University Of AL-Qadisiya

College OF Pharmacy

Effect of Some Oral Hypoglycemic Agents on Serum Lipid Profile in Patients with Type 2 Diabetes Mellitus in Al-Diwaniyah Province

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بِسْمِ اللهِ الرَّحْمنِ الرَّحِيم

(قالَ رَبِّ اشْرَحْ لي صَدْري (٢٥) وَيَسِرْ لي
أمْري (٢٦) وَاحْلُلْ عُقْدَةً مِنْ لِساني (٢٧)
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صَدق اللهُ العَلي الْعَظِيمِ

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Dedication

To my Mother

To my Father and and my brothers

Supervisor Certification

I certify that this research was carried under my supervision at the College of Pharmacy/ University of AL-Qadisiya, as a partial fulfillment of the requirement for the B.Sc in Pharmacy.

Dr. Ghufran Mohammed Hussien

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In review of the available recommendation, I forward the present research for debate by the examining committee.

Dr. Ghufran Mohammed Hussien

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Zaineb Resool

Zaineb Hur

Summary

The present study was designed to assess the serum lipid profile concentration in type 2 diabetes mellitus and control group and to evaluate the effect of Metformin tablet and Metformin + Glibenclamide tablet on serum lipid profile concentration in type 2 Diabetes Mellitus. To achieve this aim , a 60 patients with type 2 diabetes mellitus with ages range between (35-65) years are taken and 30 apparently healthy subjects with ages range between (35-65) year. Serum glucose and lipid profile concentration were determined by of enzymatic (spectrophotometric) method.

The results of present study show significant elevation in fasting glucose, total cholesterol, triglyceride, VLDL-cholesterol and LDL-cholesterol levels in type 2 diabetic patients (Metformin group and Metformin + Glibenclamide group) when compared to those of the control group. However, HDL-cholesterol was observed to be lowered significantly in type 2 diabetic patients (Metformin group and Metformin + Glibenclamide group) when compared to those of the control group.

Also, the result of present study show non significant changes in fasting glucose, total cholesterol, HDL-cholesterol, triglyceride and VLDL-cholesterol levels in type 2 diabetic patients (Metformin) group when compared to those of type 2 diabetic patients (Metformin + Glibenclamide). However, LDL-cholesterol was observed to be lowered significantly in type 2 diabetic patients (Metformin + Glibenclamide) groupwhen compared to those of the type 2 diabetic patients (Metformin) group.

In conclusion, type 2 diabetic patients (Metformin +Glibenclamide) group showed a significant reduction (favorable effect) in LDL-cholesterol when compared with type 2 diabetic patients (Metformin) group.

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Chapter One

Introduction and Literature Review

1.1-Diabetes Mellitus

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, resulting from impairment of insulin secretion and/or action (1). Hyperglycemia may lead to irreversible damage in a wide range of tissues, particularly the retina (diabetic retinopathy), the kidney glomeruli (diabetic nephropathy), the neural tissue (diabetic neuropathy) and blood vessels (2). Several characteristic symptoms may be present with DM such as polyuria (frequent urination), polydipsia (increased thirst) andpolyphagia (increased hunger). In severe cases of DM, ketoacidosis or a non ketotic hyperosmolar state may develop and it leads to coma and death in the absence of treatment (3).

1.1.1-Classification

The classification of DM based on pathophysiology (4) listed in Table 1.1.

Table 1.1: Classification of DM Based on Pathophysiology (4)

Type	Etiology
Type 1 DM	Destruction of pancreatic beta cells that lead to absolute insulin deficiency
Type 2 DM	Insulin resistance and/or impairment of insulin secretion
Gestational DM	Insulin resistance and insulin deficiency during pregnancy
Other types of DM	Diseases of pancreas: pancreatitis
	Endocrinopathies: Cushing syndrome and acromegaly
	Drug Induce DM: glucocorticoids and β-Adrenergic
	Infections: cytomegalovirus, rubella and others

1.1.1.1-Type 1 Diabetes Mellitus

Type 1 diabetes mellitus represents only 5-10% of all cases of diabetes, previously termed as insulin dependent diabetes mellitus. Type 1 diabetes mellitus resulted from pancreatic β cells destruction resulting in insulin deficiency (5). It is characterized by absolute dependence on exogenous insulin to prevent ketoacidosis (6).

Type 1 diabetes mellitus includes two forms; type 1a DM is an immune mediated disease and it is characterized by autoimmune markers such as islet cell antibodies and insulin autoantibodies. About 85-90% of type 1 diabetic patients are positive for one or more of these markers (7). Type 1b DM is also called idiopathic diabetes (unknown causes).

