya *et al.* (2022)**, variety of medicinal plants are considered to be useful in preventing and/or managing T2-DM and its problems.

Cleome droserifolia (C. *droserifolia*) is belongs to the Cleomaceae family is known traditionally as Samwa. This plant fortunately was found to be widely distributed in some Arabic countries as Egypt, Libya, Saudi Arabia, Palestine, and Syria **(Moustafa *et al.*, 2019).** Various extracts of *C. droserifolia* have been utilized for their hepatoprotective and hypoglycemic properties **(Moustafa and Mahmoud, 2023).** *C. droserifolia* extract (CDE) has shown strong anti-diabetic properties **(Ismail *et al.*, 2025).** The CDE has been found to contain a very high percentage of flavonols that showed 63.3% activity, similar to that of the Met **(Motaal *et al.*, 2014)**. Additionally, CDE is considered asistaminic, relaxant, tranquilizing, anticarcinogenic, antiparasitic, antioxidant, and antimicrobial properties **(Abdel Motaal *et al.*, 2014 and Maksoud *et al.*, 2020)**. The various biological effects are linked to the different categories of active secondary metabolites found in CDE, such as polyphenols, terpenes, flavonoids, glucosinolates, anthocyanins, and alkaloids **(Moustafa and Mahmoud, 2023)**. Consequently, this study aimed to examine the potential effects of co- treatment with CDE and Met in STZ-induced T2-DM in rats.

MATERIAL AND METHODS:

Chemicals

Streptozotocin (STZ) was purchased from MP Biomedicals Company, Illkirch, France. Metformin (Met) was purchased from Alfa Aesar Company, Haverhill, Massachusetts, USA. Glucose, C-peptide, aspartate transaminase (AST), alanine transaminase (ALT), total protein, manoaldehyde (MDA), superoxidedissmutase (SOD) and catalase (CAT) kits were purchased from Bio-diagnostic Company (Egypt). *C. droserifolia*

Collection of *C. droserifolia* and preparation of it's extract.

Samwa (*Cleome droserifolia*) plant, was obtained from the desert of North Sinai. Sinai Governorate, Egypt, in December, 2023. The collected plant was verified by the staff of the Faculty Agriculture, Sohag University, Egypt. The plant was dried in the shade ground into a powder, 50 g of powder was added to 500 ml of 70% ethanol then filtered to obtain *C. droserifolia* extract (CDE).

Phytochemicals analysis of *C. droserifolia* extract

Phytochemical analysis of CDE includes total phenolic, flavonoid, total antioxidant capacity (TAC) and DPPH scavenging capacity were determined. Total phenolic content was determined by Folin-Ciocalteau reagent according to **Miliauskas *et al.*, (2004)**. The flavonoid content was assessed according to **(Zhishen *et al.*, 1999)**. The phosphomolybednum method was used to determine TAC according to **Prior *et al.*, (2005)**. The DPPH scavenging capacity was assessed according to **El-Naggar *et al.*, (2023).** The scavenging activity on the DPPH % radical was expressed as the inhibition percentage using the following equation (1):

$$\text{DPPH \%} = [(AC - AS)/(AC)] \times 100 \quad (1)$$

Where Ac is absorbance for control, As is absorbance for sample

Normal balanced and high fat diets

The normal balanced diet (NBD), consisting of 10% protein, 10% fat, 74.4% carbs, 3.5% mineral mixture, 0.1% methionine, 1% vitamin mixture, and 1% fiber, was used to feed healthy control rats. The high-fat diet (HFD), consisting of 64 g of normal chow, 32 g of animal-sourced saturated fat, 300 IU of vitamin D3, and 15% and 12% of cholesterol, was used to treat rats **(Thomas, 1998)**.

Experimental Animals

For this study, male adult albino rats (Sprague- Dawley strain) (n=40 rats) weighing approximately (120 ±5 g.) were purchased from Helwan Experimental Animals Farm. Rats were housed in clean cages, maintained on a 12-hour light/dark cycle, and provided with free access to drinking water and a standard commercial diet.

In managing the laboratory animals, the National Institutes of Health's standards for the care and use of laboratory animals, as well as the recommendations of the National Research Centre Ethics Committee, were followed. The work was authorized by the institutional Animal Care Committee (IACUC-SCI-TU-0444).

Induction of T2-DM in rats

After 12 weeks of HFD feeding, rats were given a single intrapretonial (i.p.) injection of STZ (30 mg/kg). Blood samples were collected from the tail vein three days after the STZ injection, and the glucose level was determined using a portable glucometer (One Touch Select, Life scan, Inc., California, USA). Three days after STZ injection, blood samples were drawn from the tail vein and glucose levels were determined. Animals with a fasting blood glucose level>300 mg/dl were included in this study as T2-DM rats **(Guo *et al.*, 2011)**. Because of the ability of STZ to induce fatal hypoglycemia as a result of massive pancreatic insulin release, the rats received 10% glucose solution after 6 h of STZ injection for the next 48 hours to prevent fatal hypoglycemia **(Palsamy and Subramanian, 2008)**.

Experimental design

After T2-DM induction, the animals were divided into five groups (n = 8 rats) as follows: Group1 (Gp1): Normal control group (Ctrl), rats fed on NBD for 12 weeks and administered orally with dist. H_2O. Gp2: Diabetic group (T2-DM), rats were fed on an HFD for 12 weeks and then received a single dose of STZ 30 mg/kg i.p. (Gp3): T2-DM treated rats were given Met at a dose of 150 mg/kg by oral gavage daily for 4 weeks (T2-DM/Met). Gp4: T2-DM rats that were treated with CDE at a dose of 100 mg/k by oral gavage daily for 4 weeks (T2-DM/CDE). (Gp5): - DM rats that treated with a combination of Met and CDE as in GP3 and 4, respectively (T2-DM/Met/CDE).

Biochemical analysis

At the end of the experimental period (16weeks), rats were deprived of food overnight and sacrificed. Blood samples were collected, and then sera were separated by centrifugation for 10 minutes at 3000 rpm to separate the serum. The serum was carefully aspirated, transferred and frozen at -20°Cfor biochemical analysis. Furthermore, liver tissues were homogenized and centrifuged and then frozen at -20 C°for oxidant/antioxidant biomarkers.

Determination the total body weight changes

All rats of all groups were weighed at the beginning (initial b.wt) and at the end of the experiment (final b.wt). The percentage of the change in the total body weight (%b.wt) was calculated as follows in equation (2):

$$\textbf{b.wt\%= (final b.wt – initial b.wt / initial b.wt) × 100} \quad (2)$$

Estimation of biochemical analysis

Serum glucose and C-peptide were determined according to **Tietz, (1995)** and **Jones and Hattersley, (2013)**. Serum AST and ALT activities were determined as described by **Thomas, (1998)** and **Rei (1984)**. Serum total protein levels were determined as described by **Tietz, (1995)**. MDA was assessed based on the methods that have been previously described by **Li *et al.* (1994)**. SOD activity was determined as described by **Sun and Oberley, (1988).** CAT activities were measured following the methodology of **Aebi, (1984)**.

Histopathological investigations

Tissue specimens of the livers of all experimental groups were harvested and fixed in 10% formalin. Paraffin blocks were prepared after completing the tissue processing in different grades of alcohol and xylene. Sections (5µm) were prepared from paraffin blocks using microtomestained with hematoxylin and eosin and observed under a light microscope (Optica light microscope, B-350) to examine gross cellular damage **(Bancroft, 2008)**.

In silico study

Ligand preparation

Ligand structures were retrieved based on the results of RP-HPLC extraction **(Hashem *et al.,* 2021)** from the PubChem database. The 3D structure was optimized for energy using Avogadro 1.2.0 software (**Hanwell *et al.,* 2012**) with the MMFF94 force field.

Protein Preparation

The 3D structures of proteins (PI3K_O70173, PPAR_Q9QYK2, IRS_P35570 and glut4_P19357) were obtained from the UniProt database. The binding sites for these proteins were identified based on previously published literature and further validated using the CB-DOCK2 tool **(Liu et al .2022).** Protein preparation for molecular docking was carried out using AutoDock Tools 1.5.7 (**Morris et al., 2009**), including the removal of water molecules, addition of polar hydrogens, and assignment of Gasteiger charges.

