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Anti- diabetic activity of new Synthesized Flavonoid compound

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ABSTRACT

Background: Diabetes mellitus (DM) is associated with long-term damage, dysfunction, of various organs. **Objectives**: Study aims to assess the role of new Synthesized Flavonoid compound on experimentally induced diabetes. **Methods**: 50 adult male albino rats divided into 5 groups. Group 1 (control group, rats were orally administered with 1 ml saline daily). Group 2 (DMSO group, rats were orally administered with 0.2 % DMSO for 60 day orally). Group 3 (positive control, animals were injected intraperitoneally with 60 mg/kg b.wt streptozotocin followed by intraperitoneal injection with 120 mg/kg b.wt of Nicotinamide after 15 minute).Group 4 (standard group, diabetic animals treated with 100 mg/kg b.wt of metformin for 60 day orally). Group 5 (therapeutic group, diabetic rats treated with 50 mg /kg b.wt of Ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate for 60 day orally). At the end of experimental period blood serum & plasma samples, liver, kidney and pancreatic tissues were collected. **Results:** diabetic rats showed significant increase in plasma glucose, serum urea, creatinine, cholesterol and triglyceride . Also significant increase in mean level of Fetuin A and Netrin-1 in serum and different organs (Liver, kidney, Pancreas) in compared to control group. Oral administration of Ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate cause decrease in elevated biochemical parameters. Also, decrease Fetuin A and Netrin-1 levels when compared with diabetic rats. Molecular docking studies confirmed binding of compound with Fetuin A and Netrin-1 proteins in terms of energy and revealed of the existence of hydrogen bond ,hydrophobic interaction,Our results were confirmed by histopathological examination of pancreatic tissue. **Conclusion:** this study suggests that Ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate exihibits antihyperglycemic activity in streptozotocin- induced diabetic rats.

Introduction:

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate, proteins and fat. It resulted from impair in insulin secretion, insulin action, or both. It is symptoms was thirst, urination, impair vision, and decrease in weight **[1]**.

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Streptozotocin (STZ) is a deoxy-s [((methyl-nitrosoamino) carbonyl)-amino]-D gluco pyranose molecule that caused toxic action on β cells and induced DM in most laboratory animals **[2]**. In pancreas, STZ enters through a glucose transporter-GLUT2 and cause alkylation of genetic material DNA. Also, it caused activation of poly adenosine diphosphate (ADP) ribosylation and nitric oxide (NO) release. At the end STZ caused pancreatic β-cells destruction by necrosis. It is showed that it act on DNA. In STZ metabolism, the highly reactive carbonium ion ($CH+^{5}$) produced, that induce alkylation of DNA bases **[3]**.

Flavonoids in experimental DM exhibit antioxidation properties; it prevent generation of free radicals and affects on different detoxifying enzymes activities as well **[4]**.

Fetuin-A (FetA) is a 64-kDa glycoprotein it is produced from liver and adipose tissue. Level of FetA increased in obesity and the other related disorders as type 2 diabetes mellitus, nonalcoholic fatty liver disease**[5]**.

Netrin is classically recognized as a neural guidance cue that has been involved in various tissues including pancreas development. Because Netrin's tissue regenerative, angiogenic, and inflammatory suppression properties **[6]**.

Materials and Methods

Chemicals

All reagents were purchased from Aldrich, Fluka and Merck and were used without any further purification. All melting points are uncorrected. Elemental analyses were obtained from Microanalysis unit, Cairo University.

Synthesis of the new synthesized flavonoid (Ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3 carboxylate)

Ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate was synthesized by an efficient, solvent-free one-pot three-component cyclocondensation of ethylcyanoacetate, benzaldehyde and α-naphthol then Na_2CO_3 was added. All components were put in fusion oil path for 15-25 min at 100-120 ^{o}C. Then precipitated with ice-water bath, filtered, then after recrystallized by ethanol **[7].**

Characterization of the new flavonoid derivative.

Synthesized cromen was chemically characterized using infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopy.. IR spectroscopy analysis was done using FT-IR spectra (KBr discs, 4000–400 cm-1) by Jasco-4100 spectrophotometer, at the (IR) unit at Faculty of Science, Damietta University, Egypt. Total organic carbon (TOC) analyzer: analysis was done at Faculty of Science, Kafr El-Sheikh University, Egypt. Mass spectrum analysis was done at Faculty of Science, El-Azhar University, Egypt.

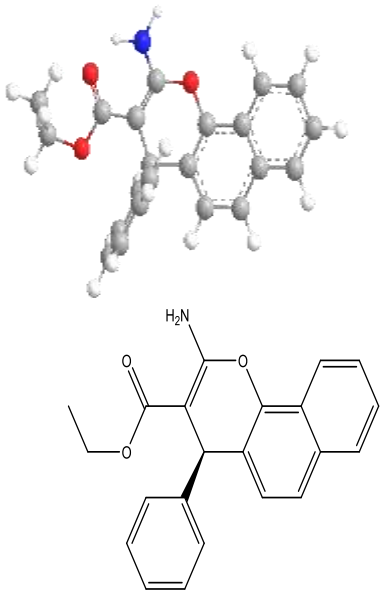

Figure 1: Structure of the new synthetic flavonoid ''ethyl 2-amino-4phenyl-4H-benzo(h)chromene-3-carboxylate''.

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Animal management

Adult male albino rats, weighing 170-200 g, were obtained from the Experimental Animal Care Center from Cairo university and were keeped in cages at experimental animal house of faculty of Science, Zagazig University under regulated environmental conditions (25°C and a 12 h light/dark cycle) 7 days before starting the experiment.

