# Synthesis of Novel Acetylinc Derivative of Metformine as a DPP-4 Inhibitors and Study its Effects on Sera of Rabbits with Diabetes

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## Background

Diabetes is a major global disease. Around 382 million people of the world's population have diabetes mellitus in 2013 and by 2035, this will rise to 592 million[1]. Therapies targeting the incretin system are important for management of type 2 diabetes. Two principal incretin hormones—glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP)—are rapidly released after meals. Both hormones augment glucosedependent insulin secretion; and GLP-1, but not GIP, also suppresses glucagon secretion, delays gastric emptying, and decreases food intake. GLP-1 and GIP are inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4).[2] Incretin-based therapies consist of two drug classes: GLP-1 receptor agonists, which have biological activity similar to GLP-1, but are resistant to DPP-4; and DPP-4 inhibitors, which prevent enzymatic inactivation of endogenous GLP-1 and GIP.[3]

The dipeptidyl peptidase (DPP)-4 inhibitors are a new class of anti hyperglycaemic agents which were developed for the treatment of type 2 diabetes by rational drug design, based on an understanding of the underlying mechanism of action and knowledge of the structure of the target enzyme[4]. As a therapeutic class, the DPP-4 inhibitors comprise a diverse group of compounds, which can be broadly divided into those that mimic the dipeptide structure of DPP-4 substrates and those which are non-peptido mimetic compounds such as sitagliptin (β-amino acid based)[5].

## Objectives

Synthesis of novel metformine derivative as dipeptidyl peptidase 4 (DPP-4) inhibitors and characterize their .biological activities in vivo

## Materials and Methods

| Instrument | kits | Chemicals |
|---|---|---|
| FT.IR470 (Infrared spectrophotometer)(Shimadzu) | Dipeptidyl peptidase – 4 (US Biological/USA) | Metformine (SDI) |
| UV-Visible Spectrophotometer(Mindray) | Insulin Kit(TOSOH/Japan) | Propargyl chloride (Fluka) |
| 1HNMR&13CNMR spectrophotometer (Perkin Elmer) | Cholesterol(Bio System/Spain) | Sodium hydroxide(Merck) |
| TOSOH/AIA360/Japan | Triglycried(Bio System/Spain) | Silica Gel (Calidon Laboratories) |
| Incubator(Memert) | HDL(Bio System/Spain) | Methanol (Merck) |
| Eliza(USA) | Glucose(Bio System/Spain) | Alloxane (Alfa Aesar) |
| Hot plat with stirrer(Labtech) | AST(Bio System/Spain) | TLC Paper(Fluka) |
| Micro pipette (Germany) | ALT(Bio System/Spain) | |

**Preparation of metformine derivative**[6]

In a round button flask 0.03 mole, 1.2 gm sodium hydroxide and 150 mL methanol were added, stirred for 10 minutes, then 2.1 mL dissolved in 21 mL methanol added to the mixture drop wise and refluxed for 24 hours, the solvent evaporated and the results collected (6.2 gm, 93.32%). The results then purified by a column chromotography height of 70 cm and diameter of 2.5 cm, and using silica gel (35-70) as stationary phase and methanol as a mobile phase.

**Sampling**

In this study, 40 rabbits has been obtained and divide it to 4 groups, (G1) control group, (G2) rabbits with diabetic wounded by 120mg/Kg alloxane, (G3) rabbits with diabetic take 10mg/Kg sitagliptin drug for 3 days and (G4) rabbits with diabetic take 8mg/Kg of the prepared derivative for 3 days.

**Estimation of biochemical parameters in sera of rabbits**

**Estimation of dipeptidyl peptidase-4 activity in blood serum**[7]

Dipeptidyl peptidase-4(DPP-4) estimated by eliza, (US Biological Kit/ USA), using the competitive between antibodies of DPP-4 on wells of the plate and antibodies at horseradish peroxidase to linked with the DPP-4 antigen in blood serum.

**Estimation of glucose in Blood Serum**[8]

Glucose has estimated by a colorimetric method, (Bio systems kit) obtain oxidation of glucose in presence of glucose oxidase by GOD-POD (Trinder) reaction.

**Estimation of insulin in blood serum**[9]

The insulin has estimated by TOSOH instrument with special kit, the insulin on serum linked with the antibodies gated from the mice frozen at magnetic granules combined with the bovine alkaline phosphatase enzyme, the magnetic granules was washed to release the non combine enzyme and incubate with the substrate(4-methyl umbelliferyl phosphate). The amount of the antibodies combined with the enzyme was directly proportional with the insulin concentration.