Molecular docking

Molecular docking studies was conducted using AutoDock Vina **(Trott and Olson, 2010)** to evaluate the binding modes and affinities of the compounds with their respective protein targets. Docking grid boxes were centered on the predicted binding sites, with the exhaustiveness parameter set to 8. The default scoring function was used for the docking calculations.

Visualization and analysis

The docked complexes were visualized and analyzed using BIOVIA Discovery Studio Visualizer 2020 **(BIOVIA Discovery Studio, 2020)**. The binding affinities (ΔG values) and intermolecular interactions, including hydrogen bonds and hydrophobic interactions, were analyzed and reported.

Statistical analysis

All data are the means of 3 replicates. The data was expressed as mean ± SD. Comparison between groups was carried out using one-way ANOVA. If there were significant differences between means, Tukey post hoc comparisons among different groups were performed. For all statistical tests P values ≤ 0.05 was considered to be statistically significant. Data and statistical analysis were performed using Excel 2013 (Microsoft Corporation, USA, and Minitab version 18) **(Kotz *et al.,* 2006)**.

RESULTS AND DISCUSSION

Phytochemicals analysis of *C. droserifolia.*

The obtained results in Table 1, revealed the phytochemical analysis of CDE. The total phenolic and total flavonoid contents were 17.54 ± 1.15 mg GAE/g DW and 29.78 ± 2.49 mg QUE/g DW, respectively. The total antioxidant capacity (TAC)of the CDE was 251.63 ± 5.82 mg AE/g DW. The DPPH scavenging activity (%) of CDE was 78% ± 1.89, the concentrations of CDE, which able to inhibit 50% of DPPH (IC_{50})was 6.95 ± 0.74 mg/ml).The obtained results are in accordance with previous studies that reported CDE contains high amount of active secondary metabolites including phenolics, carotenoids, flavonoids, anthocyanins, polysaccharides, terpenoids, triterpenoids, alkaloids and glycosides *(***Muhaidat *et al.,* 2015 and Korkor *et al.,* 2022)**

Table 1. Quantitative phytochemical analysis of *C. droserifolia*

Phytochemical analysis	CDE
Total phenolic(mg GAE/g DW)	17.54 ± 1.15
Total flavonoids (mg QE/g DW)	29.78 ± 2.49
TAC (mg AAE/g DW)	251.63 ± 5.82
DPPH scavenging%	78% ± 1.89
IC_{50} of DPPH (mg/ml)	6.95 ± 0.74

DW: Dry weight, **GAE:** Gallic acid equivalent, **QUE:** Quercetin equivalent. **TAC:** Total antioxidant capacity, **AAE**: Ascorbic acid equivalent **IC_{50}**: Inhibitory concentration 50%.

Effect of co-treatment /CDE and Met on the total body weight changes in T2-DM rats

Data presented in figure 1 show the changes in body weight durinig the experimental period in all exprimental groups; Ctrl, T2-DM,T2-DM/Met,T2-DM/CDE, and T2-DM/Met/CDE for 16 weeks. At Wk-0, all groups start at similar baseline levels (~120–130). Over time, the control group shows a slower, steady increase in body weight compared to diabetic groups. T2-DM shows the steepest increase, reaching near 280 by week 16. T2-DM/Met, T2-DM/CDE, and T2-DM/Met/CDE show slower progression in body weight than T2-DM after week 12.T2-DM/Met/CDE shows the best control after week 12, with a slight decrease, suggesting improved outcomes compared to other groups. Additionally, data illustrates the longitudinal progression of body weight in all groups. As expected, the untreated T2-DM group demonstrated a continuous and steep increase in body weight, consistent with the natural progression of uncontrolled diabetes (**American Diabetes Association [ADA], 2023**). In contrast, groups receiving Met (T2-DM/Met), a common first-line pharmacotherapy, exhibited slower progression after Week 12, supporting prior findings that Met effectively delays disease progression and improves glycemic control **(Foretz*et al.,* 2014).**The combination therapy group (T2-DM/Met/CDE) showed the most pronounced improvement post-Week 12, with the trend plateauing and even slightly declining toward Week 16. This observation aligns with previous reports emphasizing that lifestyle modifications (CDE: Counseling, diet, and exercise

interventions) synergize with Met to produce superior metabolic outcomes compared to pharmacotherapy alone (**Knowler*et al.*, 2002 and Umpierre*et al.*, 2011)**.

The Diabetes Prevention Program notably demonstrated that intensive lifestyle interventions reduced the incidence of diabetes by 58% compared to placebo, whereas Met alone reduced it by 31% (**Knowler*et al.*, 2002).**The treatment of T2-DM group with CDE alone (T2-DM/CDE) improved body weight as compared to T2-DM group but did not outperform the combined Met and CDE group, suggesting that while lifestyle changes are beneficial, their effectiveness can be potentiated when combined with pharmacological intervention **(Umpierre*et al.*, 2011)**. Ctrl. group maintained relatively stable and lower values throughout, further underscoring the pathogenic role of metabolic dysregulation in T2-DM.Overall, these findings reinforce the current understanding that a multifactorial approach, combining both medication and lifestyle interventions, is the most effective strategy for managing T2-DM progression **(ADA, 2023)**.

According to the obtained results, there was a correlation between hyperglycemia and the decreased body weight of T2-DM animals **(Carpéné*et al.*, 2022). Zafar and Naqvi, (2010)** revealed that rats treated with STZ-induced diabetes showed signs of ill health and weight loss due to the harmful effects of STZ, which resulted in DNA alkylation, hyperglycemia, and necrotic lesions. Additionally, **Wickramasinghe *et al.*, (2022)** found that a significant decrease in body weight following STZ injection in T2-DM. Recently, **Maaliah *et al.*, (2024)** demonstrated that rats given STZ saw a modest drop in body weight. Furthermore, Treatment of T2-DM rats with CDE showed a marked improvement in body weight as compared to T2-DM group as confirmed by **Hashem and Shehta, (2021)**. Additionally, these findings were in line with a recent study by **AbouHaleka*et al.*, (2023).**

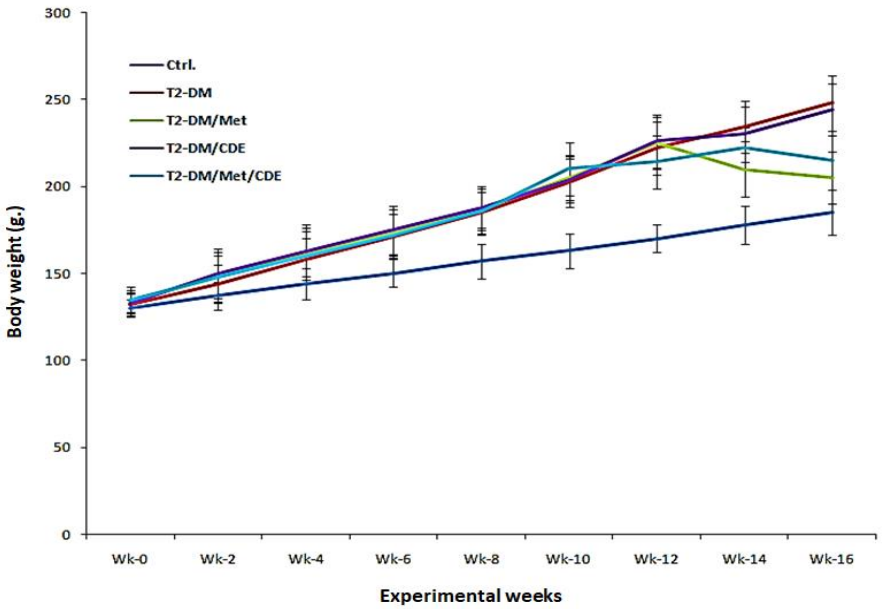

Figure 1. Ctrl.: Control group; **T2-DM:** Diabetic group; **T2-DM/Met:** Diabetic group treated with Metformin; **T2-DM/CDE:** Diabetic group treated with *C. droserifolia* extract; **T2-DM/Met /CDE:** Diabetic group treated with *C. droserifolia* extract.