Diabetic model

Diabetes was induced by STZ, purchased from Sigma Chemical Co. (St. Louis, MO, USA). For DM induction, STZ was used at a single dose of 60 mg / kg b.wt in 18 hour fasted rats followed by the i.P injection of nicotinamide (NIC) at a dose of 120 mg/kg b.wt after 15 minutes of STZ injection. STZ was dissolved in freshly prepared cold citrate buffer (100 mM, pH= 4.5) to be immediate use through five minutes. While nicotinamide was dissolved in 0.9% saline of sodium chloride **[8]**. Blood glucose level in all animals were measured after 72 hours of drug administration and rats of fasted blood glucose levels more than 250 mg/dl were considered to be diabetic and used for the further study **[9]**.

Toxicity Study:

Determination median lethal dose (LD_{50}) of 'ethyl 2-amino-4phenyl-4H-benzo(h)chromene-3-carboxylate in albino rat was determined according to previously method [**10].** The acute toxicity was estimated by orally administration of the compound to determine the median lethal dose (LD_{50}).

Experimental design

To accomplish the ultimate goal of this study, after the acclimatization period of 7 days with standard basal diet, a total of 50 adult male albino rats were classified into five groups with 10 animals in each group.

Group 1 (control Group): Rats were administrated orally with 1 ml saline single dose.

Group 2 (DMSO group): Rats were administrated by gavaging 1 ml of 0.2% DMSO for 60 day.

Group 3 (Positive control): Rats received STZ (60 mg/kg b.wt) followed by the i.p administration of Nicotinamide (120 mg/kg b.wt) after 15 minutes.

Group 4 (standard therapeutic): were induced for DM. After 1 week of DM induction, animals were post treated with metformin (100 mg/kg daily for 60 day orally **[11]** .

Group 5 (Therapeutic Group): Rats were induced for DM. After 1 week of DM induction, animals were post treated with "ethyl 2-amino-4phenyl-4H-benzo(h)chromene-3-carboxylate" (50 mg/kg daily for 60 days orally).

Doses of "ethyl 2-amino-4phenyl-4H-benzo(h)chromene-3-carboxylate" and metformin were adjusted every week according to any change in body weight to maintain the same dose per each kg body weight of rat during the entire period of study for each group.

Collection and sampling of blood

At the end of the study and after last treatment, rats were fasted for 12 hours; blood samples were collected from the retro-orbital venous plexus under light ether anesthesia. where, blood samples were collected in three different tubes, first tube containing sodium fluoride for blood glucose estimation, second tube containing EDTA to obtain plasma and third empty tube to obtain serum by centrifugation at 4000 rpm for 20 min. Serum and plasma were transferred into eppendorff tubes and stored frozen at -20 ºC til analysis of different biochemical measurements.

Tissue sample

After blood collection animals were killed by cervical decapitation and different tissues (liver , kidney , and pancrease) were excised from animals , rinsed in with ice-cold phosphate-

buffered saline (pH 7.4) to flush out any blood.

First part of different tissue samples were homogenized with ice-cold phosphate-buffered saline (pH 7.4) to prepare a 10% (w/v) tissue homogenate for determination of Fetuin A and Netrin-1 levels .

Second part the pancreatic tissue samples were used for histopathological study.

Biochemical analysis

Estimation of biochemical parameters

Plasma glucose was performed by glucose oxidase peroxidase activity using commercial kit derived from Elitech clinical system, france **[12]**. Serum urea was measured by Berthelot emzymatic colorimetric method **[13]** and creatinine was measured by Buffered kinetic jaffè reaction without deproteinization **[14]**. Also serum cholesterol concentration was determinated by CHOD-POD enzymatic colorimetric method **[15]** while serum triglyceride concentration was measured by GPO-PAP enzymatic colorimetric method **[16]**.

Estimation of Fetuin-A and Netrin-1 levels.

Fetuin-A and Netrin-1 were determined by enzyme – linked immunosorbant assay (ELISA) according to (Cloud – Clone Corp, USA/ cat no: SEB860Ra) and (Cloud – Clone Corp, USA/ cat no: SEB827Ra) respectively.

Molecular Docking:

Molecular docking studies was performed to investigate the binding modes of ’ethyl 2-amino-4phenyl-4H-benzo(h)chromene-3-carboxylate targeting the crystal structure of Fetuin-A and Netrin-1 using Autodock vina 4. 2 **[17]**. The 3D structures of ’ethyl 2-amino-4phenyl-4H-benzo(h)chromene-3-carboxylate was obtained by ChemBioDraw Ultra 14.0 andChemBio3D Ultra 14.0 software's . The 3D structures of Fetuin-A and Netrin-1 were obtained from protein data bank (http://www.rcsb.org//pdf). The MMFF94 force field **[18]** was used for energy minimization of ligand molecule. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Affinity (grid) maps of 20×20×20 Å grid points and 0.375Å spacing were generated using the Autogrid program **[19]**. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method **[20]**

Histopathological examination

Pancreatic tissue samples were then immersed with molted paraffin wax, then embedded and blocked out. Paraffin sections (4–5 um) were stained with hematoxylin and eosin then examined through light electric microscope **[21].**

Statistical analysis

All results were analyzed by SPSS software (SPSS, ver.14.00, USA). Data were expressed as mean ± SEM. Comparison of mean values of studied variables among different groups was done using ANOVA test. $P<0.05$ was considered to be significant **[22]**.

