Preparation of nitrile derivative and study its effect as a possible novel drug for diabetes

Nadia Y Al-Tikrity¹*, Firas SH- Abdulrazzaq¹, Ahmet Beyatli²

¹ College of Science, Tikrit University, Iraq
² University of Health Sciences, Iraq

*Corresponding author: nadiayousif89y@gmail.com (Nadia)

Abstract

The aim of the research is to synthesis one of the captopril derivatives by converting the carboxyl group to the nitrile group, and characterized by (FTIR, 1H-NMR, 13CNMR, Mass spectroscopy and CHNS). This process convert captopril structure design to a compound similar to the modern diabetes drugs (vildagliptin), with continuation of its effectiveness as an antihypertensive drug. After preparation of the modified drug, the effectiveness confirmed by some related enzymes, hormones, and some histological pictures of experiment animals (rabbits). The effects of the derivative on rabbits were studied. Sixty rabbits with weights (1500-1800)g, were divided into six groups (10 rabbits for each group). The first group, G1 consisting of 10 rabbits, is a healthy control group that has not been given any substance. The second group, G2 alloxan group (infected group). The third group gets the alloxan and vildagliptin. The fourth group gets the alloxan and the modified drug compared to vildagliptin dose. The fifth group gets the alloxan and the modified drug compared to captopril dose. The sixth group gets the alloxan and the captopril. After two weeks, the samples were withdrawn after 2 hours from the last dose, the serum was separated and the biochemical and enzymatic variables were studied including (glucose, insulin hormone, glucagon hormone, dipeptidyl peptidase-4, angiotensin converting enzyme, prostaglandin I2, alkaline phosphatase, aspartate amino transferase, alanine amino transferase).

Keywords: nitrile derivative, novel drug, diabetes

How to cite this article: Al-Tikrit NY, Abdulrazzaq FSH, Beyatli A (2020): Preparation of nitrile derivative and study its effect as a possible novel drug for diabetes, Ann Trop & Public Health; 23 (7): 1080-1106. DOI: http://doi.org/10.36295/ASRO.2020.23734

INTRODUCTION:

Diabetes mellitus is the most general metabolic disease and becomes a load burden of public health systems (Ke, 2016), is well known as a chronic metabolic disease that is characterized by a relative or absolute lack of insulin, resulting in hyperglycemia. A variety of complications arises from chronic hyperglycemia such as neuropathy, nephropathy, and retinopathy and increased risk of cardiovascular disease (Al-Awar, 2004). In China, the prevalence of diabetes and prediabetes in adults was 11.6% and 50.1%, respectively (Li, 2004). Damage of beta-cell function and insulin resistance are two essential pathophysiological complications of type 2 diabetes mellitus (T2DM). It has been sure that at the time when T2DM was established, the loss of beta-cell function was shown to reduce by 50% and this decline of beta-cell function progressed over time although traditional antihyperglycemic therapy had been applied (Weng ,2008). In order to postpone the progress of the disease, new therapies are required to persistently act on beta-cell failure and...
insulin resistance. In our previous studies, intensive insulin interventions, especially continuous subcutaneous insulin infusion (CSII), stimulate near-normal glycemia over 1 year without antihyperglycemic agents in nearly half of the patients with newly diagnosed T2DM with the favorable recovery of beta-cell function (Hu, 2011)(chen, 2012). Loss of glucose without compensation leads to depletion of carbohydrate stocks, which causes the body's cells to rely on proteins and fats stored as alternative sources of energy (Bayens, 2015). The normal glucose level is 80-120 mg/dl (Gosh, 2014).

Captopril: Capoten (captopril tablets, USP) is a specific competitive inhibitor of angiotensin I-converting enzyme (ACE), the enzyme responsible for the conversion of angiotensin I to angiotensin II. A white or almost white, crystalline powder, freely soluble in water, dichloromethane R, and methanol R (Ganesan, 2010). Captopril is a white to off-white crystalline powder that may have a slight sulfurous odor; it is soluble in water (approx. 160 mg/mL), methanol, and ethanol and sparingly soluble in chloroform and ethyl acetate. Capoten is designated chemically as 1-[(2S)-3-mercapto-2 methylpropionyl]-L-proline [wm 217.29] and has the following structure: (Akif, 2010)

![Figure (1): chemical structure to the captopril (Belboukhari, 2019)](image-url)

Cardiovascular agent; angiotensin-converting enzyme inhibitor, Captopril may exist in different polymorphic forms, Captopril contains not less than 98.0% and not more than 102.0% of C9H15NO3S, calculated with reference to the dried substance. Captopril derivatives In the present study, several molecules have been designed and synthesized as imidazole derivatives of captopril in such a way that cover those factors necessary for tight binding to ACE with acceptable oral absorption and fewer side effects comparing to captopril. In the captopril molecule, the SH group has a key role in the interaction with the zinc ion of ACE (Masoudi, 2010). However, the alkylthio group on the designed molecules should play that role since the lone pair electrons of sulfur atom is still available. On the other hand, the alkylthio is not expected to produce side effects like the free SH group on the captopril molecule. This study also showed that the synthesis strategy for the imidazole derivatives of captopril, as depicted in Figure (1-1), is quite applicable. That is because it consists of only a few steps that are feasible and afford overall acceptable yields. The present new imidazole derivatives of captopril are promising to give more potent ACEI compounds with better oral bioavailability. Angiotensin-converting enzyme (ACE) plays an important role in the control of arterial blood pressure. The enzyme is responsible for the conversion of the decapeptide angiotensin I into the vasopressor agent, angiotensin II. It is a zinc-containing enzyme that cleaves dipeptide units from its peptide substrate. An important competitive inhibitor of ACE is the captopril, which inhibits the conversion of the relatively inactive angiotensin I to the angiotensin
II. According to the mechanism proposed by Ondetti and colleagues, captopril interacts with the enzyme through several bonds, i.e. electrostatic, hydrogenic and lipophilic connections.