Effect of co-treatment with CDE and Met on T2-DM rats on serum glucose and C-peptide levels

It has been reported that, DM considered one of the most common endocrine diseases that affects negatively on human health. Induction of DM causes various metabolic disorders, including hyperglycemia, hyperlipidemia, hypertension, atherosclerosis, retinopathy, neuropathy, and nephropathy (**Jörns *et al.,* 2014 and Krajewski *et al.,* 2016).** Similarly**, Alrasheedi *et al.*, (2024)** mentioned that DM induced by STZ produced significant increase in serum glucose levels.

Data are presented in Table (2) illustrated that the mean values of serum glucose(mg/dl) and C-peptide (ng/ml). It was remarkable that T2-DM, exhibited a significant increase in glucose levels. The mean values of glucose of T2-DM rats were significantly increased as compared to normal rats. The data showed a significant increase in the serum glucose level in T2-DM group (295.23 ± 4.32 mg/dl) compared to control group (89.87 ± 2.55 mg/dl) ($P< 0.05$). STZ is a glucosamine-nitrosourea compound that causes hyperglycemia by alkylating DNA and destroying pancreatic β cells. The impairment of β-cell function resulted in a decline of glucose homeostasis (**Zafar and Naqvi, 2010).** Recently, **Maaliah *et al.,* (2024)** reported that STZ injection led to a significant increase in serum glucose levels in experimental animals. The obtained results showed that administration of Met or CDE to T2-DM rats led to a significant reduction of serum glucose level (161.44±3.15 or 157.25±3.41mg/dl, respectively). Co-treatment with Met/CDE showed the most pronounced decrease in glucose level (119.97 ± 2.66 mg/dl) compared to diabetic group (295.23±4.23 mg/dl).

Consuming natural antioxidants lowers the risk of diseases such as diabetes, cancer, cardiovascular disease, and other aging-related conditions (**Greenwell and Rahman, 2015).** Phytochemicals, also known as phytonutrients, are compounds derived from plants that are known to be good sources of natural antioxidants (**Sharma*et al.,*2018).**Previously, it has been reported that bioactive compounds particularly derived from plant resources acts as antitumor and antidiabetic agents **(Tran *et al.,*2020).**Polyphenolics, the main constituents of certain ancient medicinal herbs, have captured attention for their biological properties **(Xiao, 2022).**These findings are consistent with previous studies on Samwa extracts and powders (**Abdelfattah *et al.,* 2019; Abdel Maksoud *et al.,* 2020 and Ismail, 2022).**

Recently, **Ismail *et al.,* (2025)** reported that treatment with samwa extract showed improvements included reductions in serum glucose, oxidative stress in T2-DM rats induced by alloxan. C-peptide level, however, was significantly decreased in T2-DM rats (0.05 ± 0.014 ng/ml) as compared to negative group (0.16± 0.013 ng/ml). Treatment with Met or CDE in T2-DM rats have significant increase in their levels (0.09 ± 0.012 or 0.08 ± 0.009 ng/ml, respectively). Co-treatment with Met/CDE, however, in T2-DM rats showed the mose significant increases in C-peptide level (0.12 ± 0.008 ng/ml) (P< 0.05) (Table 2).C-peptides, which are released from pancreatic β-cells during the biosynthesis of insulin, are an indicator of endogenous insulin production **(Landreh and Jörnvall, 2021).**

Table 2. Glucose and C-peptides levels in the different groups.

Groups	Glucose (mg/dl)	C-peptide (ng/ml)
Ctrl	89.87 ± 2.55 [e]	0.16 ± 0.013 [b,d]
T2-DM alone	295.23 ± 4.32 [a]	0.05 ± 0.014 [a]
T2-DM /Met	161.44 ± 3.15 [b]	0.09 ± 0.012 [d]
T2-DM CDE	157.25 ± 3.41 [b]	0.08 ± 0.009 [d]
T2-DM /Met/CDE	119.97 ± 2.66 [c]	0.12 ± 0.008 [d, e]

The values represented mean ± SD. **Ctrl.:** Control group; **T2-DM:** Diabetic group; **T2-DM/Met:** Diabetic group treated with metformin; **T2-DM/CDE:** Diabetic group treated with *C. droserifolia* extract; **T2-DM/Met/CDE**: Diabetic group treated with *C. droserifolia* extract. Means that do not share the same letter are significantly different (P-value< 0.05).

Co-treatment with CDE and Met improve liver functions in T2-DM rats

As indicated in Table (3), the obtained results illustrated that serum AST, ALT activities were significantly increased in T2-DM when compared to the corresponding values of negative control group. Treatment of T2-DM rats with Met or CDE led to significant decreases in ALT and AST activities when compared with T2-DM rats alone ($P< 0.05$). T2-DMrats showed significant decrease in the total protein levels (6.12 ± 0.16 g/dL) as compared to normal group (8.95 ± 0.19g/dL). However, treatment ofT2-DMrats with Met or CDE led to significant increase in the total protein levels ($P< 0.05$) as compared to T2-DM rats (Table 3). The liver is the vital organs involved in the regulation and homeostasis of blood glucose (**Röder *et al.,* 2016).** AST and ALT activities were important in the hepatic function diagnosing for various diseases **(Thakur *et al.*,2024).** Therefore, the presence of elevated transaminases can be an indicator of liver damage. Furthermore, **El-Khawaga *et al.*, (2010**) reported that diabetes-induced liver disorders which led to an increase in liver transaminases. Liver disorders in diabetes probably cause cell lysis resulting in release the of intracellular enzymes into the blood (**El-Khawaga *et al.,* 2010and Sayed-Ahmed *et al*., 2020).**

These results are in accordance with previous study by **Ahmed *et al*. (2001)** whoreported that the treatment with CDE resulted in significant dose-dependent decreases in the activities of liver enzymes. (**AbouHaleka *et al.* (2023)** reported that CDE pre-treatment significantly decreased AST, ALT, and alkaline phosphatise (ALP) of rats exposed to adrenaline. The hepatoprotective effect of CDE may be due to its high amount of active secondary metabolites including phenolics, carotenoids, flavonoids, anthocyanins, polysaccharides, terpenoids, triterpenoids, alkaloids and glycosides (**Muhaidat *et al.,* 2015; Korkor *et al.,* 2022 and Elhassaneen *et al.,* 2024).**This effect of the phytochemical compounds of CDE may be due to their antioxidant and scavenging properties, inhibition of lipid oxidation, consequentially, they plays an important role in protecting the liver from many complications resulting from many diseases including diabetes (**AbdElalal *et el.,* 2022; El-Hawary, 2023; Mahmoud, 2023).**With the same context, many studies indicated that plant parts contain phenolics, carotenoids, anthocyanins, polysaccharides and terpenoids such as those present in CDE, which demonstrated protection against liver injuries induced by

toxic chemicals (**Mahran and Elhassaneen, 2023; Elhassaneen and Mahrran, 2024).** Recently, **Ismail *et al.* (2025)** confirmed hepatoprotective effect of CDE.

Table 3.ALT, AST activities, and total proteins levels in the different groups

Groups	ALT (U/L)	AST (U/L)	Total protein (g/dL)
Ctrl	34.55 ± 2.72^{b}	$187.21 \pm 7.41^{b,c}$	$8.95 \pm 0.19^{a,b,c}$
T2-DM alone	56.72 ± 2.79^{a}	227.14 ± 8.93^{a}	6.02 ± 0.16^{a}
T2-DM /Met	$44.65 \pm 3.41^{a,b}$	$208.23 \pm 10.44^{b,c}$	7.32 ± 0.29^{c}
T2-DM CDE	$42.52 \pm 2.21^{a,b}$	$201.75 \pm 9.65^{b,c}$	$8.02 \pm 0.20^{b,c}$
T2-DM /Met/CDE	39.60 ± 1.96^{b}	$195.15 \pm 8.76^{b,c}$	$8.56 \pm 0.21^{b,c}$

The values represented mean ± SD. **Ctrl.:** Control group; **T2-DM:** Diabetic group; **T2-DM/Met:** Diabetic group treated with metformin; **T2-DM/CDE:** Diabetic group treated with *C. Droserifolia* extract; **T2-DM/Met/CDE**: Diabetic group treated with *C. droserifolia* extract. **ALT**: Alanine transaminase; **AST**: Aspartate transaminase. The means that do not share the same letter are significantly different (*P*-value < 0.05).