1.1.1.2-Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is a non autoimmune, complex and polygenic metabolic disease. It previously termed as non insulin dependent diabetes mellitus. It's essential defects resulted either from insulin resistance with relative insulin deficiency or from insulin secretary defect with insulin resistance (8,9).

The majority of type 2 DM patients do not primarily need insulin therapy. The frequency of type 2 DM is more occurrence in adults than in children. The incidence of this disease is increased with age, particularly after 40 years old (10).

1.1.1.3-Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is any degree of glucose intolerance with first diagnosis during pregnancy. Gestational diabetes mellitus generally is diagnosed during the second or third trimester of pregnancy (11).

It occurs in about 4% of pregnancies. After pregnancy ends, the glucose tolerance generally returns to normal within six weeks, at this time the woman should be reclassified. The majority of GDM patients do not develop DM but some will develop type 2 DM (12)

1.1.2-Epidemiology

Type 2 diabetes mellitus is the most prevalent form when it is compared to other two major types of diabetes. It is responsible for 90% of the overall diabetes prevalence (13). The incidence of the disease has been rising rapidly in the past years. In the western world, type 2 DM has virtually become epidemic due to the typical western life style of sedentary behavior and high calorie diet. However, the diabetes rates are increasing also in non western countries (14)

Diabetes mellitus is one of the most common chronic diseases in human populations. The prevalence of DM are 6.5% that represent 285 million of adults in 2010. Furthermore, the diabetes prevalence persists to increase in both the western world and in the developing countries, this increase in prevalence of DM resulted from change in lifestyles that lead to reduce physical activity and increase obesity. Therefore, predictions for the next 20 years show that the prevalence of DM are 7.7% that represent 439 million of adults in 2030 (15).

1.1.3-Pathophysiology

Type 2 diabetes mellitus is characterized by the combination of disturbances in insulin secretion by pancreatic β cells and peripheral insulin resistance (16). Insulin resistance is caused by defects in the signaling pathways that process the insulin signal in its target tissues (2). Normally, plasma glucose levels are maintained within a narrow and well balanced range, known as glucose homeostasis (16).

The rise in blood glucose level after meal stimulates insulin secretion from β cells of pancreas. Insulin lower the blood glucose level by increasing the glucose uptake and utilization by several tissues such as skeletal muscle and fat cells. This rise in glucose level also inhibits glucagon release and inhibit glucose production from other sources,i.e,inhibiting glycogenolysis and gluconeogensis (17). The liver is a major organ that consumes glucose and regulates blood glucose level. It receives glucose rich blood from the digestive tract directly via the portal vein and it rapidly dispose large amounts of glucose from the circulation after a meal (18).

In type 2 DM, as a consequence of impaired insulin secretion and/or resistance, glucose uptake and release by essential tissues is disturbed, which eventually results in hyperglycemia (16, 19). It has been suggested that the disease begin with insulin resistance and is followed by increased insulin production by the pancreatic β cells to maintain glucose homeostasis. At a later stage, due to the long term compensation mechanism by the β cells tokeep up with the higher insulin demand, these cells ultimately undergo further damage. When the ultimate demand of

insulin release cannot be satisfied, the resultare higher plasma glucose levels (2, 20).

1.1.4-Etiology

Type 2 DM causes involve both genetic and environmental factors. Furthermore, the overall disease risk is determined by the interaction of the genetic background with a variety of environmental exposures encountered during each individual's life (21). Several factors are recognized.

1. Environmental factors

A number of environmental factors are recognized to be important to the development of type 2 diabetes. The environmental factors include obesity, reduced physical activity, diet, stress, environmental toxins (21).

2. Genetics

The common form of polygenic type 2 diabetes is a complex disease, the genetic risk being influenced by the conjoint effects of variation at an undetermined number of genomic sites, some with a predisposing and some with a protective effect (22). Also type 2 DM is multigenic, meaning that many different combinations of gene variants may exist among type 2 DM patients, leading to a similar disease phenotype (23). Most diabetic cases involve many genes.

3. Medical conditions

Several medications and health problems have been indicated to prompt DM (24) like glucocorticoids (25). A woman who has formerly had GDM are with greater risk for development of type 2 diabetes (26). Further health problems are also associated with diabetes such as

Cushing syndrome and acromegaly. Certain types of cancers such as glucagonomascan cause diabetes (24). Testosterone deficiency is also associated with type 2 diabetes (27).

1.1.5-Signs and Symptoms

Both types of diabetes have similar symptoms but they differ in their aggressiveness. Type 1 diabetes developed more quickly and more typically while in type 2 diabetes, the symptoms are insidious in onset and the majorityof cases may be diagnosed because of complications or incidentally (28, 29). The symptoms include polyphagia, polydipsia, polyuria, dehydration, unexplained weight loss, blurred vision, fatigue, drowsiness, irritability, dizziness and pain in the feet, legs or hands (30).