Results:

Spectral analyses of the new synthesized compound:

IR spectroscopy results of the new synthesized compound showed a number of peaks at different positions with different intensities as follow; IR (KBr. cm^{-1}): 3487 (NH_2), 3057 (Ar-H), 1658 (C=O), 1588 (C=N), 1563 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 6.82 (d, 2H, NH_2), 7.37-8.27 (m, 1H, Ar-H), 4.74 (s, 1H, CH), 4.08 (q, 2H, CH_2), 1.16 (t, 3H, CH_3). m/z: 345.14 (100.0%), 346.14 (23.8%), 347.14 (2.7%). Mass spectrum analysis of the new synthesized compound showed that the melting point is 165-170 ^oC, its color is brown, it yielded 67%. Its chemical formula is $C_{22}H_{19}NO_3$ and Anal. Calcd. C, 76.50; H, 5.54; N, 4.06; O, 13.90 Found:

C, 75.40; H, 6.04; N, 4.56; O, 13.20. Its molecular weight is 345.40.

Toxicity Study:

It was found that ethyl 2-amino-4phenyl-4H-benzo(h)chromene-3-carboxylate" being safe until 200 mg/kg; as the selective dose was 50 mg/kg**.**

Effect of synthetic compound on body weight :

The initial and final body weight was givin in table (1). There was significant decrease ($p<0.001$) in finial body weight of diabetic induced group (positive group) which amounted to 36.6 % when compared to control group, while groups treated with synthesized compound and metformin showed slight decrease in the final body weight which was statistically non-significant in compared to control group which amounted to -21.6 % & -20.5 % respectively ($P>0.05$).

Effect of synthetic compound on biochemical parameter:

Result presented in table (2) & (3) declared that positive control group showed high elevation in plasma glucose, serum urea, creatinine , cholesterol and triglyceride which amounted to 281.5%, 21.4 %, 27.6 %, 36.8 % & 56.82% respectively when compared to control group . On the other hand groups treated with compound (therapeutic) and metformin (standard) showed good improvement in these parameters but still statistically significant in comparison with control group which amounted to 120.4%, 3.8 %, 6.3 %, 11.8% & 27.06 % respectively in metformin and 125.5%, - 1.7 % , 8.5 % , 8.2 % & 23.98 % respectively in therapeutic group.

Effect of synthetic compound on level of Fetuin A in serum and different organs (Liver, kidney and Pancreas):

Results in Table (4) showed that the mean level of Fetuin A in serum, liver, Kidney and pancreas showed high significant elevation in positive group which amounted 30.31 %, 24.40%, 26.54 % and 108.57% respectively in compared to control group, while treated groups showed good improvement which amounted 1.91%, 8.89%, 12.83 % and 58.57% respectively in therapeutic group and 5.85%, 9.09%,16.04 and 57.14 % respectively in metformin when compared in control group.

Effect of synthetic compound on level of Netrin-1 in serum and different tissue organs (Liver, kidney and Pancreas):

Results in Table (5) showed that the mean level of Netrin-1 in serum, Liver , Kidney and pancreas showed significant elevation in positive group which amounted 21.84 %, 28.04 %, 28.37% and 49.77% respectively in compared to control group. While treated groups showed good improvement which amounted to 5.46%, 4.87 %, 8.10% and 13.06% respectively in therapeutic group and 9.04%, 7.80%, 13.51% and 11.11 % respectively in metformin when compared in control group.

Molecular docking:

To find the correlation between the experimental and computational data, the docking study of the synthesized compound was performed against Fetuin-A and Netrin-1 activities to understand the ligand –protein interaction. The results showed apossible revealed favorable interactions between the compound and the receptor of Fetui-A and Netrin-1 (Figure 2).

In agreement with our results, HB plot curves indicated that compound bind to the proteins with hydrogen bond, electrostatic and Vander- walls interaction (Figure 3). Interactions with decomposed interaction energies in kcal/mol exist between compound and proteins. Thus the decreae in binding energy due to the mutation will increase the binding affinity of the comound towared the receptor

ligand showed binding energy of - 6.11 kcal/mol and - 3.33 kcal/mol with Fetuin-A and Netrin-1 respectively (Table 6 & 7). Also, 2D plot curve of doking with the ligands showed in (Figure 4).

Histopathological examination:

Histology of pancreatic tissue was studied. The normal pancrease section of control & DMSO group showed healthy parenchyma , normal pancreatic acini and islets. In positive group showed congestion of the blood vessels with thickened wall with fibrous connective tissue proliferation. Treapeutic and standard group showed healthy parenchyma , normal acini and islets (Figure 5) .

Discussion:

Diabetes mellitus (DM) is one of the worledwide enduring metabolic disorders characterized by continoued elevation of plasma sugar level **[23].** Previous studies showed that DM progression was rapid due to different complications such as heart, renal, nerve injury, retinopathy, developing in early stages of the disease **[24].** Identifying newly biomarkers for T2D and its complications remains challenging due to the heterogeneous nature of T2D. The heterogeneity relates not only to glycemic control or treatment response **[25].**

The present work investigate the role of synthesized compound "ethyl 2-amino-4phenyl-4H-benzo(h)chromene-3 carboxylate" in treatment of type 2 diabetes induced by STZ in rats.

Our data illustrated that significant decrease ($p<0.001$) in finial body weight of diabetic induced group (positive group) when compared with control group. while therapeutic and metformin group showed non significant difference in final body weight in treated groups in compared to control group.

The decrease of insulin secretion by pancreas caused disorders in metabolism of glucose, lipids and protein. The decline and inadequacy of insulin changed over anabolism to catabolism of proteins and lipids **[26].** The good improvement of mean body weight due to administration of metformin as type 2 diabetic drug. It cause diabetics to respond normally to insulin. Like most diabetic drugs, it helps on reduce metabolic disorders of glucose and also lipids and loss protein tissue **[27].**

Our result in agreement with who showed significant decrease in body weight of STZ-induced diabetic rats in compared to control group **[28]& [29]**.