Co-treatment with CDE and Met improved hepatic antioxidants

The obtained results from table (4) showed that mean values of hepatic antioxidants enzymes were MDA, SOD and CAT. It was noticed that MDA level was significantly increased in the T2-DM group (76.54 ± 3.18nmol/mg tissue) as compared to normal group (36.94 ± 2.92 nmol/mg tissue). The serum level of MDA in the treated diabetic groups decreased after Met or CDE administration. T2-DM that receiveda combination Met/CDE showed a marked decrease in their MDA levels up to 47.26 ± 3.54 nmol/mg tissue compared to T2-DM group alone ($P< 0.05$).

Diabetes is accompanied by a decreased level of antioxidant enzymes SOD and CAT.The T2-DM group showed a significant decrease in their SOD and CAT activities (6.95 ± 0.57 and 54.39 ± 3.18 U/mg tissue, respectively) when compared to the normal control group (15.62 ± 1.14 and 88.59 ± 3.26 U/mg tissue, respectively) ($P< 0.05$) (Table 4). The antioxidant effect of Met and/or CDE administration showed significant increase in the SOD and CAT activities of the treated T2-DM rats. T2-DM group that was co-treated with Met/CDE showed notable increase in their SOD and CAT activities (12.75 ± 1.04 and 80.64 ± 3.79 U/mg tissue, respectively) compared to the T2-DM alone ($P< 0.05$) (Table 4). Additionally, rats' serum MDA level was reduced in a dose-dependent manner by CDE therapy. These findings are mostly in line with a number of earlier investigations (**El-Khawaga *et al.*, 2010; Arafa, 2021 and Elhassaneen *et al.* 2024).**

Increased lipid peroxidation disrupts the functions of the cell membrane by decreasing membrane fluidity, which changes the activities of enzymes and receptors that are boun d to the membrane **(Baynes, 1991).** These lipid peroxidation products are extremely cyt otoxic and react with the cell of organelles like mitochondria, lysosomes, and cell wall membrane (**Badawy, 2017**). In the same context, **Grune *et al.* (1997)** found

that MDA is a modulator of signal transduction pathway that disrupt cellular functions in the same setting.

Table 4. Activities MDA, SOD, and CAT in the different groups

Groups	MDA (nmol/mg tissue)	SOD (U/mg tissue)	CAT (U/mg tissue)
Ctrl	36.94 ± 2.92^{c}	$15.62 \pm 1.14^{a,b}$	88.59 ± 3.26^{b}
T2-DM alone	76.54 ± 3.18^{a}	6.95 ± 0.57^{d}	54.39 ± 3.18^{e}
T2-DM/Met	60.27 ± 2.82^{b}	$9.78 \pm 0.83^{c,d}$	66.95 ± 3.64^{d}
T2-DM/CDE	$53.67 \pm 2.34^{b,d}$	$10.68 \pm 0.76^{b,c}$	$71.27 \pm 3.25^{c,d}$
T2-DM/Met/CDE	$47.26 \pm 3.54^{c,d}$	$12.75 \pm 1.04^{a,b}$	$80.64\ 3.79^{b,c}$

The values represented mean ± SD. **Ctrl.:** Control group; **T2-DM:** Diabetic group; **T2-DM/Met:** Diabetic group treated with metformin; **T2-DM/CDE:** Diabetic group treated with *C. droserifolia* extract; **T2-DM/Met/CDE**: Diabetic group treated with *C. droserifolia* extract. **MDA**: Malondialdehyde; **SOD**: Superoxide dismutase; **CAT**: Catalase. The means that do not share the same letter are significantly different (P-value < 0.05).

The obtained results in table 4 revealed activities of antioxidant enzymes (MDA, SOD and CTA). MDA activity is the highest in T2-DM alone group, suggesting elevated oxidative stress as compared to control group. SOD and CAT activities are lowest in the T2-DM alone group, indicating impaired antioxidant defenses. Treatment with Metformin (T2-DM/Met), CDE (T2-DM/CDE), and combined Metformin + CDE (T2-DM/Met/CDE) improved antioxidant enzyme activities and reduced oxidative stress compared to T2-DM alone. T2-DM/Met/CDE group showed the most significant improvement, nearly approaching the control values, suggesting a synergistic or additive therapeutic effect.

The current findings align with previous studies that highlight increased oxidative stress in T2-DM, reflected by elevated MDA levels and decreased activities of antioxidant enzymes like SOD and CAT (**Maritim *et al.,* 2003).** The significant reduction of MDA and the restoration of SOD and CAT activities following Met treatment are consistent with reports demonstrating Metformin's antioxidative effects beyond its glucose-lowering action (**Viollet *et al.,* 2012).** Moreover, the use of CDE independently and in combination with Met appears to enhance antioxidant defenses further, in agreement with studies on phytochemicals that reveal their potent antioxidative properties (**Lobo *et al.,* 2010).** The synergistic effect observed in the T2-DM/Met/CDE group supports earlier evidence that combining conventional drugs with plant-based therapies can lead to superior therapeutic outcomes by simultaneously targeting different pathogenic mechanisms (**Yuan *et al.,* 2016).**The near-normalization of oxidative stress markers in the T2-DM/Met/CDE group indicates that the combined therapy not only mitigates oxidative stress but also likely offers broader protective

effects against diabetes-related complications, corroborating findings from integrative therapeutic approaches in diabetic models (**American Diabetes Association, 2022**). Recently, **Ismail *et al.*, (2025)** found that by enhancing insulin sensitivity and lowering oxidative stress in diabetic rats, CDE therapy of the alloxan diabetic rats eliminated some metabolic abnormalities brought on by diabetes in many cells by lowe ring ROS (H2O2), MDA production, and the hypoglycaemic impact.

Treatment with CDE/Met improves hepatic histopathological changes in T2-DM rats

The histopathological investigations of liver sections from the normal control group showed normal hepatocytes with blood sinusoids and centrally located nuclei (Figure 2A). The liver section of T2-DM rats displayed a congested and dilated central vein; some degenerated hepatocytes with vacuolated cytoplasm and nuclear changes (Figure 2B). Liver section of T2-DM groups that were treated with Met or/and CDE exhibited an improvement in the hepatic architectures that represented by regular central vein, with fewer congestion, and less cellular infiltrations (Figure 2C-E).