1.1.6-Diagnosis

There are four standard ways used for diagnosis of DM which are based on values of fasting blood glucose, random blood glucose, oral glucose tolerance test (OGTT) and Hb A1C (Table1.2) (31, 32, 33).

Table 1.2: Criteria of the Diagnosis of Diabetes Mellitus (32, 33)

Condition	Type of test	Plasma glucose concentrations mmol/L (mg/dL)
Normal	Fasting blood glucose	< 5.6 (< 100)
	Oral glucose tolerance	< 7.8 (< 140)
Diabetes Mellitus	Fasting blood glucose	≥ 7.0 (≥ 126)
	Oral glucose tolerance	≥ 11.1 (≥ 200)
	Random blood glucose	≥ 11.1 (≥ 200)
	Hb A1C	≥ 6.5%

1.1.7-Complications

The complications of DM include acute and chronic complications, the acute complication include, diabetic ketoacidosis (DKA) (34), hyperosmolar nonketotic (HONK) syndrome (35) and hypoglycemia (36) while Chronic complications of DM have an effect on several organ systems and these complications are responsible for the morbidity and mortality (37).

They are classified as vascular and nonvascular. The vascular complications are further classified into micro and macro vascular. The micro vascular complications such as retinopathy, neuropathyand nephropathy while macro vascular complications such as coronary artery disease, cerebro vascular disease and peripheral vascular disease. Nonvascular complications include several problems such as infection and skin damage (38, 39).

1.1.8-Treatment of DM

1-Insulin:mainly used for type 1 and Gestational DM.

2-Oral hypoglycemic agent: It include biguanide, sulfonylurea, alpha-glucosidase inhibitors, thiazolinedione & DPP-4inhibitors (40).

1.1.8.1-Biguanide

Mechanism of action of metformin

Several different mechanisms are included in the reduction of serum glucose level by metformin without increasing insulin secretion, predominantly via non-pancreatic pathways. The compound is often called insulin sensitizer as it increases the effects of insulin (41).

Metformin also suppresses the endogenous glucose production in the liver by reducing the rate of gluconeogenesis with a little impact on cellular ATP levels. AMP-activated protein kinase (AMPK) represents a target capable of mediating the beneficial metabolic effects of metformin. AMPK is a multisubunit enzyme that is recognized as a major regulator of lipid biosynthetic mechanisms due to its role in the phosphorylation and subsequent inactivation of pivotal enzymes (such as acetyl-CoA carboxylase) (41).

Recent research strongly suggests that AMPK has a wider role in metabolic regulation, which includes muscle glucose uptake, fatty acid oxidation. Thus it is an ideal therapeutic target for type 2 diabetes mellitus. Chronic activation of AMPK may also induce the expression of muscle hexokinase and glucose transporters, mimicking the effects of extensive exercise training. Metformin also showed protective properties against diabetic complications, especially by reducing the diabetes-related death rate (41).

Pharmacokinetics

The absolute bioavailability of metformin hydrochloride 500 mg tablet given under fasting conditions is approximately 50-60%. Studies using single oral dose of metformin tablet of 500 mg and 1500 mg, and 850 mg to 2550 mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination (42).

Food decreases the extent of and slightly delays the absorption of metformin, as shown by approximately a 40% lower mean peak

concentration and 25% lower area under the plasma concentration versus time curve (42).

Metformin is negligibly bound to plasma proteins in contrast to sulfonylureas which are more than 90% protein bound. Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans. nor biliary excretion (42).

Side effect

Abdominal pain, constipation, distention abdomen, dyspepsia/heartburn, flatulence, dizziness, headache, upper respiratory infection, taste disturbance. Liver function test abnormalities or hepatitis, resolving upon metformin (42).

1.1.8.2-Sulfonylurea: It include **(** glyburide)**,** (glipizide**),**(glimepiride**).**

Glibenclamide

Mechanism of action

The drug works by binding to and inhibiting the ATP-sensitive potassium channels (K_{ATP}) inhibitory regulatory subunit sulfonylurea receptor 1 (SUR1) in pancreatic beta cells. This inhibition causes cell membrane depolarization, opening voltage-dependent calcium channels. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release (43).

Side effect

Hypoglycemia, sulfonylureas can induce weight gain, mainly as a result of their effect to increase insulin levels and thus utilization of glucose and other metabolic fuels. Other side-effects includes gastrointestinal upset, headache and hypersensitivity reactions (43).

1.1.8.3-combines glibenclmide and metformin

The combination of two antihyperglycemic agents (glibenclmide and metformin) with complementary mechanisms of action, to improve glycemic control in patients with type 2 diabetes (43).