**Dipeptidyl peptidase-4 (DPP-4):**

is a complex enzyme expressed in epithelial cells, capillary endothealias and lymphocytes of the gastrointestinal tract, kidney, liver, and brain.1 It is a ubiquitous serine protease responsible for the degradation of several endogenous peptides including the L-cell-derived peptide glucagon-like peptide-1 (GLP-1).2 DPP-4 transforms the active form of GLP-1 (GLP-1(7-36)) to an inactive form (GLP-1(9-36)) by removing the N-terminal dipeptide, making it incapable of activating its receptor (Rotondo,2019). Dipeptidyl peptidase-4 (DPP-4) inhibition is an established glucose-lowering therapy in type 2 diabetes. It has a low risk of hypoglycemia and other adverse events and is not associated with weight gain. It is used mainly as an add-on to metformin when metformin alone is insufficient for glycemic control, particularly when there is a desire to minimize the risk for hypoglycemia. It is also used as first-line therapy when metformin is not tolerated, in subjects with renal insufficiency and in combination with thiazolidinediones, sodium-glucose transport protein 2 (SGLT2) inhibitors, and insulin. It may also be a possibility for DPP-4 inhibition as first-line glucose-lowering therapy when an islet-directed approach is desirable. The development of the DPP4 inhibition concept for glucose-lowering therapy in type 2 diabetes originated on the fundament of the incretin concept. The term incretin was coined by Starling in the early 1900s to mean a gut hormone that stimulates the internal secretion of the pancreas (Ahren,2019).

**Angiotensin-converting enzyme:**

Angiotensin-converting enzyme (EC 3.4.15.1) or ACE is a central component of the renin-angiotensin system (RAS), which controls blood pressure by regulating the volume of fluids in the body. It converts the hormone angiotensin I to the active vasoconstrictor angiotensin II. Therefore, ACE indirectly increases blood pressure by causing blood vessels to constrict. ACE inhibitors are widely used as pharmaceutical drugs for the treatment of cardiovascular diseases. The enzyme was discovered by Leonard T. Skeggs Jr. in 1956 (Skeggs,1956). It is located mainly in the capillaries of the lungs but can also be found in endothelial and kidney epithelial cells (Abrahum,2007). Other less known functions of ACE are the degradation of bradykinin (Filardi,2015) and amyloid beta-protein.

**Insulin hormone**

(From Latin insula, island) is a peptide hormone produced by beta cells of the pancreatic islets; it is considered to be the main anabolic hormone of the body (Voet, 2011). Insulin is a polypeptide with a molecular weight of 6000 Dalton containing 51 amino acids arranged in two series, A and B. The two chains are linked by disulfide bridges Series A consists of 21 amino acids and B series of 30 amino acids (Smith,2015).
This series is known as the first insulin Pro Insulin is the ineffective form of the hormone It is transferred to the Golgi apparatus and then stored in granules in the cell wall and then excreted from the cell. There are types of insulin used as a medicine divided according to the speed of action and these types are (Hering, 2016):

1- Rapid-Acting Analogue Insulin
2- Short-Acting regular human Insulin
3- Intermediate-Acting (basal) human Insulin
4- Long-Acting (basal) Analogue Insulin

**Blood Glucose**

Polysaccharides are the basic food of humans and are the main source of energy supply to the living cell. Structure of blood groups, enzymatic aid, and nucleic acids, Glucose is the final product of carbohydrate digestion as it is broken down into single sugars absorbed by the body and converted into glucose by several processes, part of which occurs in the liver (Fox,2009), Glucose is a source of energy for tissues and is directly related to many metabolic processes within the body, Any defect in the metabolism of glucose leads to damage and change in many of these metabolic processes (Slatter, 2000). The level of glucose is regulated in the blood by a number of hormones that the pancreas secretes the most important insulin hormone, which works to reduce the level of glucose in the blood and glucagon Glucagon, which works in contrast to insulin and increases the level of glucose in the blood, as with the hormone Adrenaline Adrenaline and Glucocorticoids and growth hormone (Gow,1999).

**Glucagon hormone**

Glucagon is a peptide hormone is a 29-amino acid polypeptide. Its primary structure in humans is NH2-3-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg- Arg-Ala-Gln- Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-COOH., produced by alpha cells of the pancreas. It works to raise the concentration of glucose and fatty acids in the bloodstream and is considered to be the main catabolic hormone of the body (Sandoval,2015).
Prostacyclin

Prostacyclin (also called prostaglandin I2 or PGI2) is a prostaglandin member of the eicosanoid family of lipid molecules. It inhibits platelet activation and is also an effective vasodilator. When used as a drug, it is also known as epoprostenol. (Badesch, 2000) The terms are sometimes used interchangeably (Kermode, 1999). Prostacyclin (PGI2) chiefly prevents the formation of the platelet plug involved in primary hemostasis (a part of blood clot formation). It does this by inhibiting platelet activation.