**Estimation of aspartate aminotransferase(AST) in blood serum**[10]

Aspartate aminotransferase(AST) catalyzes the transfer of the amino group from aspartate to 2-oxaloacetate and glutamate. The catalytic concentration was determined from the rate of decrease of NADH, measured at 340nm by means of the malate dehydrogenase(MDH) coupled reaction.

**Estimation of alanine aminotransferase(ALT) in blood serum**[11]

Alanine aminotransferase(ALT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration was determined from the rate of decrease of NADH, measured at 340nm, by means of the lactate dehydrogenase(LDH) coupled reaction.

**Estimation of total cholesterol(Ch) in blood serum**[12]

The principle of measurement was based on the enzymatic hydrolysis according to the following reaction:

**Estimation of triglyceride(TG) in blood serum**[13]

Triglyceride in the sample originates, by means of the coupled reactions described below:

A colored complex can be measured by spectrophotometer at 500nm.

**Estimation of high density lipoprotein-cholesterol(HDL-Ch) in blood serum**[14]

Very low density lipoproteins(VLDL-Ch), low density lipoproteins(LDL-CH) and chylomicrons were precipitated by addition of phosphotungstic acid and magnesium chloride. The supernatant fluid after centrifugation contains (HDL) fraction, which was assayed for (HDL-Ch) with the cholesterol liquicolor test kit.

**Estimation of low density lipoprotein-cholesterol(LDL-Ch) and very low density lipoprotein-cholesterol(VLDL-Ch) in blood serum**[15]

The (VLDL-Ch) concentration was measured from the triglyceride (TG) and the (LDL-Ch) concentration was measured from the total cholesterol concentration(TC), (HDL-Ch) and (VLDL-Ch) concentrations, described by the following equations:

**VLDL-CH(mg/dL) = Triglyceride / 5**

**LDL-Ch(mg/dL) = Total cholesterol-(HDL-Ch + VLDL-Ch)**

## RESULTS

### Organic part

The synthesis of acetylinc derivative of metformen has been obtained by the reflux of metformine with propargyl chloride in methanol
according to the reaction below:

The derivative was purified by a column chromatography(silica gel as stationary phase and methanol as a mobile phase) and follow up by TLC. The characterization of the acetylinc derivative was accomplished by measuring spectral data, FT-IR which showed the presence of the absorbance band at 3178 $cm^{-1}$ for H acetylinic, and at 2191 $cm^{-1}$ for acetylinc group, while the results of $^{1}$HNMR and $^{13}$C-NMR showed by the following table:

**Table(1): $^{1}$HNMR and $^{13}$CNMR of metformin derivative**

| $^{1}$HNMR | | $^{13}$CNMR | |
|---|---|---|---|
| A | 73.20 – 66.10 | A | 2.70 – 2.65 |
| B | 82.01 – 80.10 | B | 3.66 – 3.20 |
| C | 36.09 – 33.30 | C | 5.02 – 5.00 |
| D | – 160.01<br>166.09 | D | 2.97 – 2.83 |
| E | 38.60 – 37.80 | E | 4.74 – 4.70 |
| F | – 172.16<br>173.01 | F | 5.02 – 5.00 |

### Biochemistry part

The results of DPP-4 at table (2) showed a significant decrease in the activity of enzyme by the effect of G3[16] and G4 compared with the G2[17], and the values were 55.32± 26.96, 31.64±12.65 and 137.65±30.90 U/L respectively, and a significant decrease in G3 compared with G4, so, the difference between the two group, which means that the effect of the prepared derivative record the strongest inhibitory effect than sitagliptin, which was clear in the results of blood glucose at the same table, table(2) showed a significant decrease in the glucose level by the effect of G3 and G4 compared with the G2, and the values were 150.70± 11.96, 124.30± 25.88 and 214.90± 27.67 mg/ dl respectively, and the effect of the prepared derivative decrease the blood sugar more than the effect of sitagliptin, and showed a significant decrease in the insulin level by the effect of G3 and G4 compared with the G2, and the values were 9.48±1.48, 8.67±1.70 and 13.07±1.67 μU/mL respectively. The DPP-4 inhibition with sitagliptin improved the expression of GLP-1 and GLP-1R in pencreas. Since, GLP-1R stimulate the adenylyl cyclase pathway, and increase the insulin synthesis in langerhans islet, so sitagliptin can restore damaged pencreas[18]. The change in all hormones with respect to expression and blood glucose level indicate that sitagliptin may cause the regulation of hyperglycemia and hypoglycemia in type-2 diabetes[19].