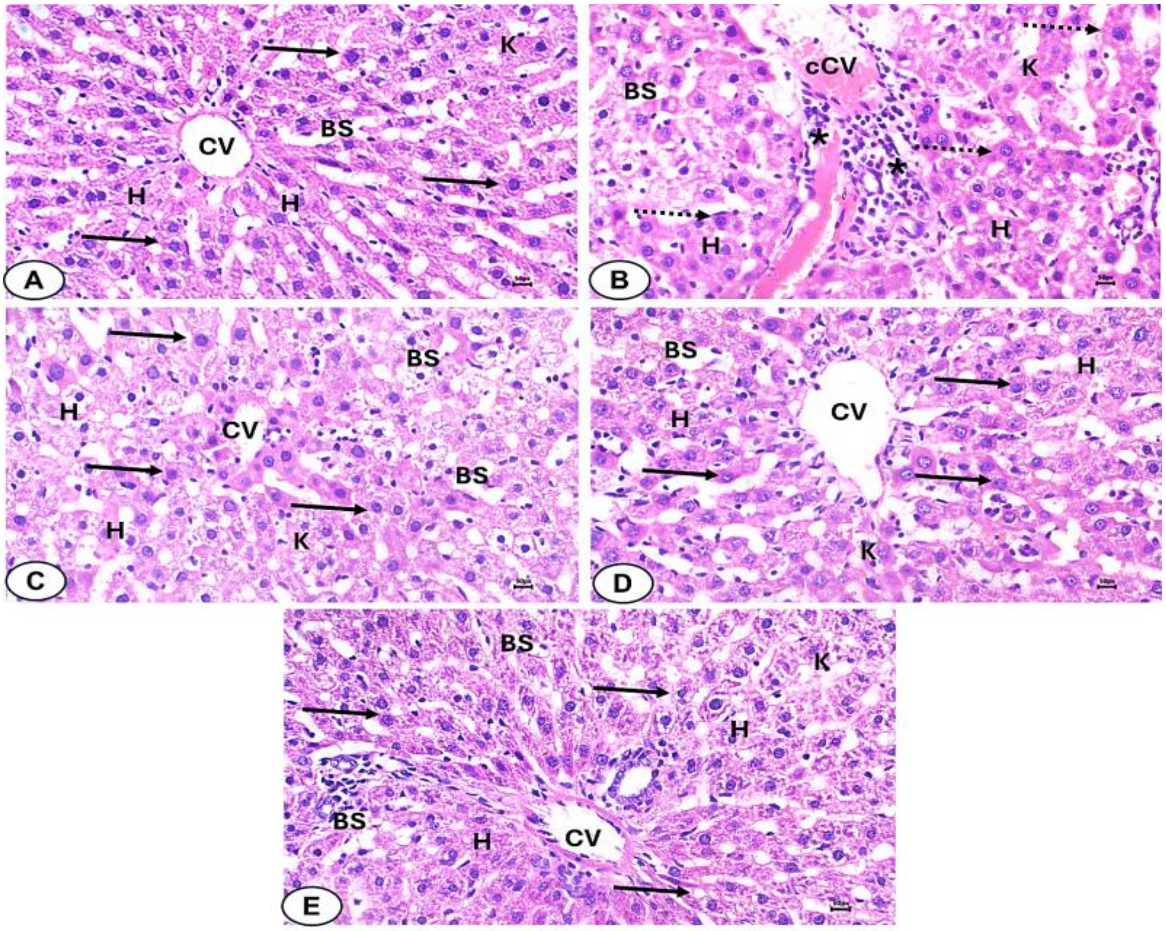

Figure 2.(A) Photomicrograph of liver section of **Ctrl:**Normal control group shows normal hepatic structure; central veins (CV), hepatocytes (H), with normal blood sinusoids (Bs) nucleus (arrows), and Kupffer cells (K) **(B)** Liver section of **T2-DM** group shows disorganization of hepatic tissues, congested and dilated (cCV), cellular infiltrations (*), and pyknotic nuclei (dotted arrows). **(C)** Liver section of **T2-DM/Met** group shows improvement in the hepatic organization with fewer congestion. **(D)**Liver section of **T2-**

DM/CDE group shows improvement in the hepatic structures with fewer congestion.**(E)** Liver section of **T2-DM/Met/CDE** group shows significant improvement in the hepatic architectures, less cellular infiltrations, and almost with normal Kupffer cells (X 400).

In silico study

Molecular docking analysis of *C. droserifolia* phytochemicals with anti-diabetic targets revealed significant interactions between various phytochemicals and key proteins involved in glucose homeostasis and insulin signaling pathways. The molecular interaction analysis demonstrates that rutin binds within the glucose transport channel of GLUT4, potentially modulating glucose uptake into cells, which is a critical mechanism for maintaining glucose homeostasis (Figure 3). These interactions with IRS suggest that Chlorogenic acid may enhance insulin signaling by stabilizing or modulating IRS activity, which is crucial for proper insulin-mediated glucose metabolism (Figure 4). The interaction profile suggests that Quercetin may modulate PI3K activity, potentially enhancing the insulin signaling cascade and promoting glucose uptake (Figure 5). The extensive interaction network between Rutin and PPAR suggests potential modulation of PPAR activity, which could enhance insulin sensitivity and lipid metabolism (Figure 6).

The phytochemicals in Cleome droserifolia exhibit potential anti-diabetic effects by modulating key proteins involved in glucose homeostasis and insulin signaling, as revealed by molecular docking studies: Rutin showed the strongest binding affinity (-9.1 kcal/mol), stabilizing GLUT4's active conformation via hydrogen and hydrophobic interactions (e.g., LYS50, TRP81), potentially enhancing glucose transport. Rutin also bound strongly to PPAR (-7.0 kcal/mol), forming hydrogen bonds with residues (e.g., CYS749, ARG751), suggesting agonist activity to enhance genes involved in glucose/lipid metabolism and insulin sensitivity. Compounds like rutin target multiple pathways (GLUT4, PPAR), while others (Chlorogenic Acid, Quercetin) enhance insulin signaling (IRS, PI3K). This multi-target approach addresses diabetes complexity by improving glucose uptake, insulin sensitivity, and metabolic regulation. Rutin is one such plant flavonoid that has anti-diabetic activity induced via reducing the expression of insulin-resistant molecules, enabling the interaction between insulin and its receptors, inducing adipose tissue peroxisome proliferator-activated receptor (PPAR) expression and adipocyte-derived protein production, according to studies through molecular and physiological mechanisms **(Savych and Milian, 2021). Chen *et al.* (2020)** demonstrated that rutin-containing formulations significantly improved serum insulin and C-peptide concentrations, hexokinase, hyperlipidemia, liver glycogen content, hyperglycemia, glucose-6-phosphatase, and glycogen phosphorylase activities in type 2 diabetes mellitus (DM) rats that were orally treated with rutin.

Other compounds like ellagic acid, rosmarinic acid, and chlorogenic acid, also strongly bind to GLUT4, suggesting synergistic enhancement of glucose uptake. Chlorogenic acid boundstrongly to IRS (-7.5 kcal/mol), stabilizing critical residues (e.g., LYS21, ASP236), which may protect IRS phosphorylation and improve insulin sensitivity. These results are in accordance with previous study that reported that ellagic acid has antidiabetic effect **(Amor *et al.*, 2020).** Additionally, **Inui *et al.*, (2016)**reported that rosmatic acid enhances insulin sensitivity by reducing glucose levels through upregulation of GLUT4 and downregulation of phosphoenolpyruvate

carboxykinase (PEPCK), The uptake of glucose in muscles can be boosted by treating L6 muscle cells with rosmarinic acid through the activation of AMPK Similarly, Chlorogenic Acid was reported which improved insulin sensitivity **(Chen *et al.*, 2019)** It also reduced the decrease in IRS-1 expression caused by high insulin concentration, prevented inactivation of the PI3K/Akt pathway, and also prevented GLUT4 level reduction observed after high glucose exposure. These results are consistent with those of **(Liang *et al,* 2013).** Also, the obtained results showed that Quercetin exhibited the highest affinity for PI3K (-6.8 kcal/mol), interacting with catalytic domain residues (e.g., SER977), potentially amplifying insulin signaling to activate GLUT4 translocation.

These results are in line with previous study that reported Quercetin also induces the AMPK activity in hepatocytes and inhibits glucose 6 phosphatase (Eid et al., 2015). AMPK and CaMKII are key signalling molecules that regulate cellular GLUT4 expression. Exercise is also a potent stimulator of GLUT4 expression which improves insulin action and muscle glycogen storage (**Dhanya, 2022).**

The combined action of these phytochemicals aligns with traditional use of *C. droserifolia* extracts, offering a holistic strategy to counteract core diabetic pathologies: impaired glucose uptake, insulin resistance, and dysregulated metabolism. The phytochemicals in *C. droserifolia* demonstrate a promising multi-mechanistic and synergistic approach to diabetes management, potentially surpassing single-target therapies by addressing glucose transport, insulin signaling, and metabolic gene regulation simultaneously.