Aspartate aminotransferase AST

GOT is also called glutamic oxaloacetate transaminase belonging to the class of enzymes [EC.2.6.1.1] transporter of the amine group called transferase, one of the enzymes that catalyze the transfer of the amine group from specialized amino acids (L-glutamate, L-aspartate) into specialized ketoacids (Ketoglutarate, Oxaloacetate). This shift is a key function of protein metabolism (Hall, 2012) This enzyme is present in high concentrations in the heart muscle. The liver and skeletal and renal muscles contain large amounts of it. The clinical significance of the AST level in clinical diagnosis of heart disease, is increased in patients with myocardial infarction due to myocardial infarction AST proceeds from the heart muscle cells to the blood and the level rises significantly during the hours 6-4 hours from the beginning of pain centered in the thoracic region and reaches its peak after 24 hours 36-36 from the beginning of chest pain, where the level reaches 5-8 times more than normal and then starts Gradually decrease to normal levels at the fourth or fifth day of onset. The enzyme level also rises in cases where liver tissue is affected, such as viral hepatitis, liver cancer, cirrhosis, obstructive jaundice, and bile duct obstruction. The high enzyme is attributed to the release of the enzyme from the liver cells to the blood circulation and also rises in the case of muscle atrophy disease. (Thapa, 2007)

Alanine Aminotransferase ALT

This enzyme is a carrier enzyme [EC.2.6.1.2] that stimulates the transfer of the group of amine (glutamate, L-alanine (L-to ketone acids) Ketoglutarate, Pyrurate) both skeletal muscle, heart, and kidney. The clinical significance of ALT in serum lies in its direct relationship to liver disease. Although both AST and ALT are elevated when hepatic cell damage occurs, ALT is the most specific enzyme because of its high levels in liver disease last longer than AST (Abdulrazzaq, 2014).
Alkaline phosphatase ALP

The basal phosphatase belongs to the class of hydrolyzed enzymes [EC.3.1.3.1] that decompose a number of phosphate esters. This enzyme is present in the plasma membrane of most body tissues. Placenta, kidney, brain tissue, liver tissue, and lungs (Narisawa, 2007). The enzyme is also found in animal tissues such as cattle breast tissue and serum (Tajik, 2011), and in microorganisms such as E-coli and Rhizobium sp They are rich sources of ALP (Kumar, 2008) It is also found in plants and their seeds (Kumar, 2010) and when purified it remains active for 6 months at 4 °C and in the presence of a structured solution (5 mM Tris-HCl) and when stored at 25 °C (kept active for ten days) The clinical significance of ALP measurement is to indicate hepatic and bone disease and prevent osteoporosis and children have high levels of enzyme because their bones are growing and ALP activity increased in the case of atherosclerotic and vascular diseases (Junior, 2008). The large part of the enzyme (ALP) is formed in the bone and hepatic tissue and is excreted into the circulatory system and its elevation is observed in pathological conditions: viral hepatitis, biliary tract infection, rickets, osteomalacia, diabetes mellitus (Lamashvili, 2008).

The aim of this study is to synthesis one of the captopril derivatives by converting the carboxyl group to the nitrile group to be a compound similar to modern diabetes drugs

Materials and Methods:

Organic part:

Preparation of captopril Derivative

In a one necked round bottom flask equipped with amagnetic bar, a mixture of (12.2g, 0.1 mol) captopril and (10g,0.11 mol) ethyl carbamate were heated at 75°C with stirring for several minutes, then dropwise addition of (7.3ml, 0.1 mol) SOCl2 over a period of about 0.5 h. Then (20 ml) of chloroform has been added to the mixture and refluxed with a stirrer at 75-80°C for 4 h. The progress of the reaction was monitored by TLC. Upon the completion, the solids were filtered and the organic phase was evaporated under reduced pressure, purified by column chromatography to obtain the final purely. The compound was characterized by FTR, 1HNMR, 13C NMR, mass spectroscopy and eliminated analysis (CHNS) to confirm the final composition of the product, and purified by column chromatography to obtain the final product purely.

Biochemistry Part

Local male rabbits were used in this study obtained from local markets, with ages ranged between 6-8 months, and weights ranged between (1000-1800 g), were used in this study. It was left for two weeks to adapt to new environmental conditions. The experimental animals were divided randomly into six groups, each group of 10 animals, and were treated (10) days after the onset of diabetes as follows:

Group1: The control group was given normal drinking water and no substance was given.

Group 2: a group of diabetic rabbits injected with alloxan.
Group 3: a group of diabetic rabbits dosed with vildagliptin in a dose of 2 ml (0.00417 gm / kg).

Group 4: a group of rabbits with diabetes dosed in the compound prepared with a dose of 2 ml per rabbit (0.0015 gm / kg) compared with the vildagliptin.

Group 5: Diabetes rabbits group prepared with the compound prepared at a dose of 2 ml per rabbit (distilled) (0.068441 gm / kg) compared to captopril.

Group 6: a group of diabetic rabbits dosed with captopril in a dose of 2 ml (0.07497 gm / kg).

At the end of the treatment which lasted for week, the blood sample was withdrawn on the seventh day from each rabbit and the serum was separated to study Biochemical variables and enzymatic.