All these results confirm the ability of the prepared derivative to treating the hyperglycemic, and its results similar to the results of sitagliptin which mean that may be have similar properties. Figure(1) show the proposed designing model of the combination between the prepared derivative and the active site of DPP-4.

The results of table(3) show inhibitory effect by G3 and G4 comparing with the injury group G2 in the liver enzymes AST and ALT, and the values were 52.500± 8.50, 50.700±12.18 and 76.700± 16.36 U/L for AST and 43.100b± 8.87, 44.600b±12.20 and 77.700c±15.46 U/L for ALT respectively.

Table (4) illustrate the comparing between the divided groups on lipid profile, there are a significant decreases in the levels of cholesterol and triglyceride in G3 and G4 comparing with G2, and the values were 144.08±18.78, 152.34±25.11 and 255.25±18.28 mg/dL for Ch, 154.70±30.38, 158.08±52.11 and 299.92±28.73 for TG respectively. Furthermore, table (4) showed a significant increased in HDL-Ch between G3 and G4 comparing with G2, and the values were 31.20±3.61, 33.50±4.62 and 27.80±2.29 mg\dL respectively, and a significant decreases in LDL-Ch and VLDL-Ch between G3 and G4 comparing with G2, and the values were 84.64±19.10, 88.05±20.22 and 136.66±20.8 mg/dL for LDL-Ch, and 30.94±6.07, 31.60±10.06 and 61.98±5.82 mg/dL respectively.

The level of free fatty acids in blood serum increase because of the low levels of glucose in diabetic patients,so the free fatty acids are catabolized In liver to result acetyl CoA, the excess of acetyl CoA is convert to cholesterol, triglyceride and ketone bodies and other lipoproteins like LDL and VLDL. The abnormally high concentration of serum lipoprotein in the diabetic patients may also be due to the increase in the mobilization of free fatty acids from the peripheral fat depots by glucagon in the absence of insulin [20].

**Table (2) : The comparing between the divided groups on DPP-4, Glucose and Insulin in sera of rabbits**

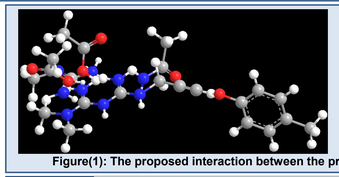
| Mean ±SD | N | Group | Parameters |
|---|---|---|---|
| 29.55$^{a}$± 8.33 | 10 | G1 | DPP-4 pg/ mL |
| 137.65$^{c}$± 30.90 | 10 | G2 | |
| 55.32$^{b}$± 26.96 | 10 | G3 | |
| 31.64$^{a}$± 12.65 | 10 | G4 | |
| 106.50$^{a}$± 19.173 | 10 | G1 | Glucose mg/ dl |
| 214.90$^{b}$± 27.67 | 10 | G2 | |
| 150.70$^{b}$± 11.96 | 10 | G3 | |
| 124.30$^{a}$± 25.88 | 10 | G4 | |
| 6.03$^{a}$± 1.75 | 10 | G1 | Insulin μU/mL |
| 13.07$^{c}$± 1.67 | 10 | G2 | |
| 9.48$^{b}$± 1.48 | 10 | G3 | |
| 8.67$^{b}$± 1.70 | 10 | G4 | |

**Table (3) : The comparing between the divided groups on liver enzymes AST and ALT in sera of rabbits**

| Mean ±SD | N | Group | Parameters |
|---|---|---|---|
| 28.000$^{a}$± 9.23 | 10 | G1 | AST U/L |
| 76.700$^{c}$± 16.36 | 10 | G2 | |
| 52.500$^{b}$± 8.50 | 10 | G3 | |
| 50.700$^{b}$± 12.18 | 10 | G4 | |
| 27.300$^{a}$± 11.28 | 10 | G1 | ALT U/L |
| 77.700$^{c}$± 15.46 | 10 | G2 | |
| 43.100$^{b}$± 8.87 | 10 | G3 | |
| 44.600$^{b}$± 12.20 | 10 | G4 | |