Synthesized compound administration in diabetic rats improved body weight and this could be because of a superior control of the hyperglycaemic state in the diabetic rats and it is synergistic anti-inflammatory and against oxidative Properties. These results in line with who said that Arylchromenes as naturally occurring flavonoids analogs were reported with the outstanding inhibitory potential of α-glucosidase **[30]**.

Streptozotocin administration to rats indicated critical ($P< 0.001$) elevation blood glucose **[28]**. Metformin and therapeutic treated groups exhibited a decline in plasma glucose when compared with control group.

Studies have shown that the use of glibenclamide increases the sensitivity of β-cells and activates insulin synthesis and secretion from the β-cells of the pancreas that might be associated with its anti-hyperglycemic activity**[31]**.

Renal illness is one of the most widely recognized and extreme difficulties of diabetes **[32]**.

Our data found that positive control group showed high elevation ($p< 0.001$) in serum urea, creatinine , cholesterol and triglyceride ($p< 0.001$) when compared to control group . On the other hand , therapeutic and metformin groups showed significant decrease in comparison with positive group.

Past investigations announced that diabetic rats indicated essentially elevated

serum uric acid, serum creatinine and blood urea nitrogen levels **[33]**.

These results were in line with other previous studies where it has been observed that increase in circulating free fatty acids enhances hepatic TGs production. High triglycerides (TGs) content associated with formation of an atherogenic dyslipidaemia consisting of an elevation in high TG content, low HDL cholesterol and increased LDL or apo lipoprotein-B **[34]**.

Previous studies investigated that, there was high significant increase in serum cholesterol, triglycerides in STZ-induced diabetic rats, accompanied by a decrease in high density lipoprotein **[35].**

Favonoids have been reported as scavenger of free radicals that shows strong antioxidant activity by donating hydrogen group, as synthesized compound is one of synthetic flavonoids so it able to restore renal and lipid function tests **[36]**.

Our results showed that the mean level of Fetuin-A and Netrin-1 in serum, Liver , Kidney and pancreas showed significant elevation in positive group. While therapeutic and metformin groups showed good improvement in compared control group.

Previous results showed that Fetuin-A significantly elevated in overweight persons with kidney disease. Fetuin-A value is also related to impaired insulin secretion and glucose tolerance. Increased FetA level caused impaired glucose control, as FetA was implicated in impairment of insulin receptor signaling, toll-like receptor 4 activation, macrophage migration and polarization, adipocyte dysfunction, hepatocyte triacylglycerol accumulation and liver inflammation and fibrosis. Weight loss, aerobic exercise **[5]**.

Studis showed that the high expression of Netrin-1 supports defective adipose tissue migration and retention which, in turn, enhance the progression of chronic inflammation, insulin resistance, and metabolic dysfunction **[37]**

Molecular doking is a crucial element when designing computer-assisted drugs. Molecular docking is known as getting an optimized modification for both the protein and drug with relative direction between them such that the free energy of the framework is minimized **[38].** Our molecular docking results was found that interactions with decomposed interaction energies in kcal/mol exist between compound and proteins. Thus the decreae in binding energy due to the mutation will increase the binding affinity of the comound towared the receptor ligand showed binding energy of - 6.11 kcal/mol and - 3.33 kcal/mol with Fetuin-A and Netrin-1 respectively. This suggesting the ability of the compounds to be good inhibitor of Fetuin-A and Netrin-1 receptors

Our histopathological examination showed Severe alterations of pancreatic tissues were observed in untreated diabetic rats. Our results are in accordance with who stated that Pancreatic tissues of diabetic control rats showed a decrease of Langerhans islet size and multiple degeneration and injuries. In addition to, the number of β-cells was reduced, and some necrosis and destruction were found **[39]**.

Reactive oxygen species produced with diabetes mellitus. Damage to pancreas, resulted in impaired insulin secretion can be caused by an increase in concentration of free radicals or decrease of antioxidant defenses. In therapeutic group, synthetic compound as one of flavonoid compounds which have antioxidant activity where it was reported that antioxidants have reverse some of the damaging effects of free radicals on the pancreatic tissue **[40]**.

Conclusion:

The current study designated that the anti hyperglycemic and hypolipidemic ability of synthesized compound "ethyl 2-amino-

4phenyl-4H-benzo(h)chromene-3-carboxylate" exhibits a protective effect on various parameters in the STZ induced diabetic rats and helped in relieving the related complications. Results of the histopathological examination confirmed that. Also, the molecular docking study was suggested the ability of the compounds to be good inhibitor of Fetuin-A and Netrin-1 receptors.

References:

[1]. Trivedi, B., Mazumdar, J., Bhatt, D. and Hemavathi, **K.G.(2004):** Effect of Shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. Indian J. Pharmacol, 36(6): 373-376.

[2]. Doux, S.P. (1986): Mechanism of nitrosourea induced β cell damage: Alteration in DNA Diabetes. 35:866-72.

[3]. Portha, B. (1989): The rat models of non-insuin dependant diabetes induced by neonatal Streptozotocin Diabete Metab. 15:61-75.

[4]. Lukacinova, A., Mojzis, J., Benacka, R. (2008): Preventive Effects of Flavonoids on Alloxan-Induced Diabetes Mellitus in Rats. Acta Vet Brno. 77: 175-182.

[5]. Malin, S.K., Del Rincon, J.P., Huang, H., Kirwan, J.P. (2014): Exercise-Induced Lowering of Fetuin-A May Increase Hepatic Insulin Sensitivity. Med Sci Sports Exerc. 46: 2085–2090.