**Estimation of Biochemical Variables**

The concentration of hormone Glucagon, ACE, DPP4, PGI	extsubscript{2} was determined by Elisa Technology according to the Bioassay Technology Laboratory from (China) and Insulin hormone serum was determined by Elisa Technology according to the Elabscience from (USA). The concentration of ALP, ALT, AST were estimated using a Special analysis by optical spectroscopy technique from Linear (Spain).

**Statistical Analysis**

The purely results were analyzed using and determined the differences between groups using the one-way analysis of Anova Duncan test (Duncan, 1955).

**RESULTS AND DISCUSSION**

**Organic part:**

synthesis one of the captopril derivative by converting the carboxyl group to the nitrile group to afford a compound similar to modern diabetes drugs like (gliptins, sitagliptin, saxagliptin, vildagliptin, linagliptin, and alogliptin, or in addition with metformin, sulfonylurea, or Thiazolidinedione, this treatment is similar to the other oral antidiabetic drugs) (Chaudhury, 2017)(Pareek, 2019). The captopril derivative (1-(3-mercapto-2-methyl propanoyl) pyrrolidine -2-carbonitrile) was prepared by the reacted of urithane with captopril in chloroform as a gum as a solo product, m.p 75°C. The reaction has been confirmed by the thin layer chromatography (TLC) through using a thin paper of silica gel as a stationary phase, while the mobile phase consists a mixture of n-hexane and ethyl acetate in aration of (1:4) It was valuable Rf=0.9 to the captopril derivative, As the following equation:

The chemical structure of the prepared compound was confirmed using spectral methods (FTIR, 1H-NMR, 13CNMR, Mass spectroscopy and CHNS) and as shown in the following forms:
Where the FTIR spectrum of the compound prepared is noted in figure (4):

![Fig 4: IR spectrum of the captopril derivative](image)

The most noteworthy spectroscopic feature is the unexpected observation of a strong absorption band at 2133 cm$^{-1}$ assigned to $\nu$(C$\equiv$N) the cyanide group (Nakamoto, 1986) (Silverstein, 1997). Such compound result from the converted the carboxyl group to nitrile group. The stretching vibration of $\nu$(C=O) appeared in the IR spectrum at 1718 cm$^{-1}$. The spectrum also displayed a strong band at 1467 cm$^{-1}$, 1573 cm$^{-1}$ and (2960, 2875) cm$^{-1}$ for the $\nu$(C=N), $\nu$(C=C) and $\nu$(CH)aliphatic respectively (Nakamoto, 1986)(Badertcher, 2009). A band at 2557 cm$^{-1}$ attributed to the stretching vibration of the thiol group $\equiv$(S-H)

The spectrum of the 1H-NMR was studied as shown in Figure(5) using DMSO as a solvent. It was measured in units (ppm).

![Fig. 5: 1H NMR spectrum of the captopril derivative](image)

The 1H NMR spectrum of the captopril derivative (Fig. 5) displayed a doublet peak at $\delta$ 1.06 ppm with coupling constant to the neighboring proton (CH) ($3J_{H-H}$ = 4.25 Hz) assigned to the protons...
of the methyl group. The signals of the protons of the CH and CH2 groups for the side chain appeared as unresolved multiplets at □2.14ppm and □2.42ppm, integrations indicate that each represents one and two protons, respectively.

Also, the spectrum clearly showed the protons of the heterocyclic ring as four signals, three of these signals are as unresolved multiplets at □2.19ppm, □2.70ppm and □3.57ppm, assigned to the protons in positions 3, 2 and 4 respectively. Whereas the proton in position 1 appeared as triplet peak at □2.25ppm (3JH-H= 8.00Hz). And the proton of the thiol group (SH) appeared as a single peak at □3.40ppm.

13C-\{1H\} NMR spectrum of the captopril derivative

![13C-\{1H\} NMR spectrum of captopril derivative in CDCl3](image)

The 13C-\{1H\} NMR spectrum of the captopril derivative (Fig. 6) is in good agreement with the suggested structures. The spectrum showed a characteristic peak for the carbon of (C□N) group at □88.59 ppm \(^{40,42}\) Whereas the carbon of the carbonyl group (C=O) appeared at □172.02 ppm. And the CH3, CH2 and CH of the side chain showed at □15.99, □26.57, and □40.57 ppm respectively. While the peaks of the carbon atoms of the heterocyclic ring appeared at □23.84, 28.17, 45.97 and 57.72 ppm assigned to C3, C2 C4 and C1 respectively.

The elemental analysis (CHNS) is listed in Table 1, and the experimental analysis is agreed well with the calculated date of the captopril derivative.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calc. %</td>
<td>54.520</td>
<td>7.120</td>
<td>14.130</td>
<td>16.170</td>
</tr>
<tr>
<td>Found %</td>
<td>54.010</td>
<td>7.561</td>
<td>14.56</td>
<td>15.975</td>
</tr>
</tbody>
</table>

Table 1: The elemental analysis of the captopril derivative

ESI-Mass spectra of captopril derivative
The ESI-Mass spectrum of captopril derivative showed the m/z (relative intensity) at 198.963 (198.08) g/mole found (calculate), agree well with the calculated molecular weight (Fig. 1-5)

Biochemical variables

Concentration of Glucose and Insulin

The concentration of (Glucose mg/dl) (Insulin µu/ml) was measured as showed in Fig 8 control groups (healthy) G1, Alloxan treatment group (infected group) (G2), vildagliptin treatment group (G3). The Prepared treatment compared to vildagliptin (G4). The Prepared treatment compared to captopril (G5), and captopril treatment group (G6), respectively.