**Table (4) : : The comparing between the divided groups on lipid profile in sera of rabbits**

| Mean ±SD | N | Group | Parameters |
|---|---|---|---|
| 131.26$^{a}$± 14.08 | 10 | G1 | Cholesterol(Ch) mg\dL |
| 255.25$^{c}$± 18.28 | 10 | G2 | |
| 144.08$^{ab}$± 18.78 | 10 | G3 | |
| 152.34$^{b}$± 25.11 | 10 | G4 | |
| 116.89$^{a}$± 25.87 | 10 | G1 | Triglyceride(TG) mg\dL |
| 299.92$^{c}$± 28.73 | 10 | G2 | |
| 154.70$^{b}$± 30.38 | 10 | G3 | |
| 158.08$^{b}$± 52.11 | 10 | G4 | |
| 41.60± 3.77 | 10 | G1 | HDL- Ch mg\dL |
| 27.80± 2.29 | 10 | G2 | |
| 31.20± 3.61 | 10 | G3 | |
| 33.50$^{a}$± 4.62 | 10 | G4 | |
| 71.18$^{a}$± 14.41 | 10 | G1 | LDL- Ch mg\dL |
| 136.66$^{b}$± 20.84 | 10 | G2 | |
| 84.64$^{a}$± 19.10 | 10 | G3 | |
| 88.05$^{a}$± 20.22 | 10 | G4 | |
| 23.37$^{a}$± 5.18 | 10 | G1 | VLDL- Ch mg\dL |
| 61.98$^{b}$± 5.82 | 10 | G2 | |
| 30.94$^{a}$± 6.07 | 10 | G3 | |
| 31.60$^{a}$± 10.06 | 10 | G4 | |



**Figure(1): The proposed interaction between the prepared derivative and the active site of DPP-4**

**Correlations between biochemical parameters for G1**

| | Glu mg/dL | VLDL mg/dL | LDL mg/dL | TG mg/dL | HDL mg/dL | Ch mg/dL | GOT U/L | GPT U/L | DDP-4 Pg/mL | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | -0.491 | GPT U/L |
| | | | | | | | | 0.020 | -0.125 | GOT U/L |
| | | | | | | | -0.121 | -0.339 | -0.073 | Ch mg/dL |
| | | | | | | -0.273 | -0.529 | 0.092 | 0.024 | HDL mg/dL |
| | | | | | -0.137- | -0.141 | 0.079 | 0.013 | -0.434 | TG mg/dL |
| | | | -0.231 | -0.635** | 0.828** | 0.222 | -0.341 | -0.128 | LDL mg/dL | |
| | | -0.389 | 0.605 | 0.137 | -0.518 | -0.025 | 0.360 | -0.710** | VLDL mg/dL | |

**Correlations between biochemical parameters for G2**

| | Glu mg/dL | VLDL mg/dL | LDL mg/dL | TG mg/dL | HDL mg/dL | Ch mg/dL | GOT U/L | GPT U/L | DDP-4 Pg/mL | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | -0.491 | GPT U/L |
| | | | | | | | | 0.020 | -0.125 | GOT U/L |
| | | | | | | | -0.121 | -0.339 | -0.073 | Ch mg/dL |
| | | | | | | -0.273 | -0.529 | 0.092 | 0.024 | HDL mg/dL |
| | | | | | -0.137- | -0.141 | 0.079 | 0.013 | -0.434 | TG mg/dL |
| | | | -0.231 | -0.635** | 0.828** | 0.222 | -0.341 | -0.128 | LDL mg/dL | |
| | | -0.389 | 0.605 | 0.137 | -0.518 | -0.025 | 0.360 | -0.710** | VLDL mg/dL | |

**Correlations between biochemical parameters for G3**

| | Glu mg/dL | VLDL mg/dL | LDL mg/dL | TG mg/dL | HDL mg/dL | Ch mg/dL | GOT U/L | GPT U/L | DDP-4 Pg/mL | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 0.228 | GPT U/L |
| | | | | | | | | -0.094 | 0.703** | GOT U/L |
| | | | | | | | 0.495 | 0.085 | -0.607 | Ch mg/dL |
| | | | | | | -0.698** | -0.792** | -0.264 | 0.822** | HDL mg/dL |
| | | | | | 0.016 | 0.089 | -0.186 | 0.551 | 0.346 | TG mg/dL |
| | | | | -0.012 | -0.548 | 0.857** | 0.414 | -0.163 | -0.657** | LDL mg/dL |
| | | | -0.513 | 0.294 | -0.118 | -0.199 | -0.156 | 0.515 | 0.114 | VLDL mg/dL |