[6]. Breuck, S., Lardon, J., Rooman, I. and Bouwens, L. (2003): "Netrin-1 expression in fetal and regenerating rat pancreas and its effect on the migration of human pancreatic duct and porcine islet precursor cells," Diabetologia. 46(7): 926–933.

[7]. Gholipour, S., Davoodnia, A., Nakhaei-Moghaddam, M. (2015): Synthesis, characterization, and antibacterial evaluation of new alkyl 2-amino-4-aryl-4H-chromene-3-carboxylates. Chem Heterocycl Compd; 51: 808.

[8]. Kim, M.J. and Lim, Y., (2013): Protective effect of short-term genistein supplementation on the early stage in diabetes-induced renal damage. Mediators. Inflamm. 510212.

[9]. Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M. Ribes, G. (1998): Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes*; 47: 224-229.

[10]. Meier, J., and Theakston, R.D.G. (1986): Approximate LD50 determination of snake venuoms using eight to ten experimental animals. Toxicon, 24 (4), 395-401.

[11]. Kiliari, E.K., Mullapudi, B., Moka, P.V., Silakabattini, K. and Nelli, G. (2014):inhibition of DPP-IV Activity and Enhancement Of GLP-1Expression By Aqueous Peel Extract Of *Punicagranatumin* Albino Wistar rats. Journal of Globale Trends in Pharmaceutical Science ; (5)2: 1528-1541.

[12]. Pruden, E.L., Mc Pherson, R.A., Fuhrman, S.A., (1995): Clinical guide to laboratory test-Ed. Tiet N.W. / Saunders W.B. Company. 3th ed. Section 1:general clinical test; 268-273.

[13]. Kaplan, A. (1984): Urea ,Kaplan A.et al. Clin Chem the C.V. Mosby Co.St Louis.Toronto. Princeton 1257-1260 and 437 and 418.

[14]. Kaplan, A., (1984): Urea ,Kaplan A.et al. Clin Chem the C.V.

Mosby Co.St Louis.Toronto. Princeton 1257-1260 and 437 and 418.

[15]. Young, D.S. (2001): Effects of disease on Clinical Lab. Tests, 4th ed., AACC 2001.

[16]. Stein, E.A. (1987): Lipids, lipoproteins, and Apo lipoproteins. In:Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed . Philadephia: WB Saunders. 448-481.

[17]. Tachakittirungrod, S., Okonogi,S. and Chowwanapoonpohn, S. (2007): Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chem. 103, 381–388.

[18]. Bikadi, Z. and Hazai, E. (2009): Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock J. Cheminf. 1: 15

[19]. Halgren Merck, T.A. (1998): Molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94 Journal of Computational Chemistry.17 (5-6), 490-519.

[20]. Morris , G.M., Goodsell, D.S., et al. (1998): Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function Journal of Computational Chemistry.19 (14), 1639-1662.

[21]. Bancroft, J. and Gamble, M. (2008): Theory and Practice of Histological Technique 4th Ed., Churchill Livingston, New York, London, San Francisco, and Tokyo.

[22]. SPSS. (2008): Statistical package for social science, computer software, Ver. 16. London, UK: SPSS Company.

[23]. Yimer, E.M., Zewdie, K.A. and Hishe, H.Z. (2018): Netrin as a Novel Biomarker and Its Therapeutic Implications in Diabetes Mellitus and Diabetes-Associated Complications.

[24]. Keskin, M., Çulha, C., Gülçelik, N.E., Ademoğlu, E., Keskin, A. and Aral, Y. (2017): Fetuin-A levels determine cardiovascular risk in young diabetic patients. Biomedical Research.28 (15): 6767-6772.

[25]. Ahluwalia, T.S., Kilpeläinen, T.O., Singh, S.P. and Rossing, P. (2019): Editorial: Novel Biomarkers for Type 2 Diabetes 10: 649.

[26]. The global diabetes community (**Diabetes.co.uk**) 15th January 2019.

[27]. Guex, C.G., Reginato, F.Z., Jesus, P.R-D.,Brondani, G.C. (2019): Antidiabetic effects of Olea europaea L. leaves in diabetic rats induced by high-fat diet and low-dose streptozotocin.

[28]. Guex, C.G., Reginato, F.Z., de Jesus, P.R., Brondani, J.C., Lopes, G.H.H. and Bauermann, L.F. (2019): Antidiabetic effects of Olea europaea L. leaves in diabetic rats induced by high-fat diet and low-dose streptozotocin. J Ethnopharmacol. 10(235):1-7.

[29]. Nazir, N., Zahoor, M., Ullah, R., Ezzeldin, E. and Gamal, A.E. (2021): Curative Effect of Catechin Isolated from Elaeagnus Umbellata Thunb. Berries for Diabetes and Related Complications in Streptozotocin-Induced Diabetic Rats Model. Molecules. 26: 137.

[30]. Spasov, A. A., Babkov, D. A., Osipov, D. V., Klochkov, V. G., Prilepskaya, D. R., Demidov, M. R. (2019): Synthesis, *in vitro* and *in vivo* evaluation of 2-aryl-4H-chromene and 3-aryl-1H-benzo [f] chromene derivatives as novel α-glucosidase inhibitors. Bioorg. Med. Chem. Lett. 29: 119–123.

[31]. Alam, F., Saqib, Q.N., Ashraf, M. (2018): Zanthoxylum armatum DC extracts from fruit, bark and leaf induce hypolipidemic and

hypoglycemic effects in mice- in vivo and in vitro study. BMC Complement. Altern. Med.18: 68.

[32]. Tierney, L. M., McPhee, S. J., Papadakis, M. A. (2002): Current medical Diagnosis and Treatment. International edition. NewYork: Lange Medical Books/McGraw-Hill. 1203–1215

[33]. Jia, Q., Yang, R., Liu, X-F., Ma, S-F. and Wang, L. (2019): Genistein attenuates renal fibrosis in streptozotocin-induced diabetic rats. Molecular Medicine Reports. 19: 423-431.