The results showed a significant differences between G1,G2,G3,G4,G5 and G6 and the levels of them (120.20±7.20) (178.50±69.94) (122.30±24.08) (122.70±21.33 ) ( 112.10±18.84) (130.06±39.94) respectively. It was noted that the level of glucose in the G2 is significantly higher compared to the (G1) because alloxan is characterized by its high toxicity, which works to break down the cell of the pancreas (inhibits the secretion of insulin) which is responsible for the provide the glucose to the cells (Musa,2015), or because of an imbalance in the responsible receptors of cells for the entry the glucose into the cells, due to the oxidative intensity of free radicals represented by unstable oxygen molecules; causes increasing in the level of blood glucose after treatment with alloxan (Ahmed,2015). The results showed no significant differences between (G3), and the (G4), which confirms the success of the desired target.

The results showed no significant differences between (G5), compared with G1, this result consistent with a study of diabetic rats that have been given captopril sins the captopril inhibits intracellular accumulation of glucose (Wakisaka,2019), also the results showed that there were no significant differences between (G5) compared with group (G6) means that the modification of the antihypertensive drug Achieve the target and work as anti diabetes drug too.
The results of insulin showed a significant differences between G1, G2, G3, G4, G5 and G6, and the levels of them (17.18±6.33), (11.18±5.77), (5.94±3.91), (4.09±2.34), (1.57±0.84), (5.62±2.98) respectively. We noted that the level of insulin concentration in the (G2) (diabetic group) is significantly lower compared to the (G1), because alloxan has destroyed the beta cells in the pancreas, so it is unable to produce enough insulin, and thus reduces its secretion and instead of transferring sugar to the cells it needs to obtained the energy, sugar builds up in the bloodstream that leads to diabetes (Etuk, 2010). In the G3, insulin concentration was significantly lower compared to (G2), this is confirmed by some studies using vildiglipatin as a treatment for insulin resistance and improve its sensitivity, as it is used as a concomitant treatment to reduce the concentration of insulin, and keep low level of glucose in the blood (Lavine, 2010). As for Treatment of (G4) there was no significant difference compared to the group (G3) (vildiglipatin) which acted to reduce the level of insulin concentration and thus reduce the level of glucose which confirms the success of the desired target derivative prepared to using it as a drug for diabetes. We noted that the level of insulin concentration in the (G5) is significantly lower to the G1 indicates that the prepared treatment improves cell sensitivity to insulin by decreasing the level of it.
Figure 8b: Insulin concentration between study group

The Concentration of activity (ALP, ALT, and AST)

The Activity of (ALP U/L), and (ALT,AST U/L) was measured as shown in Fig 9a control groups (healthy) G1, Alloxan treatment group (infected group) (G2), vildagliptin treatment group (G3). The prepared treatment compared to vildagliptin (G4). The prepared treatment compared to captopril (G5), and captopril treatment group (G6), respectively. The result of the ALP enzyme activity showed a significant difference between (G1, G2, G3, G4, G5 and G6), (31.00 ± 12.63), (61.20 ± 13.83), (47.50 ± 14.54), (50.80 ± 11.78), (38.10 ± 16.16) and (43.20 ± 12.54) respectively, our results were consistent with the results of (Motshakeri, 2014), as that the effectiveness of the enzyme in the group with diabetes (G2) increased compared to the (G1).

Recent studies showed that the activity of ALP increases with the increase in blood sugar, this increase in the activity of ALP is usually due to many disease conditions such as liver, or bile duct disease or the presence of gallstones. The rise of ALP may indicate bone disease such as Osteopenia or Osteoporosis as well as Blood disorders also cause an increase in the activity of this enzyme, in addition to some cancerous conditions and high levels of glucose lead to liver damage (Guimaraes, 2007)

The increase in serum alklin phosphatase activity in the case of diabetes can be the result of increased enzyme activity rather than glycolysis and the phosphate-6-Glucose oxidation pathway which causes liver cell degradation and damage (Elhabashy, 2011) and blockage of bile ducts inside the liver. It leads to a high activity of the alkalin phosphatase enzyme, the alloxan lead to break down the membrane phospholipids by oxidizing the fats present in the membrane of the hepatic cells which leads to the release of enzymes (ALP, AST,ALT) in blood stream (Abdulazeez, 2015). ALP Among the evidence widely used for bone metabolism, it provides a good impression of the extent of new bone formation, and the activity of osteoblasts of the bone, Osteoblast, and was not consistent with the results of the study, which indicated that no change in the activity of the enzyme occurred in the healthy G1 compared to the G2 (ISSA, 2011) and G3, we note that there is a significant difference between it and the G1, our result did not agree with the researcher’s findings, (Ajaet, 2015), as they indicated that the level of effectiveness of the alkalin phosphatase enzyme did not change in rats with diabetes.