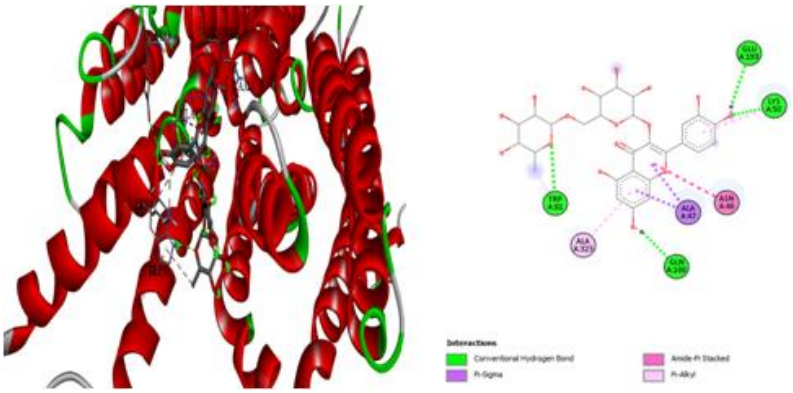

Figure (3): Mechanism of interaction 3D and 2D of Glut4

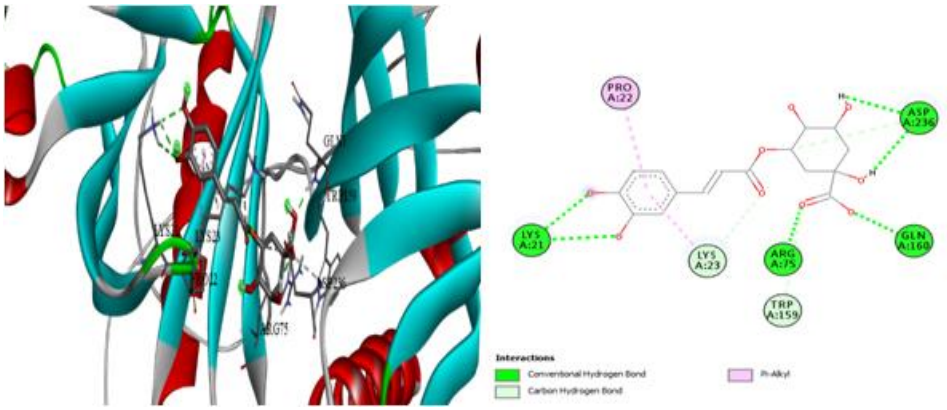

Figure (4): Mechanism of interaction 3D and 2D of IRS

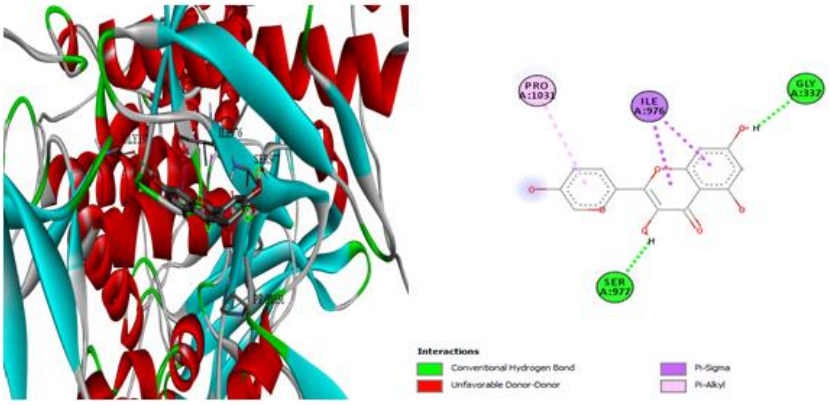

Figure (5): Mechanism of interaction 3D and 2D of PI3K

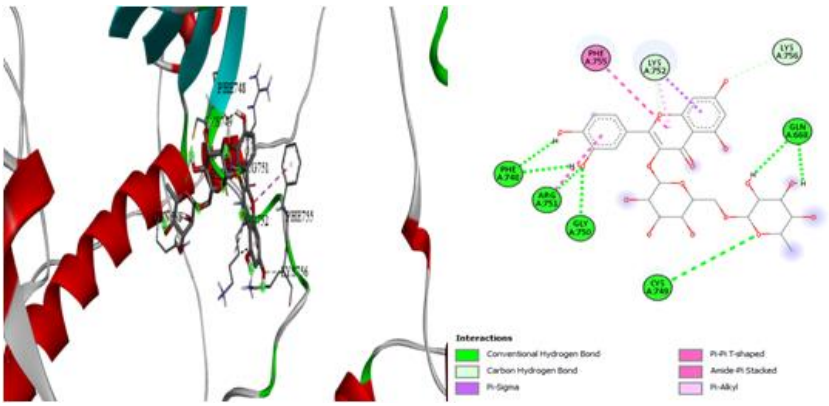

Figure (6): Mechanism of interaction 3D and 2D of ppra

REFERENCES

AbdElalal, N. S.; Elsemelawy, S. A. and Elhassaneen, Y. A. (2022). Potential Effects of Wild Milk Thistle (Silybummarianum L) Seed Extract Intervention on Oxidative Stress Induced by Busulfan Drug in Different Organs of Rats. International Journal of Healthcare and Medical Sciences, 8(3): 19-34.

Abdel Maksoud, H.A.; AbouZaid, O.A. Elharrif, M. G. and Omnia, M. (2020). Selenium Cleome droserifolia nanoparticles (Se-CNPs) and it's ameliorative effects in experimentally induced diabetes mellitus. Clin.Nutr., 40: 383-391.

Abdelfattah, E.; Rizk, M., Elregal, N. and Amin, A. (2019). Antidiabetic activity of callus extract of Cleome- droserifolia in rats. J. Mater. Environ. Sci., 10 (11): 1083-1097.

Aboraya, A. O.; Elhassaneen, Y. A. and Nassar. O. M. (2022). Reishi Mushroom (Ganoderma lucidum) intervention improves lipids profile and paraoxonase/arylesterase activities in serum as well as enhances haemostatic effects in streptozotocin-induced diabetic rats. Alex. Sci. Exch.J.43. (4): 593-608.

AbouHaleka, S.A.; Rashwan, H.M.; Ebaid, H. M.; Gad El Hak, H.N.; Alkadri, D. and Abdelrazek, H. M.A. (2023). The Reno and Hepatoprotective Effects of SAMWA Plant (Cleome droserifolia) Methanolic Extract against Adrenaline-Induced Adverse Effect to Male Rats. Journal of Advanced Veterinary Research; 13(10): 2085-2089.

Aebi, H. (1984). Catalase in vitro. Methods Enzymol 105:121-126.

Ahmed, I.; Lakhani, M. S.; Gillett, M.; John, A. and Raza, H. (2001). Hypotriglyceridemic and hypocholesterolemic effects of antidiabeticMomordicacharantia fruit extract in streptozotocin induced diabetic rats.Diabetes Res. Clin. Pract. 51(3): 155-161.

Alrasheedi, A. A.; Basnawi, A.I. andAlthaiban, M. A. (2024). Effects of Spirulinaplatensis microalgae on renal function and antioxidant defense in rats with streptozotocin-induced diabetes.Journal of Functional Foods.;122, 106485.

American Diabetes Association. (2022). Standards of medical care in diabetes—2022.Diabetes Care, 45(Supplement_1), S1-S264.

American Diabetes Association. (2023). Standards of medical care in diabetes—2023.Diabetes Care, 46(Supplement_1), S1–S291.

Amor AJ, Gómez-Guerrero C, Ortega E, Sala-Vila A, Lázaro I. (2020). Ellagic Acid as a Tool to Limit the Diabetes Burden: Updated Evidence. Antioxidants (Basel). 3;9(12):1226.

Arafa, S. G. M. (2021). Chemical and Biological studies on Extracts of periwinkle (Catharanthusroseus L.) ". Ph.D. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt.

Badawy, R. M. H. (2017). The effect of phytochemical extracts of some plant parts in liver cancer initiation induced by benzo(a)pyrene ". Ph.D. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.

Bancroft, J.and Gamble, M. (2008). Theory and Practice of Histological Techniques; Elsevier Health Sciences: Philadelphia, PA, USA.

Baynes, J.W. (1991). Role of oxidative stress in development of complications in diabetes. Diabetes, 40: 405-412.

BIOVIA Discovery Studio (2020). DassaultSystèmes BIOVIA, San Diego, CA, USA

Carpéné, C.; StiliyanovAtanasov, K.; Les, F. andMercaderBarcelo, J. (2022). Hyperglycemia and reduced adiposity of streptozotocin-induced diabetic mice are not alleviated by oral benzylamine supplementation. World J Diabetes. Sep 15;13(9):752-764.

Chen L.; Teng H. and Cao H. (2019). Chlorogenic acid and caffeic acid from Sonchus oleraceus Linn synergistically attenuate insulin resistance and modulate glucose uptake in HepG2 cells. Food and Chemical Toxicology.;127:182–187.

Chen, L.Y.; Huang, C.N.; Liao, C.K.; Chang, H.M.; Kuan, Y.H.; Tseng, T.J.; Yen, K.J.; Yang, K.L.; Lin, H.C. (2020). Effects of rutin on wound healing in hyperglycemic rats. Antioxidants, 9, 1122.

Dhanya, R. (2022). Quercetin for managing type 2 diabetes and its complications, an insight into multitarget therapy,Biomedicine & Pharmacotherapy, 146, 112560.