**Correlations between biochemical parameters for G4**

| | Glu mg/dL | VLDL mg/dL | LDL mg/dL | TG mg/dL | HDL mg/dL | Ch mg/dL | GOT U/L | GPT U/L | DDP-4 Pg/mL | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 0.045 | GPT U/L |
| | | | | | | | | 0.629 | -0.439 | GOT U/L |
| | | | | | | | -0.340 | -0.339 | 0.018 | Ch mg/dL |
| | | | | | | 0.361 | -0.589 | -0.522 | 0.126 | HDL mg/dL |
| | | | | | -0.147 | 0.448 | 0.364 | 0.142 | 0.176 | TG mg/dL |
| | | | | 0.044 | 0.359 | 0.900** | -0.474 | -0.400 | -0.158 | LDL mg/dL |
| | | | 0.043 | 1.000** | -0.148 | 0.447 | 0.364 | 0.143 | 0.176 | VLDL mg/dL |
| | | 0.223 | 0.341 | 0.222 | -0.106 | 0.342 | 0.165 | 0.627 | -0.126 | Glu mg/dL |
| | 0.106 | 0.548 | 0.173 | 0.548 | 0.451 | 0.439 | -0.279 | -0.221 | 0.354 | Insulin uU/mL |

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**. Correlation is s

## SUMMARY

The study aimed to prepare novel derivative of metformin similar to the work of the enzyme inhibitors dpp-4 and so the need for new inhibitors may be a side effect with less addition to linking types of drugs have a stronger effect on patients with diabetes and to study the impact of this derivative inside the living cell has been prepared derived through interaction metformin with propargyl chloride. It was to make sure the chemical structure by using analytical and spectral methods (FT-IR, $^{1}$HNMR, and $^{13}$CNMR), and the results confirming the obtained structures, then purified by column chromatography by using silica gel as stationary phase and methanol as a mobile phase. The study is derived on the impact of rabbits where they were taking the 40 rabbits with similar weights and were divided into four groups (10 rabbits per group) were as follows, the first group G1 obtained as a control group, which did not gave any things. The second group G2 has injected by aloxane a concentration of 120 mg / kg using syringes medical capacity of 3 ml to inject rabbits in the vein ear and after two hours of injection they were given glucose solution of 10%, the confirmed they injured rabbits diabetes by measuring blood sugar to 10 rabbits have been selected randomly and then it was taken two sets of this group, the third group G3 were given a drug sitagliptin concentration of 10 mg / kg, and the fourth group G4, were given the prepared derivative record a concentration of 8 mg / kg for 3 days and pulled blood samples after the last dose on the third day serum to isolate and carried out the study of biochemical and enzymatic changes were the results of statistical analysis showed a significant decrease in the level of glucose and inhibition for DPP-4 impact of the prepared derivative, and also showed a decrease in the level of cholesterol, triglyceride, LDL and VLDL, while the results showed increase in HDL compared with diabetic group

## CONCLUSIONS

DPP-4 inhibitors are a novel class of orally available molecules for the treatment of type 2 diabetes. they potently reduce blood glucose levels which was obtained by the results below

1. Significant decrease in the concentration of DPP-4 by the effect of G3 and G4 compared with the G2.
2. Significant difference in the concentration of DPP-4 between G3 and G4, this difference due to the activity of the prepared derivative compared with sitagliptin drug toward the inhibition of DPP-4.
3. Significant decrease in the glucose level by the effect of G3 and G4 compared with the G2,
4. The prepared derivative decrease the blood sugar more than the effect of sitagliptin.
5. Significant decrease in the insulin level by the effect of G3 and G4 compared with the G2,
6. The most results of the prepared derivative similar to the results of sitagliptin which means they have a similar properties.
7. inhibitory effect by G3 and G4 comparing with the injury group G2 in the liver enzymes AST and ALT.
8. significant decreases in the levels of cholesterol and triglyceride when in G3 and G4 comparing with G2.
9. significant increased in HDL-Ch level between G3 and G4 comparing with G2.
10. significant decreases in LDL-Ch and VLDL-Ch between G3 and G4 comparing with G2.

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