[34]. Bae, J., Kim, N., Shin, Y., Kim, Y.S., Kim, J.U. (2020): Activity of catechins and their applications. biomed. dermatol.4: 8.

[35]. Vergès, B. (2015): Pathophysiology of diabetic dyslipidaemia: Where are we? Diabetologia.58: 886–899.

[36]. Fang, X., Azain, M., Crowe-White, K., Mumaw, J., Grimes, J.A., Schmiedt, C., Barletta, M., Rayalam, S. Park, H.J. (2019): Effect of Acute Ingestion of Green Tea Extract and Lemon Juice on Oxidative Stress and Lipid Profile in Pigs Fed a High-Fat Diet. Antioxidants. 8: 195.

[37]. Yang, Y. H. C., Szabat, M. Bragagnini, C. (2011): Paracrine signalling loops in adult human and mouse pancreatic islets: netrins modulate beta cell apoptosis signalling via dependence receptors," Diabetologia. 54(4): 828–842.

[38]. Hosny, N.M., Hussien, M.A., Radwan, F.M., Nawar, N. (2014): Synthesis, spectral characterization and DNA binding of Schiff-base metal complexes derived from 2-amino-3-hydroxyprobanoic acid and acetylacetone. Spectrochim. Acta A Mol. Biomol. Spectrosc. 132: 121-129.

[39]. Almalki, D.A., Alghamdi, S.A. and Al-Attar, A.M. (2019): Comparative Study on the Influence of Some Medicinal Plants on Diabetes Induced by Streptozotocin in Male Rats. BioMed Research International. 1-11.

[40]. Maritim, A.C. Sanders, R.A. Watkins, J.B. (2003): Diabetes, Oxidative Stress, and Antioxidants: A Review. J. Biochem. Mol. Toxicol. 17: 24- 37.

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Table 1: The initial and final body weight of rats in all studied groups.

Groups	Initial Body weight (g)	Final body weight (g)
Control Mean ± SEM	216.5± 8.5	322±12.3[C]
DMSO Mean ± SEM % change	227± 1.06 4.8 %	330.6±1.14 [C] 2.6 %
Positive **(STZ-induced)** Mean ± SEM % change	224.4± 18.9 3.6 %	204±3.8*** -36.6 %
Metformin Mean ± SEM % change	208± 12.7 -0.04 %	256±16.6 [b] -20.5 %
Compound Mean ± SEM % change	212.7± 25.9 -1.7 %	252.3±42.8 [b] -21.6 %
P value	**P > 0.05**	**P< 0.001**

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group

Table 2: Mean level of glucose in different studied groups.

Groups	Glucose (Initial) (mg/dl)	Glucose (final) (mg/dl)
Control Mean ± SEM	99±2.2	107.2±4.5[c]
DMSO Mean ± SEM % change	95±0.84 -4.123%	122.3±3.7[c] 14.08%
Positive **(STZ-induced)** Mean ± SEM % change	332±8.1*** 240.2%	409±15.0*** 281.5%
Metformin Mean ± SEM % change	333.5±5.6*** 241.75%	236.3±4.8***[c] 120.4%
Compound Mean ± SEM % change	329.1±4.8*** 237.21%	241.8±2.1***[c] 125.5%
P value	**P< 0.001**	**P< 0.001**

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group

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Table 3: Mean level of urea, creatinine, cholesterol, triglyceride in all studied groups.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Control Mean ± SEM	11.2±0.9	0.47±0.02[c]	73.3±2.9[c]	133.4±7.2 [c]
DMSO Mean ± SEM % change	11.8±0.7 5.4 %	0.48±0.02[c] 2.1 %	76±3.4 [c] 3.6 %	148.5±5.4 [c] 11.31%
Positive (STZ-induced) Mean ± SEM % change	13.6±5 21.4 %	0.60±0.007*** 27.6 %	100.3±3.6** 36.8 %	209.2±10.2*** 56.82%
Metformin Mean ± SEM % change	11.6±0.8 3.8 %	0.50±0.016[c] 6.3 %	82.0±3.9[c] 11.8 %	169.5±5.7**[b] 27.06%
Compound Mean ± SEM % change	11.0±0.7 - 1.7 %	0.51±0.01[c] 8.5%	79.3±4.4[c] 8.2 %	165.4±7.5* [b] 23.98%
P value	**P > 0.05**	**P< 0.001**	**P< 0.05**	**P= 0.001**

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table 4: Mean levels of Fetuin A in serum and different organs of all studied groups.

Groups	Fetuin A (ng/ml)			
	Serum ng/ml	**Liver ng/ml**	**Kidney ng/ml**	**Pancreas ng/ml**
Control Mean ± SEM	9.40±0.11 [c]	10.45±0.72 [b]	8.10±0.34 [a]	3.5±0.2 [c]
DMSO Mean ± SEM % change	9.76±0.02 3.82%	10.49±0.015 [c] 0.38%	8.70±0.1 7.40%	4.0±0.24[c] 14.28%
Positive (STZ-induced) Mean ± SEM % change	12.25±0.45*** 30.31%	13.00±0.10** 24.40%	10.25±0.15* 26.54%	7.3±0.3** 108.57%
Metformin Mean ± SEM % change	9.95±0.02 [c] 5.85%	11.40±0.089 [a] 9.09%	9.40±0.04 16.04%	5.50±0.15** [a] 57.14%
Compound Mean ± SEM % change	9.58±0.43 [c] 1.91%	11.38±0.36 [a] 8.89%	9.14±0.34 12.83%	5.55±0.32* [b] 58.57%
P value	**P< 0.001**	**P< 0.01**	**P< 0.001**	**P< 0.001**

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table 5: Mean levels of Netrin-1 in serum and different organs of all studied groups.