In the G4, we notice a significant difference between it and the G1. The result for G6, we noticed that there were no significant differences between it and the G1. We also note that there were no significant differences in the G5 with the (G1) which means the prepared derivative and vildagliptin improve the liver tissue that affected by alloxan.
The activity of AST enzyme, the results showed significant differences between (G1, G2, G3, G4, G5 and G6) respectively (11.77 ± 5.85), (38.95± 6.52),(12.90 ± 6.25),(14.78 ± 8.57), (15.15 ± 7.92), and (15.72±7.56). The rate of enzyme efficacy in the (G2) diabetic group was significantly higher compared to the (G1). This is mainly due to the breakdown of the hepatocytes, which is usually accompanied by a high level of enzyme, this observation corresponds to many studies conducted on laboratory animals developed with diabetes by alloxan, where there are increase in the level of this enzyme causing diabetic development; leading to increased metabolism and enlargement of hepatocytes, and stimulation of the endoplasmic reticulum to produce a larger amount of enzyme commensurate with cell size, hormonal disturbance and impaired metabolism in a diabetic patient, usually accompanied by activity of Enzyme ,( Ajaet,2015).

(G3), we noticed a significant decrease in the activity of the enzyme compared to the (G2), as it effect to regulate the proportion of the enzyme and repair the damage that occurred in the liver. (Tung,2011) We noted that there were no significant differences for the activity of the enzyme of the group (G4) compared (G3), which indicates that the compound prepared worked to repair the damage to the liver and the enzyme-release systems outside the cell are similar to the action of vildagliptin. The results in G6, showed no significant differences between it and the G1. Also note that there were no significant differences in the G5 with the G1.

![Fig9b: AST concentration between study group](image_url)

The activity of the enzyme (ALT), no significant difference was observed between (G1, G2, G3, G4, G5and G6) the levels of them ((18.61 ± 15.60), (15.40 ± 6.22) (21.28 ± 11.14) (14.55 ± 6.61) (13.80 ± 4.37), and (16.94 ± 6.51) respectively. It was observed from the table that there were no significant differences in the rate of enzyme activity in the (G2) compared to the G1. These results
are incompatible with a study conducted high enzyme level in diabetic animals compared to healthy animals where alloxan function to increase the activity of Transaminase enzymes in both the liver and serum (Nishra, 2017). The activity of enzyme has not affected with vildagliptin and the G4. It was noted that there were no significant differences in the activity of the enzyme in (G6) and (G5) compared to the (G1).

![Fig 9C: ALT concentration between study group](image)

**Concentration of Glucagon and PGI2**

The concentration of (Glucagon µu/ml) (PGI2 µu/ml) was measured and Fig 10a demonstrates a levels of it in in control groups (G1), the Alloxan treatment group (G2), and vildagliptin treatment group (G3). Prepared treatment compared to vildagliptin (G4), prepared treatment compared to captopril (G5), and that captopril group (G6).

The results of the concentration of the hormone glucagon showed no significant differences between (G1, G2, G3, G4, G5, and G6), the levels of them (374.67 ± 137.11), (273.03 ± 73.10), (210.85 ± 100.05), (279.47 ± 113.73), (233.48 ± 102.09), and (233.48 ± 102.09) respectively finding, as shown in Figure 10a, consistent with the results of researchers Campbell and Drucker and Compbell, 2015 (Wang, 2013), and inconsistent with the results of Beljic, 2016 which they indicated that the level of glucacone decreased in the group with diabetes induced by alloxane compared to the control group. Moran (Moran, 2011) have indicated that an increase in the level of glucagon can cause stimulation of the production of new glucose and the process of glycogenolysis increased concentrations of circulating plasma glucose (Lee, 2011). The results also showed that there were no significant differences between (G3) and G1, as well as the (G4). There were no significant differences between it and G1.
The results also showed no significant differences between (G6) and (G5) compared G1.

Figure 10a: Glucacon concentration between study group

The results of the (PGI$_2$ µu/ml) showed a significant difference between G1, G2, G3, G4, G5, and G6, the level them (81.86±20.89), (124.81±66.59), (39.85±19.34), (65.79±22.79), (28.97±20.22) and (77.72±22.10) respectively. Noted from the table the presence of significant differences in the concentration of PGI$_2$ in G2 compared to G1. PGI$_2$ is produced by endothelial cells and a lowering of release has been reported in diabetic (Colwell, 1988) vascular injury and glycemic control may also influence PGI$_2$ synthesis (Jeremy, 1983)(Jeremy, 1984)(Jeremy, 1985). Prostacyclin have been investigated in a few studies that assessed their role in the pathogenesis of diabetic and were significantly reduced in patients with type 2 diabetes (Kalogeropoulou, 2002). There are a direct relationship between insulin levels and blood pressure through a broad range of insulin levels, mediated by the inhibitory effect of insulin on production prostaglandins PGE$_2$ and prostacyclin PGI$_2$, two potent vasodilators by adipose and possibly other tissue (Vong, 2018) PGE$_2$ and PGI$_2$ production by normal adipose tissue in vitro is increased in the absence of insulin and decreased in a dose-response manner by insulin (Axelrod, 1983)(Willis, 2017) these findings and a variety of in vivo observations in rats and in humans suggest that adipose tissue is the principle source of the elevated levels (Axeirod, 1982)(Axeirod, 1986) of these two potent vasodilators in diabetic a condition caused by sever insulin deficiency increased production of PGI$_2$ and PGE$_2$ by adipose tissue may explain the decreased vascular resistance and hypotension of diabetic (Vong, 2018). The results also showed that there were no significant differences between (G3), and (G1), as well as (G4), no significant differences between it and compared (G1). The results showed there is no significant difference between (G6) and G5 compared (G1) that caused by the damaging in the endothelial cell, which may lead to increased synthesis of PGI2 in blood.
Concentration of (ACE, and DPP4)