Elhassaneen, Y. A. and Mahran, M. Z. (2024). Potential Protective Effects of Milk Thistle (SilybumMarianum L.) Seeds Against Benzo[a]Pyrene-Induced Hepatic and Nephritic Injuries in Rats: Biochemical and Histopathological Studies. Alex. Sci.Exch.J. 45, (1): 131-152.

Elhassaneen, Y. A.; ElBassouny, G. M.; Emam, O. A. and Ismail, S. I. (2024). Potential Effects of Samwa (*Cleome Droserifolia*) Ethanol Extract on Hyperglycemia, Oxidative Stress and Inflammation in Diabetic Rats Induced by Alloxan. American Journal of Medical and Biological Research, 12(1): 13-26.

El-Hawary, E. M. M. (2023). Studies on the potential effects of brown algae (Sargassumsubrepandum) on hyperglycemia and oxidative stress induced by alloxan in diabetic rats ", MSc. Thesis in Nutrition and Food Science, Faculty of Specific Education, Benha University, Benha, Egypt.

El-Khawaga, O.; Abou-Seif, M.A.; El-Waseef, A.and Negm, A.A., (2010). Hypoglycemic, hypolipidemic and antioxidant activities of Cleome droserifolia in streptozotocindiabetic rats. Journal of Stress Physiology and Biochemistry 6, 28–41.

El-Naggar, S. A.; Basyouny, M. A.; Amin, S. E. and Elwan, M. (2023). Phoenix dactylifera seeds extract ameliorates the hepato-renal toxicities that induced b cyclophosphamide in male mice. Biological and Biomedical Journal, 1(1), 1-10.

El-Said, K. S.; Hussein, S.; Alrashdi, B. M.; Mahmoud, H.A.; Ibrahim, H. A.; Elbakry, M.; El-Tantawy, H.; Kabil, D.I. and El-Naggar, S. A. (2022). Musa sp.Leaves Extract Ameliorates the Hepato-Renal Toxicities Induced by Cadmiumin in Mice. Molecules; 27 (2), 559.

Foretz, M.; Guigas, B.; Bertrand, L.; Pollak, M. and Viollet, B. (2014). Metformin: From mechanisms of action to therapies. *Cell Metabolism*, 20(6), 953–966.

Greenwell, M.and Rahman, P. (2015). Medicinal Plants: Their Use in Anticancer Treatment. Int. J. Pharm. Sci. Res., 6(10), 4103. DOI: 10.13040/IJPSR.0975-8232.6(10).4103-12.

Grune, T.; Siems, W. G. and Petras, T. A. (1997). Identification of metabolic pathways of the lipid peroxidation product, 4-hydroxynonenal, in situ perfused rat kidney. J Lipid Res. 38(8): 1660-1665.

Guo, H.; Chi, J. and Xing, Y. (2011). Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. Indian J. Med. Res; 129: 279–284.

Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E. and Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. Journal of Cheminformatics, 4(1), 1-17.

Hao, H.; Jung, H.; Chen, L.; Chia, C.; Yueh, L.and Jang, S. (2018). Effect of metformin on kidney function in patients with type 2 diabetes mellitus and moderate chronic kidney disease. Oncotarget.; 9(4):5416-23.

Hashem, N.M. and M.G. Shehata. (2021). Antioxidant and Antimicrobial Activity of Cleome droserifolia (Forssk.) Del. and Its Biological Effects on Redox Status, Immunity, and Gut Microflora. Animals: an open access journal from MDPI., 11(7), p:1-14

Harding, J.L.; Pavkov, M. E.; Magliano, D. J.; Shaw, J. E. and Gregg, E. W. (2019). Global trends in diabetes complications: a review of current evidence. *Diabetologia* 62(1):3–16.

Hashem, N.; Soltan, Y.; El-Desoky, N.; Morsy, A. andSallam, S. (2019). Effects of *Moringa oleifera* extracts and monensin on performance of growing rabbits. Livest. Sci.; 228:136–143.

Hashem, N. M., and Shehata, M. G. (2021). Antioxidant and Antimicrobial Activity of *Cleome droserifolia* (Forssk.) Del. and Its Biological Effects on Redox Status, Immunity, and Gut Microflora. Animals: an open access journal from, 11(7), 1929.

Hsu, W. H.; Hsiao, P. J.; Lin, P. C.; Chen, S. C.; Lee, M. Y.and Shin, S. J. (2017). Effect of metformin on kidney function in patients with type 2 diabetes mellitus and moderate chronic kidney disease.Oncotarget. 17;9(4):5416-5423.

Hundal, R. S.;Krssak, M.;Dufour, S.; Laurent, D.; Lebon, V.; Chandramouli, V.; Inzucchi, S. E.; Schumann, C. W.; Petersen, . F.; Landau R. B. and Shulman, I. G. (2000): Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* 49, 2063–2069.

IDF, International Diabetes Federation (2021). IDF Diabetes Atlas 2021, 10th edition scientific committee.

Inui, A.; Cheng, K.-. C.; Asakawa, A.; Amitani, H.; Amitani, M.; Morinaga, A.; Takimoto, Y.; Kairupan, B. H. R. and Runtuwene, J. (2016). Rosmarinic acid ameliorates hyperglycemia and insulin sensitivity in diabetic rats, potentially by modulating the expression of PEPCK and GLUT4. Drug Design, Development and Therapy, 10, 2193–2202.

Ismail, S. A. E. (2022). Potential effects of Al-Samwa (Cleome droserifolia) extract on hyperglycemia and oxidative stress in diabetic rats induced by alloxan", MSc. Thesis in Nutrition and Food Science, Faculty of Specific Education, Benha University, Benha, Egypt

Ismail, N. S.; Elhassaneen, Y. A. and Gouda, D. O. (2025). Oxidative Stress, Hyperglycemia, Hyperlipidemia, and Hemostasis Impairment as Risk Factors for Diabetes in Rats: Investigating the Therapeutic Potential of Samwa (*Cleome droserifolia*). Alexandria Science Exchange Journal,46, (1) 57-80.

Jones, A. G. and Hattersley, A. T. (2013). The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med; 30:803–17.

Jörns, A.; Akin, M.; Arndt, T.; Terbish, T.; ZuVilsendorf, A. M.; Wedekind, D.; Hedrich, H. J. and Lenzen S. (2014). Anti-TCR therapy combined with fingolimod for reversal of diabetic hyperglycemia by β cell regeneration in the LEW.1AR1-iddm rat model of type 1 diabetes. J Mol Med (Berl). Jul;92(7):743-55.

Khan, M. A. B.; Hashim, M. J.; King, J. K.; Govender, R. D.; Mustafa, H. and Al Kaabi J. (2020). Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. J. Epidemiol Glob Health. Mar;10 (1):107-111.

Knowler, W. C.; Barrett-Connor, E.; Fowler, S. E.; Hamman, R. F.; Lachin, J. M.; Walker, E. A. and Nathan, D. M. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or Metformin. *The New England Journal of Medicine*, 346(6), 393–403.

Korkor, A. M.; Mansourm, A. M. and Abbass. H.S. (2022). Evaluation of Antidiabetic and Anti-Obesity Potential and Safety of A Poly Herbal Remedy. Az. J. Pharm Sci., 65. 229:245.

Kotz, S.; Balakrishnan, N.; Read, C. B. and Vidakovic, B. (2006) Encyclopedia of statistical sciences.2nd ed. Hoboken, Wiley-Inter. science, N.J.

Krajewski, W.; Bolanowski, M.; Krecicki, T. and Zatoński, T. (2016). "Diabetes and cancer: a review of current knowledge," Experimental and Clinical Endocrinology and Diabetes, vol. 124, no. 5, pp. 263–275.

Landreh, M.andJörnvall, H. (2021). Biological activity versus physiological function of proinsulin C-peptide. Cellular and Molecular Life Sciences, 78(3), 1131-1138.

Li, X. Y. and Chow, C. K (1994). An improved method for the measurement of malondialdehyde in biological samples. Lipids 29:73-75.

Liang X. C., Meng W., Zhong Y. L., Huang L., He W. Y., Wang Z. (2013). Effects of chlorogenic acid on mouse insulin resistance development induced by high fat emulsion. Chinese Pharmacological Bulletin.;29(5):654–658.