Aims of the study

The main objectives of the present study can be summarized as follows:

1- To assess the serum lipid profile concentration in type 2 Diabetes Mellitus and control group.

2- To evaluate the effect of some oral hypoglycemic drugs on serum lipid profile concentration in type 2 Diabetes Mellitus.

Chapter Two

Materials and Methods

2.1-Materials

2.1.1-Subjects

The study included two groups (type 2 diabetic patients and control group). All samples were collected from July 2016 till October 2016. The work was carried out in the biochemistry laboratory in ALdiwaniyah Teaching Hospital.

2.1.1.1- Patient Group

The study was performed on 60 type 2 diabetic patients (31male and 29 female). The patient ages ranged between 35-65year. Patients were diagnosed by specialist physicians. They were selected from AL-diwaniyah Teaching Hospital Province. Any subject suffered from the following health problems were excluded from the current study:

- Cardiovascular diseases
- Heart diseases and hypertension
- Patient with renal dysfunction
- Patients with malignancies
- Drug dependency such as glucocorticoid
- Patient on insulin therapy

2.1.1.2- Control Group

The control group included 30 apparently healthy subjects (22 male and 8 female). The ages of the control individuals ranged between 35-65 year. They were selected from medical staff and relatives. They were free from symptoms and signs of any chronic diseases such as DM, cardiac diseases, heart diseases, hypertension, renal diseases or others. Any subject suffered from diseases were excluded from the current study.

2.1.2-Blood Sampling

Five milliliters of blood was withdrawn from all subjects by vein puncture in fasting status and placed in plain tubes. It was left 10-15 minutes at room temperature for coagulation. Blood was centrifuged for10-15 minutes at 2000 xg. Sera were obtained glucose, total cholesterol, HDL-cholesterol, TGs, VLDL-cholesterol and LDL-cholesterol concentrations were measured.

2.1.3-Chemicals

Chemicals and kits used in this study were tabulated in Table 2-1.

Table 2-1: Chemicals and Kits Used

No.	Chemicals and Kits	Origin
1	Glucose kit	Plasmatic (France)
2	HDL-cholesterol kit	Biolabo SA (France)
3	Total cholesterol kit	Biolabo SA (France)
4	Triglyceride kit	Biolabo SA (France)

2.1.4-Apparatus and Equipments

Apparatus and equipments used in this study were tabulated in Table 2-2.

Table 2-2: Apparatus and Equipments Used

No.	Apparatus and Equipments	Origin
1	Centrifuge	Hettich (Germany)
2	Spectrophotometer	Apel (Japan)
3	Disposable syringes (5 mL)	Medical jet (Syria)
4	Distillator	England
5	Micropipette tips (different size)	Promega (USA)
6	Micropipettes (different size) (10100, 1000 μL)	Watson Nexty (Japan)

2.2-Methods

2.2.1-Determination of Serum Glucose Concentration

Principle:

Glucose oxidase (GOD) oxidized glucose to gluconate and hydrogen peroxide (H_2O_2). The H_2O_2 oxidized the mixture of 4-amino-antipyrine (4-AA) and phenol in the presence of peroxidase (POD) to form a red quinoneimine dye which proportional to the concentration of glucose in the sample as shown in the following reactions (44, 45):

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} H_2O_2 + \text{gluconate}$$

$$2H_2O_2 + \text{Phenol} + \text{4-AA} \xrightarrow{\text{POD}} 4H_2O + \text{Quinonimine}$$

Reagents:

Reagents	Composition	
Reagent 1 (Buffer)	Tris buffer pH 7	100 mmol/L
	Phenol	0.3 mmol/L
Reagent 2 (Enzymes)	Glucose oxidase	10000 U/L
	Peroxidase	1000 U/L
	4-AA	2.6 mmol/L
Reagent 3 (Standard)	Glucose 100 mg/dL or 5.56 mmol/L	

Procedure:

The content of vial reagent 2 (enzymes) was added to vial reagent 1 (buffer), then mixed gently until complete dissolution to prepare working reagent. The procedure was carried out as in the following:

Reagents	Blank	Standard	Sample
Working reagent	1 mL	1 mL	1 mL
Distilled water	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

The tubes were mixed, then left stand at 37˚C for 5 minutes. The absorbance was read at 505 nm at room temperature (25˚C) by using cuvett of 1 cm light path.