The concentration (ACE $\mu$u/ml), and (DPP4 $\mu$u/ml) was measured, and Fig 11a show its level for all groups (control groups G1, the Alloxan treatment group G2, vildagliptin treatment group G3, prepared treatment compared to vildagliptin G4, prepared treatment compared to G5, and that captopril group G6. The results of (ACE $\mu$u/ml) level were observed and showd a significant difference between (G1, G2, G3, G4, G5, and G6), the levels of them (57.37±20.93), (61.51±16.95), (41.41±3.96), (49.33±9.35), (42.02±4.13) and (49.58±18.01) respectively. noted through the table that there were no significant differences in ACE concentration between the (G1) and (G2), The use of angiotensin-converting enzyme (ACE) inhibitors in the treatment of diabetic nephropathy disease progression the end stage renal failure disease (ESRF) (Mogensen,1982)(Parving,1983) similar effects have been reported for other anti-hypertensive agents, but during recent years ACE inhibitors have become the drugs chosen in the treatment of diabetic nephropathy (Borch,1996). ACE have been effectively used in the treatment of high blood pressure, for the prevention of cardiovascular complication and treatment of hypertension, these drugs have shown beneficial effects in reducing macro vascular complication improving insulin sensitivity and glucose metabolism in T2DM patient (Rahmun,2017) (Malkin,2019). The results also showed that there were no significant differences between (G3), compared (G1), as well as (G4), there were no significant differences between it compared (G1),
the results show no significant differences between (G6), and (G5) comparative with (G1), this is clear evidence for drug acting as antihypertensive.

**Fig11a: ACE concentration between study group**

The result of the enzyme DPP-4 activity showed no significant differences between (G1, G2, G3, G4, G5 and G6.) and the level of them (7.84 ± 1.92), (7.23 ± 1.51), (7.55 ± 0.72) (6.85 ± 3.00), (6.00 ± 2.55), and (7.09 ± 2.11), respectively. This is inconsistent with the results of the researchers (Matteucci and Giampietro, 2009) (Varga, 2011), (Lee, 2013), (Kirino, 2012) found that when rats had exposure to induced diabetes by alloxan; the level Dpp-4 increase, and in the as indicated by the researcher. (Pala, 2003) that hyperglycemia destroys cells in the liver caused by a deficiency of the enzyme Dpp4. (Lamers, 2011) found that the level of Dpp-4 increases in obese patients with metabolic symptoms, as it assumed that the expression Dpp-4 in visceral adipose tissue is more noticeable compared to subcutaneous fat, and that visceral Dpp-4 may be a sign for insulin sensitivity. In the G3, found that there were no significant differences compared (G2), and this result does not agree with studies on diabetes rats, where there was a decrease in the level of DPP-4, due to the fact that Vildagliptin is an enzyme inhibiting drug that lowers blood sugar (Fadini,2012). In G4, there were no significant differences compared to group (G2) indicating that the derivative prepared from vildagliptin has not affective on the activity of the enzyme. In the G6, we noted that there were no significant differences between the induced diabetes group by alloxan G2, the levels of these enzymes were not affected by the drugs used.
Docking results:

Docking results of three compounds against DPP4

Table 1-2. Docking results of proposed compounds against DPP4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dipeptidyl peptidase-4 (DPP4) docking score</th>
<th>RMSD (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>-5.637</td>
<td>0.047</td>
</tr>
<tr>
<td>2nd</td>
<td>-3.902</td>
<td>0.05</td>
</tr>
<tr>
<td>3rd</td>
<td>-4.053</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Figure 12. The interaction between 1st compound and Dipeptidyl peptidase-4 (DPP4)
To distinguish the inhibitory activity by covalent bonding of three proposed compounds against both of angiotensin converting enzyme (ACE) and Dipeptidyl peptidase-4 (DPP4) molecular docking. As shown in Figure 12. That 1st formed one covalent bond with bond length of 1.38 Å between Ser630 and carbon atom that caring double bond with oxygen atom of carboxyl group via Nucleophilic Addition to a Double Bond. Besides, 1st interacted by forming four hydrogen bonds with Tyr547, Tyr631, Asn710 and His740 of DPP4. The docking score of 1st compound was the lowest value (-5.637 kcal/mol) if it compared with 2nd and 3rd compounds due to the hydrogen bandings. The results of molecular docking for 2nd reveals that a significant decrease in the docking score (-3.902 kcal/mol), resulted from the decrease in the number of hydrogen bonds (two hydrogen bonds) despite its ability to form covalent bond with Ser630 (Figure 13) via Nucleophilic Addition to a Triple bond of nitrile carbon. What is interesting about the data in this table 1 is that 3rd has the lowest number of hydrogen bonds but its docking scores lower than 2nd compound. It can be concluded that 1st compound much more promising compound than 1st and 2nd compounds against DPP4.