Liu, Y.; Yang, X.; Gan, J.; Chen, S.; Xiao, Z. X. and Cao, Y. (2022). CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic acids research, 50(W1), W159–W164

Lobo, V.; Patil, A.; Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews, 4(8), 118-126.

Maaliah, M.S.; Haddadin, M. and Abdalla, S. (2024). Hypolipidemic and hypoglycemic effects of Silybummarianum (L.) Gaertn. (Milk Thistle) Ethanol Seed Extract in Streptozotocin-induced Diabetes in Rats. Pharmacognosy Magazine 20(3) 841–852

Mahmoud, S. A. E. (2023). Effect of some Phyto-extracts on hyperglycemia and its complications in alloxan-induced diabetic rats ". Ph.D. Thesis in Nutrition and Food Science, Faculty of Specific Education, Port Saied University, Port Saied, Egypt.

Mahran, M. Z. andElhassaneen, Y.A. (2023). Attenuation of Benzo[a]pyrene-Induced Oxidative Stress and Cell Apoptosis in Albino Rats by Wild Milk Thistle (SilybumMarianum L.) Seeds Extract. Egypt. J. Chem. 66 (SI: 13): 1671 - 1687.

Maksoud, H.A.; Zaid, O.A.A.; Elharrif, M.G.; Omnia, M. and Alaa.E. (2020). Selenium *Cleome droserifolia* nanoparticles (Se-CNPs) and its ameliorative effects in experimentally induced diabetes mellitus. Clin.Nutr. ESPEN. 40: 383–391.

Miliauskas G.; Venskutonis P.R. and Van Beek T.A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem.; 85:231–237.

Maritim, A. C.; Sanders, R. A., and Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: A review. Journal of Biochemical and Molecular Toxicology, 17(1), 24-38.

Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S. and Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry, 30(16), 2785-2791.

Motaal, A.; Ezzat, S. and El-askary, H. (2014). Antihyperglycemic Activity and standardization of the bioactive extract of Ceomedroserifolia growing in Egypt. Pharmacogn. J.; 6:15–21.

Moustafa, A. A. and Mahmoud, M. A. (2023). Importance of *Cleome droserifolia* as an endangered medicinal plant species in the Sinai Peninsula and the need for its conservation. Advancement in Medicinal Plant Research, 11(3): 43-51.

Moustafa, A.; Sarah, R.; Qiqa, S.; Mansour, S. and Alotaibi, M. (2019). *Cleome droserifolia*: An Egyptian natural heritage facing extinction. Asian J. Plant Sci. Res.; 9:14–21.

Muhaidat, R.; Al-Qudah, M.A.; Samir, O.; Jacob, J.H.; Hussein, E.; Al-Tarawneh, I. N.; Bsoul, E. and Abu Orabi, S.T. (2015). Phytochemical investigation and in vitro antibacterial activity of essential oils from *Cleome droserifolia* (Forssk.) Delileand C. trinervia Fresen. (Cleomaceae) South African Journal of Botany., 99: 21- 28.

Nuckols, T. K.; Keeler, E.; Anderson, L. J. Green, J.; Morton, O. S.; Doyle, J. B.; Shetty, K.; Arifkhanova, A.; Booth, M.; Shanman, R. and Shekkelle, P. (2018). Economic evaluation of quality improvement interventions designed to improve glycemic control in diabetes: a systematic review and weighted regression analysis, Diabetes Care, 41 ,985.

Palsamy, P. and Subramanian, S. (2008). Resveratrol, a natural phytoalexin, normalizes hyperglycemia in streptozotocin nicotinamide induced experimental diabetic rats. Biomed Pharmacother; 47:1–8.

Prior, R. L., Wu, X. and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of agricultural and food chemistry, 53(10), 4290-4302.

Rei, R. (1984). Measurement of aminotransferase: Part I. Aspartate aminotransferase. CRC Crit. Rev. Clin Lab Sci 21:99-186.

Röder, P. V.; Wu, B.; Liu, Y. and Han W. (2016). Pancreatic regulation of glucose homeostasis. Exp Mol Med. Mar 11;48(3): e219.

Savych, A. and Milian, I. (2021). Total flavonoid content in the herbal mixture with anti-diabetic activity. Pharmacology On Line, 2, 68–75. 43

Sayed-Ahmed, S.; Shehata, N. and Y. Elhassaneen. (2020). Potential Protective Effects of Ganodermalucidum Powder against Carbon Tetrachloride Induced Liver Disorders in rats: Biological, Biochemical and Immunological Studies, Egypt. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt 56(2): 99-132.

Shi, G.J.; Zhou, J.Y.; Zhang, W. J.; Gao, C.Y.; Jiang, Y. P, Zi, Z.G.; Zhao, H. H.; Yang, Y. and YU, J.Q. (2018). Involvement of growth factors in diabetes mellitus and its complications: a general review, Biomed. Pharmacother., 101, 510.

Simos, Y.V.; Spyrou, K.; Patila, M.; Karouta, N.; Stamatis, H.; Gournis, D.; Dounousi, E. and Peschos, D. (2020). Trends of nanotechnology in type 2 diabetes mellitus treatment. Asian J Pharm Sci. Jan;16(1):62-76.

Sun, Y.L.W. and Oberley, Y.L.I. (1988). Simple method for clinical assay of superoxide dismutase, Clin. Chem., 34, 497–500.

Thomas, L. (1998). Clinical Laboratory Diagnostics. 1st ed. Frankfurt: Verlagsgesellschaft. 27:644-647.

Tietz, N. W., (1995). Clinical guide to laboratory tests. 3rd Ed. Philadelphia: WB Saunders: 268-273.

Tillman, L.; Tabish, T.A.; Kamaly, N.; Moss, P.; El-briri, A.; Thiemermann, C.; Pranjol, M. Z.I. and Yaqoob, M.M. (2022). Advancements in nanomedicines

for the detection and treatment of diabetic kidney disease, Biomaterials and Biosystems, 6 ,100047.

Thakur, S., Kumar, V., Das, R., Sharma, V., & Mehta, D. K. (2024). Biomarkers of hepatic toxicity: an overview. Current Therapeutic Research, 100737.

Trott, O. and Olson, A. J. (2010). AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455-461.

Tran N.; Pham, B. and Le, L. (2020). Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. Biology (Basel). 28;9(9):252.

Umpierre, D.; Ribeiro, P. A. B.; Schaan, B. D. and Ribeiro, J. P. (2011). Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: A systematic review and meta-analysis. *JAMA*, 305(17), 1790–1799**.**

Vial, G.; Detaille, D. and Guigas, B. (2019). Role of mitochondria in the mechanism(s) of action of metformin. *Front. Endocrinol.* 10, 294.

Viollet, B., Guigas, B., Garcia, N. S., Leclerc, J., Foretz, M., &Andreelli, F. (2012). Cellular and molecular mechanisms of metformin: An overview. Clinical Science, 122(6), 253-270.

Vlavcheski, F.; Naimi, M.; Murphy, B.; Hudlicky, T. Tsiani, E. (2017). Rosmarinic acid, a rosemary extract polyphenol, increases skeletal muscle cell glucose uptake and activates AMPK. Molecules, 22(10), 1669.

Wickramasinghe, A. S. D.; Attanayake, A. P. and Kalansuriya, P. (2022). Biochemical characterization of high fat diet fed and low dose streptozotocin induced diabetic Wistar rat model. Journal of Pharmacological and Toxicological Methods.113.

Xiao, J. (2022). Recent advances on the stability of dietary polyphenols. E Food. 3:(21):1-7.

Yadav, K. (2024). Nanotechnology in diabetes management: Revolutionizing treatment and diagnostics. Journal of Molecular Liquids, 126117.

Yang, D.K. and Kang, H. S. (2018). Anti-Diabetic Effect of Co treatment with Quercetin and Resveratrol in Streptozotocin-Induced Diabetic Rats. BiomolTher (Seoul). 1;26(2):130-138.

Yuan, H.; Ma, Q.; Ye, L. and Piao, G. (2016). The traditional medicine and modern medicine from natural products. Molecules, 21(5), 559.

Zafar, M. and Naqvi, S.N.; (2010). Effects of STZ-Induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. Int. J. Morphol., 28(1):135-142.

Zhishen, J.; Mengcheng, T. and Jianming W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem.; 64:555–559.