Groups	Netrin-1 (ng/ml)			
	Serum	**Liver**	**kidney**	**pancreas**
Control Mean ± SEM	293±3.4 [c]	205±1.7 [c]	148±0.4 [b]	112.5±4.5 [b]
DMSO Mean ± SEM % change	295±1.2 0.68%	215±2.2 [c] 4.87%	157.3±0.7 [b] 6.283%	114±0.7 [b] 1.33%
Positive (STZ-induced) Mean ± SEM % change	357±7.5*** 21.84%	262.5±5.5*** 28.04%	190±1.0** 28.37%	168.5±10.5** 49.777%
Metformin Mean ± SEM % change	319.5±6.1** 9.044%	221±2.9** [c] 7.80%	168±2.2** [a] 13.51%	125±11.5 [a] 11.11%
Compound Mean ± SEM % change	309.4±1.9* 5.46%	215±5.7 [c] 4.87%	160±4.4 8.10%	127.2±0.73 [b] 13.06%
P value	**P< 0.001**	**P< 0.001**	**P< 0.001**	**P< 0.01**

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table 6: Energy values obtained in doking calculations of the new synthesized compound with receptor of Fetuin-A.

Results Table

Rank	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
1	-6.11 kcal/mol	33.19 uM	-6.80 kcal/mol	-0.02 kcal/mol	-6.82 kcal/mol	100%	660.919

Table 7: Energy values obtained in doking calculations of the new synthesized compound with receptor of Netrin-1.

Rank	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
1	-3.33 kcal/mol	3.62 mM	-3.97 kcal/mol	-0.02 kcal/mol	-3.99 kcal/mol	50%	398.317

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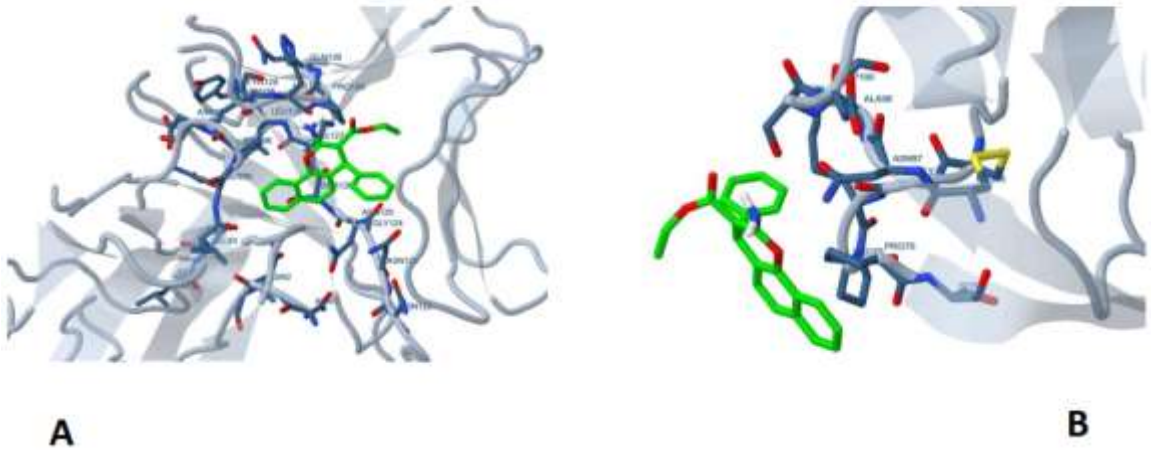

Figure 2: 3D interaction of synthesized compound (green) inside the active position of Fetuin-A (A) and Netrin-1(B).

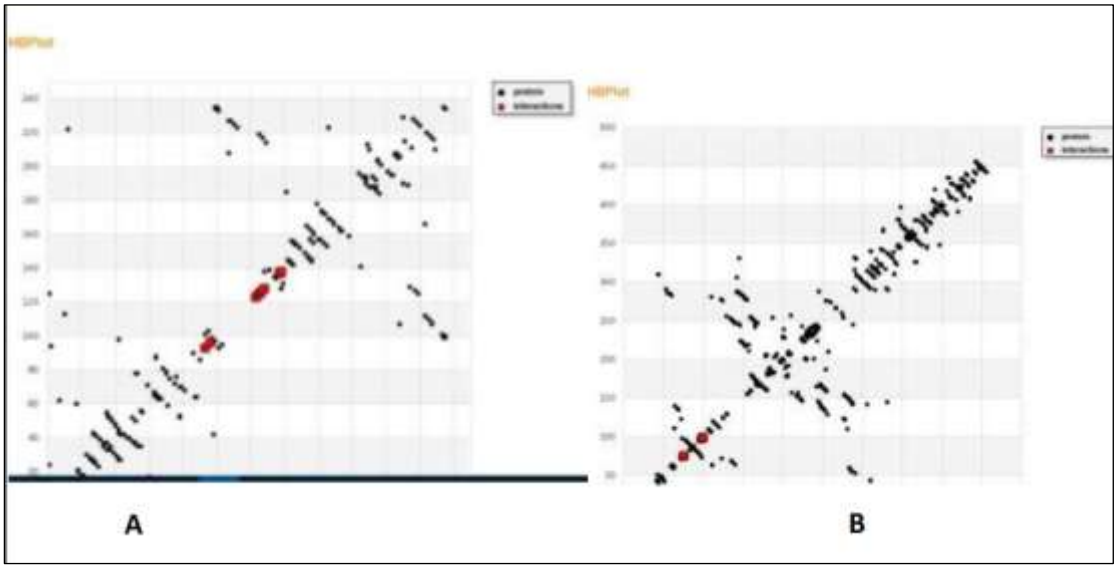

Figure 3: HB plot of the interaction between synthesized compound and receptor of both Fetuin-A (A) and Netrin-1(B).