Calculation:

$$\text{Glucose (mmol/L)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 5.56$$

2.2.2-Determination of Serum lipid Profile Concentration

2.2.2.1-Determination of Serum Total Cholesterol

Principle:

Cholesterol concentration was determined enzymatically according to the method described by Allain *et al*. (46), as shown in the following reactions:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesten 4 one 3} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{PAP} \xrightarrow{\text{Peroxidase}} \text{Quinonimine (pink)} + 4\ H_2O$$

Reagents:

Reagents	Composition	
Reagent 1 (Buffer)	Phosphate buffer	100 mmol/L
	Chloro-4-phenol	5.0 mmol/L
	Sodium chloride	2.3 mmol/L
	Triton x 100	1.5 mmol/L
	Preservative	
Reagent 2 (Enzymes)	Cholesterol oxidase	100 IU/L
	Cholesterol esterase	170 IU/L
	peroxidase	1200 IU/L
	PAP	0.25 mmol/L
	PEG 6000	167 µmol/L
Reagent 3 (Standard)	Cholesterol 200 mg/dL or	5.17 mmol/L

Procedure:

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (Buffer), then mixed gently until complete dissolution (2 minutes) to prepare work reagent. The procedure was carried out as in the following:

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Distilled water	10 µL	-	-
Standard	-	10 µL	
Sample	-	-	10 µL

The tubes were mixed, then left at 37˚C for 5 minutes. The absorbance was recorded against blank at 500 nm.

Calculation:

$$\text{Total cholesterol (mmol/L)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 5.17$$

2.2.2.2-Determination of Serum HDL-Cholesterol Concentration

Principle:

Phosphotungstic acid and magnesium chloride were used to precipitate LDL, VLDL and Chylomicron from specimens. After centrifugation, HDL-cholesterol was obtained in supernatant and measured with total cholesterol reagent (47) .

Reagents:

Reagents	Composition
Reagent 1 (precipitant)	Phosphotungstic acid 13.9 mmol/L Magnesium chloride pH 6.2 490 mmol/L
Reagent 2 (Standard)	Cholesterol 100 mg/dl or 2.58 mmol/L

Procedure:

Reagents	Volume
Serum	0.5 mL
Precipitant	50 µL

The tubes were mixed vigorously, then left at room temperature for 10 minutes and centrifuged at 3000 xg for 15 minute. The procedure for measurement of cholesterol in supernatant was used.

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Distilled water	25 µL	-	-
Standard	-	25 µL	
Supernatant	-	-	25 µL

The tubes were mixed, then left at 37˚C for 5 minutes. The absorbance was recorded against a blank at 500 nm.

Calculation:

$$\text{HDL-cholesterol (mmol/L)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 2.58$$

2.2.2.3-Determination of Serum Triglycerides Concentration

Principle:

Triglycerides concentration was determined enzymatically (48) as shown in the following reactions:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-P} + O_2 \xrightarrow{\text{Glycerol-3- p Oxidase}} \text{Dihydroxyacetone-p} + H_2O_2$$

$$H_2O_2 + \text{4-chlorophenol} + \text{PAP} \xrightarrow{\text{Peroxidase}} \text{Quinonimine (pink)} + 4\ H_2O$$

The absorbance of the colored complex (quinonimine) is proportional to TG concentration in the sample.

Reagent:

Reagents	Composition	
Reagent 1 (Buffer)	PIPES	100 mmol/L
	Magnesium chloride	9.8 mmol/L
	Chloro-4-phenol	3.5 mmol/L
	Preservative	
Reagent 2 (Enzymes)	lipase	1000 IU/L
	Peroxidase	1700 IU/L
	Glycerol-3-p-oxidase	3000 IU/L
	Glycerol kinase	660 IU/L
	PAP	0.5 mmol/L
	ATP	1.3 mmol/L
Reagent 3 (Standard)	Triglycerides 200 mg/dl or 2.28 mmol/L	

Procedure:

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (Buffer), then mixed gently until complete dissolution (2 minutes) to prepare work reagent. The procedure was carried out as in the following:

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Distilled water	10 µL	-	-
Standard	-	10 µL	
Sample	-	-	10 µL

The tubes were mixed, then left at 37˚C for 5 minutes. The absorbance was recorded against blank at 500 nm.

Calculation:

$$\text{Triglyceride (mmol/L)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 2.28$$

2.2.2.4-Determination of Serum VLDL-Cholesterol Concentration

VLDL-cholesterol concentration was calculated by dividing TGs value obtained in section (2.2.4.3) by 2.22 at which VLDL-cholesterol was measured in mmol/L (49).

$$\text{VLDL-cholesterol (mmol/L)} = \frac{\text{TG}}{2.22}$$

2.2.2.5-Determination of Serum LDL-Cholesterol Concentration

The concentration of LDL-cholesterol was calculated by using Friedewald equation (50).

$$\text{LDL-cholesterol (mmol/L)} = \text{Total-cholesterol} - \text{HDL-cholesterol} - \frac{\text{TG}}{2.22}$$

2.3.Statistical Analysis

The results were expressed as mean ± SD. Student's t-test was used for the evaluation of data. P-value of < 0.05 was considered to be statistically significant.