Figure 13. The interaction between 2nd compound and Dipeptidyl peptidase-4 (DPP4)
Docking results of three compounds against ACE

Table 3. Docking results of proposed compounds against ACE

<table>
<thead>
<tr>
<th>Compound</th>
<th>Angiotensin converting enzyme (ACE)</th>
<th>Score (kcal/mol)</th>
<th>RMSD (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td></td>
<td>-4.507</td>
<td>0.05</td>
</tr>
<tr>
<td>2nd</td>
<td></td>
<td>-4.345</td>
<td>0.033</td>
</tr>
<tr>
<td>3rd</td>
<td></td>
<td>-5.208</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The table below illustrates that the rank of docking scores of suggested three compounds against ACE, are coming in this order 3>1>2 whereas the ranking order of selected compounds was 1>3>2. (Figure 15) shows that 1st interacted through one covalent bond (1.38 Å) between Ser333 and carbon of carboxyl group, and two hydrogen bonds with both of His331 and Ala332. Despite of the length of covalent bond between 2nd compound and Ser333 of ACE (Figure 16) is shorter than the covalent bond between 1st and Ser333 of ACE. Also, the number of hydrogen bonds of 2nd compound with ACE little higher than the number of hydrogen bonds between 1st and ACE. As illustrated in the Figure 16 that 2nd formed three hydrogen bonds with Thr496, Ala332 and His331, along with covalent bond between Ser333 and 2nd compound. The 3rd compound has lowest docking score of under investigated compounds (-5.208). Where it is forming four hydrogen bonds with Gln259, Lys489, His491 and His333 in addition to covalent bond between Ser333 and carbon atom nitrile group via Nucleophilic Addition to a Triple bond (Figure 17). It can be been seen that 3rd compound interacted with the vital residues with high number of hydrogen bonds. This makes the 3rd compound more stable inside the pocket of ACE also can be good starting scaffold to design much more effective inhibitors against both of ACE and DPP4.

Figure 14. The interaction between 3rd compound and Dipeptidyl peptidase-4 (DPP4)
Finally, both of 1\textsuperscript{st} and 3\textsuperscript{rd} compounds are considered as good two starting scaffolds for design good irreversible inhibitors against both DPP4 and ACE.

Figure 15. The interaction between 1\textsuperscript{st} compound and Angiotensin converting enzyme (ACE).

Figure 16. The interaction between 2\textsuperscript{nd} compound and Angiotensin converting enzyme (ACE).
CONCLUSIONS

decrease in the level of (Insulin) at G4 and G5 compared to G2, G3 with low significant with G1 which indicates that the prepared treatment improve cell sensitivity to insulin by decreasing the level of it. no significant difference in (DPP4) concentrations in all groups, so it means that the levels of these enzymes were not affected by the drugs used. decrease in the level of (glucose) at G4 and G5 compared to G2, with no significant difference with G1 and G3, which means that the modification of the antihypertensive drug Achieve the target and work as anti diabetes drug too, significant decrease in the level of (ALP) at G3 and G5 compared with G2, and reach to the normal range which means that the prepared derivative and valdigliptin improve the liver tissue that affected by alloxan. significant decrease in the level of (AST) at G3, G4, G5 and G6 compared with G2 and return to the normal level with G1. no significant difference in the level of (ALT) between all groups. significant decrease in the level of (Glucagon) at G3, G4 and G5 compared with G1 and the G5 outperformed the other groups. significant decrease in the level of (PGI2) at G3, G4 and G5 compared with high level of G2 that caused by the damaging in the endothelial cell, which may lead to increased synthesis of PGI2 in blood decrease in the level of (ACE) at G5 compared with G1 and G2, this is clear evidence for drug acting as antihypertensive. The effectiveness of the compound prepared for use as a treatment for type 2 diabetes to reduce blood glucose levels is higher than the effectiveness of the drug alone.

REFERENCES


19- Smith C., Marks A. D. and Lieberman M. (2015). Basic medical Biochemistry . 4nd  
20- Ji, L., Min, K. W., Oliveira, J., Lew, T., & Duan, R. (2016). Comparison of efficacy and  
safety of two starting insulin regimens in non-Asian, Asian Indian, and East Asian patients  
with type 2 diabetes: a post hoc analysis of the PARADIGM study. Diabetes, metabolic  
syndrome and obesity: targets and therapy, 9, 243.
21- Hering, B. J., Clarke, W. R., Bridges, N. D., Eggerman, T. L., Alejandro, R., Bellin, M.  
D., ... & Kaufman, D. B. (2016). Phase 3 trial of transplantation of human islets in type 1  
Livingstone: p96-150.
glucagon and GLP-1 in health and disease. Physiological reviews, 95(2), 513-548.
hypertension due to the scleroderma spectrum of disease: a randomized, controlled trial.  
Annals of internal medicine, 132(6), 425-434.
27- Kermode, J., Butt, W., & Shann, F. (1991). Comparison between prostaglandin E1 and  
28- Hall, P., & Cash, J. (2012). What is the real function of the liver ‘function’tests?. The  
Ulster medical journal, 81(1), 30.
Parameters And Liver Enzymes. kufa Journal for Nursing sciences, 4(1), 204-207.
31- Narisawa, S., Harmey, D., Yadav, M. C., O'Neill, W. C., Hoyaerts, M. F., & Millán, J. L.  
(2007). Novel inhibitors of alkaline phosphatase suppress vascular smooth muscle cell  
32- Tajik, J., & Tahvili, S. (2011). Serum Alkaline Phosphatase and Amylase Activities in  
Subacute Ruminal Acidosis in Dairy Cows. Asian Journal of Animal Sciences, 5(2), 153-  
157.
34- Kumar V.P., Prasanthi S., Reddy A.C., Raj, N.D. and Anudeep L.,(2010), "Characterization  
studies of thermostable alkaline phosphatase from various plant seeds", Journal of Applied  
Biosciences,36:2403 – 2408.
M.L.,(2008),"Purification and biochemical characterization of thermostable alkaline