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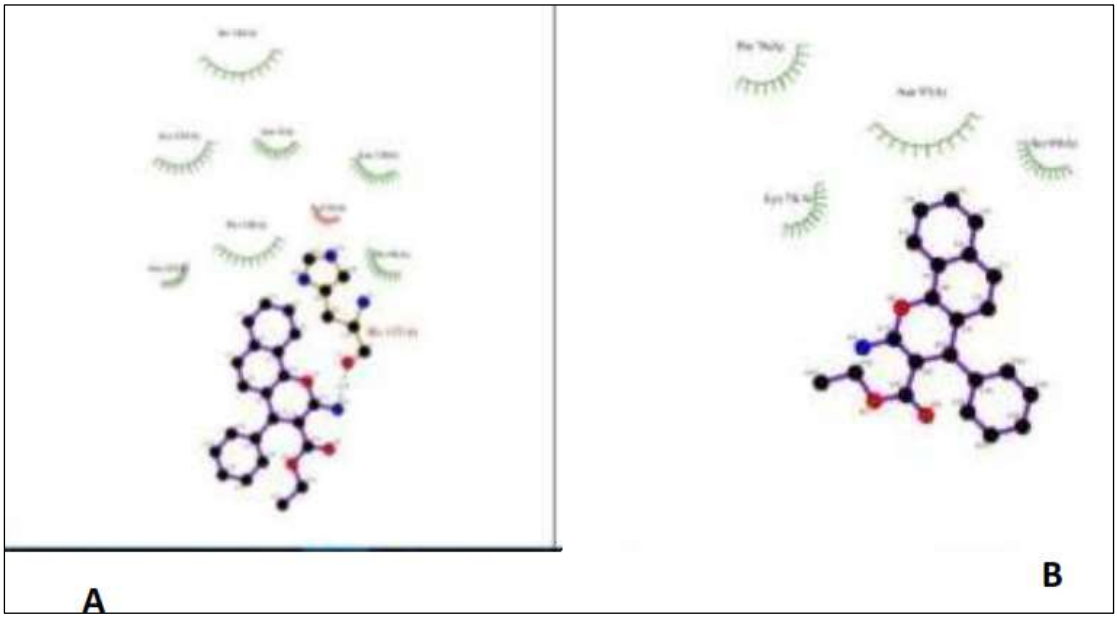

Figure 4: 2D plot interaction between synthesized compound and receptor of both Fetuin-A (A) and Netrin-1(B).

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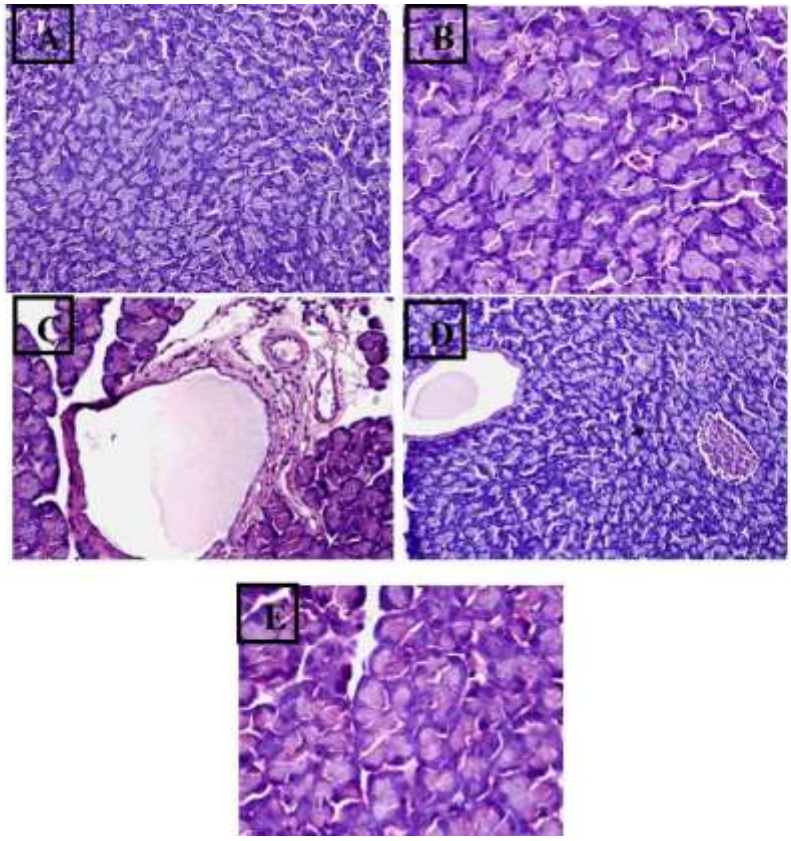

Figure 5: Histopathological examination of pancreas tissue. A (negative control)& B (DMSO) showed normal pancreatic acini, ducts, and islets, (H&E X 200) (H&E X 400) respectively, C (Positive control) showed severly dilated pancreatic duct (arrow head),and showed sever congestion in the interstitial blood vessel (arrow head) with thickened muscular wall (arrows) (H&E X 400). D (Metformin) showed pancreatic islets hyperplasia (arrow) and pancreatic duct dilatation (arrow head), (H&E X 200), E (compound) showed normal pancreatic acini, ducts, and islets, (H&E X 400).