Chapter Three

Results and Discussion

3.1-General Characteristics of Study Groups

The general characteristics of the type 2 diabetic patients and control group were shown in Table (3-1).

Table 3-1: General Characteristics of Type 2 Diabetic Patients and the Control Group

Characteristics	Group	
Number	Control	30
	Patient (Metformin)	30
	Patient (Metformin+Glibenclamide)	30
Sex Male/Female	Control	22/8
	Patient (Metformin)	12/18
	Patient (Metformin+Glibenclamide)	19/11
Duration of disease (year)	Control	-------
	Patient (Metformin)	2 year
	Patient (Metformin+Glibenclamide)	2 year

3.2-Serum Glucose and Lipid profile Concentration in Type 2 Diabetic Patients and Control Group

Fasting glucose, total cholesterol, triglyceride, VLDL-cholesterol and LDL-cholesterol levels were found to be elevated significantly ($p < 0.001$) in type 2 diabetic patients (Metformin group and Metformin + Glibenclamide group) when compared to those of the control group. However, HDL-cholesterol was observed to be lowered significantly ($p < 0.05$) in type 2 diabetic patients (Metformin group and Metformin + Glibenclamide group) when compared to those of the control group (Table 3-2).

There are different mechanisms responsible for changes of serum glucose and lipid levels in diabetes mellitus. The defect in insulin action and/or secretion and elevated plasma levels of the counter regulatory hormones are responsible for accelerated lipolysis and impaired lipids synthesis that lead to increase plasma concentration of cholesterol, triglycerides and free fatty acids (51).

The faulty of glucose utilization in diabetes, resulting in hyperglycemia and mobilization of free fatty acids from adipose tissue for energy purpose and the excess of fatty acids are accumulated in the liver and then converted to triglycerides. An increase in VLDL occurred in diabetes mellitus due to increase availability of glucose for VLDL synthesis and decrease in lipoprotein lipase activity leading to decrease the clearance of VLDL from peripheral circulation (52,53).

Lipoprotein lipase is required for chylomicrons and VLDL metabolism. This enzyme is induced by insulin and transported to the

luminal surface of capillary endothelium where it is in direct contact with the blood. Lipoprotein lipase hydrolyzes the fatty acids from triglycerides that carried by chylomicrons and VLDL (54, 55).

In diabetes, the active lipolysis increases cholesterol synthesis leading to cholesterol accumulation in the walls of blood arteries (56). These results indicated a high risk for heart diseases in those patients due to the atherosclerotic events of hypercholesterolemia(57).

The increase in LDL-cholesterol and oxidized LDL in diabetes stimulate the immune system that a harmful molecule has appeared in excessive quantities(58).They cause inflammation and promote further injury to the areas they target. Monocytes and other factors form the fatty substance, plaque (59). Lipid abnormalities are common in diabetics and frequently seen in type 2 diabetics. Dyslipidaemia make diabetics prone to develop coronary heart disease and other complications of atherosclerosis (60).

The results of the present study are in agreement with those of Sasmita*et al.* (61) who pointed out significant increases in fasting glucose, total cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol concentration in type 2 diabetic patients when compared with the control group and significant decrease in HDL-cholesterol concentration intype 2 diabetic patients when compared with the control group.

Table 3-2: Mean Fasting Serum Glucose,Total Cholesterol,HDL-Cholesterol, Triglyceride,VLDL-Cholesteroland LDL-Cholesterol Concentration in Type 2 Diabetic Patients (Metformin group and Metformin+Glibenclamide group)and the Control Group

Parameter	Group	Number	Mean ± SD	P-value
Glucose (mmol/L)	Control	30	4.99±0.70	< 0.001
	Patient	60	9.60± 3.10	
Total Cholesterol (mmol/L)	Control	30	4.10±0.35	< 0.001
	Patient	60	5.06±0.55	
HDL Cholesterol (mmol/L)	Control	30	1.09±0.27	< 0.05
	Patient	60	0.98 ±0.23	
Triglyceride (mmol/L)	Control	30	1.27±0.52	< 0.001
	Patient	60	1.97 ±0.84	
VLDL Cholesterol (mmol/L)	Control	30	0.57 ±0.23	< 0.001
	Patient	60	0.89±0.38	
LDL Cholesterol (mmol/L)	Control	30	2.45 ±0.65	< 0.001
	Patient	60	3.19±0.91	

3.3-Levels of Serum Glucose and Lipid profile in Type 2 DiabeticPatients (Metformin group and Metformin+Glibenclamide group)

The result of present study show non significant ($p > 0.05$) changes in fasting glucose (Figure 3-1), total cholesterol (Figure 3-2), HDL-cholesterol (Figure 3-3), triglyceride (Figure 3-4) and VLDL-cholesterol (Figure 3-5) levels in type 2 diabetic patients (Metformin) group when compared to those of type 2 diabetic patients (Metformin + Glibenclamide).

However, LDL-cholesterol was observed to be lowered significantly ($p < 0.05$) in type 2 diabetic patients (Metformin + Glibenclamide) groupwhen compared to those of the type 2 diabetic patients (Metformin) group (Figure 3-6).

The Metformin does not affect insulin secretion but requires the presence of insulin to be effective. It decrease hepatic glucose production and increase peripheral glucose uptake (62). The glibenclamide potentiate insulin effects, either by increasing insulin secretion or enhancing transport of blood glucose to peripheral tissues (63).

Insulin promotes LDL-cholesterol uptake by up regulating LDL receptors (64). The increasein insulin secretion lead to increase LDL-cholesterol uptake, this leading to decrease the LDL-cholesterol concentration. In this present study, treatment with metformin plus glibenclamide was associated with statistically significant and durable reductions in LDL-cholesterol concentration as compared to the in type 2 diabetic patients treated with metformin, this is may be resulted from increasinginsulin secretion by glibenclamide. Dailey *et al.* (65) found that

combination therapy of metformin and glibenclamide shows a favorable effecton LDL-cholesterol levels.

The results of the present study are in agreement with those of Alaa*et al*. (66) who pointed non significant changes in fasting glucose, total cholesterol, HDL-cholesterol, triglyceride and VLDL-cholesterol levels in type 2 diabetic patients (Metformin) group when compared to those of type 2 diabetic patients (Metformin + Glibenclamide). However, LDL-cholesterol was observed to be lowered significantly ($p<0.05$) in type 2 diabetic patients (Metformin + Glibenclamide) group when compared to those of the type 2 diabetic patients (Metformin) group .

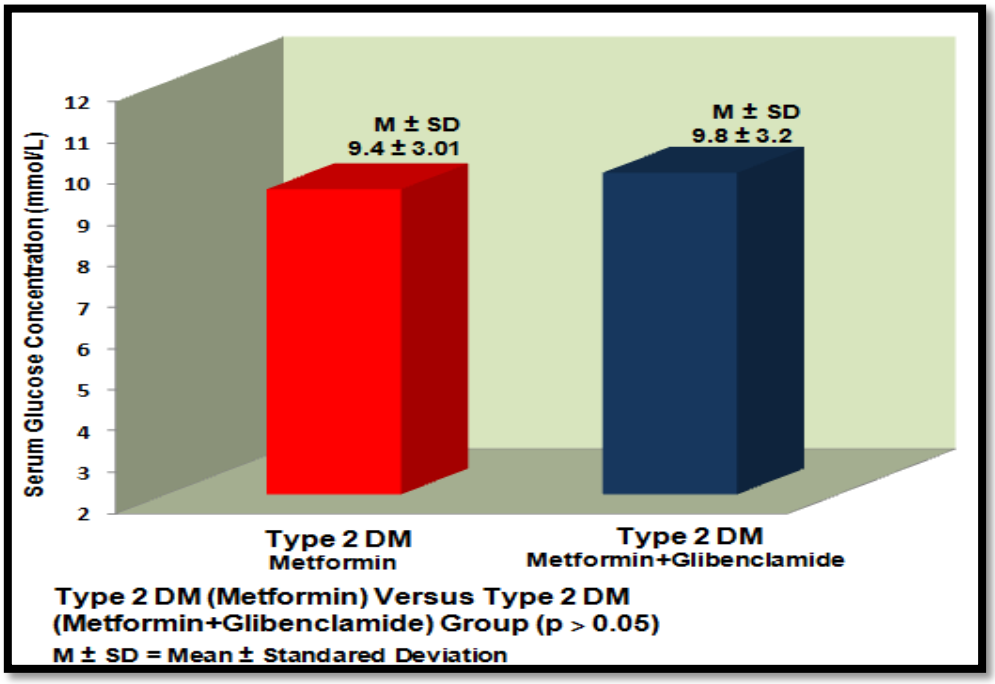

Figure 3-1: Mean Fasting Glucose Concentration in Sera of Type 2 Diabetic Patients (Metformin group and Metformin + Glibenclamide group)

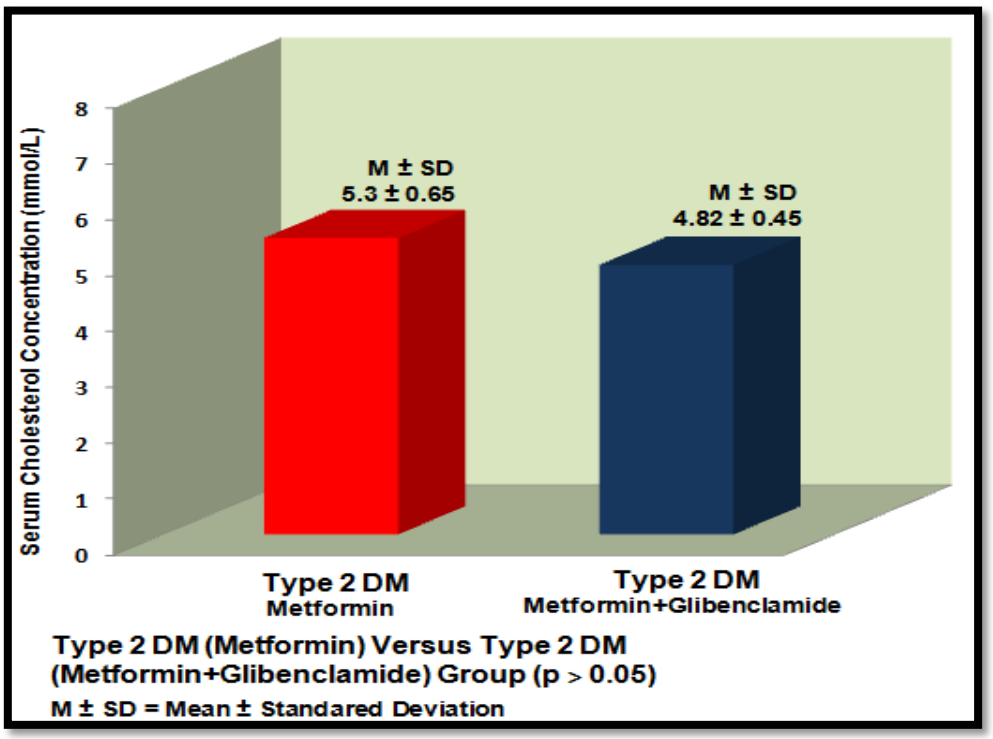

Figure 3-2: Mean Fasting Total Cholesterol Concentration in Sera of Type 2 Diabetic Patients (Metformin group and Metformin + Glibenclamide group)

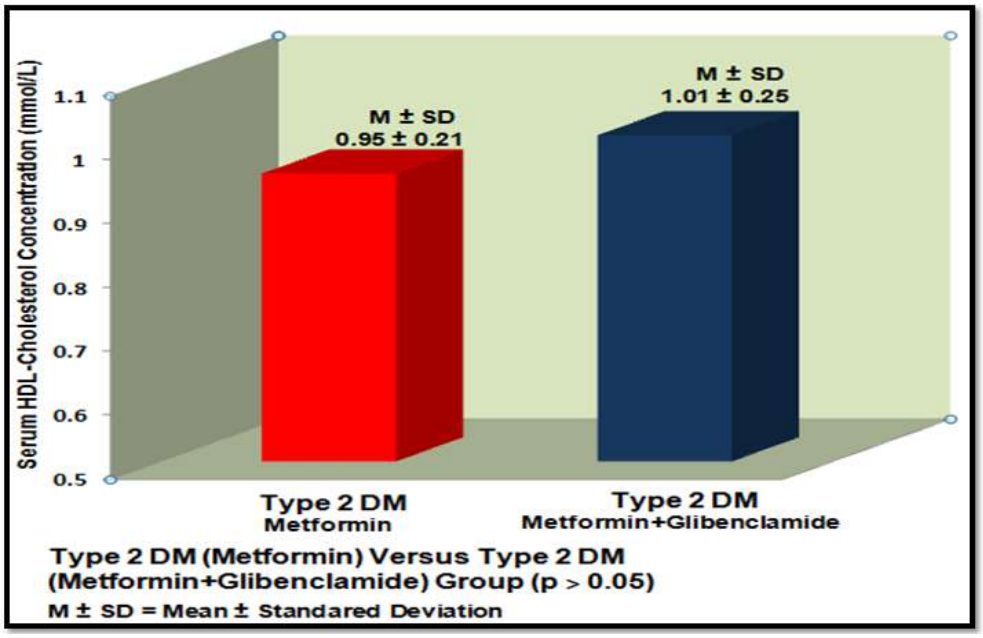

Figure 3-3: Mean Fasting HDL-Cholesterol Concentration in Sera of Type 2 Diabetic Patients (Metformin group and Metformin + Glibenclamide group)

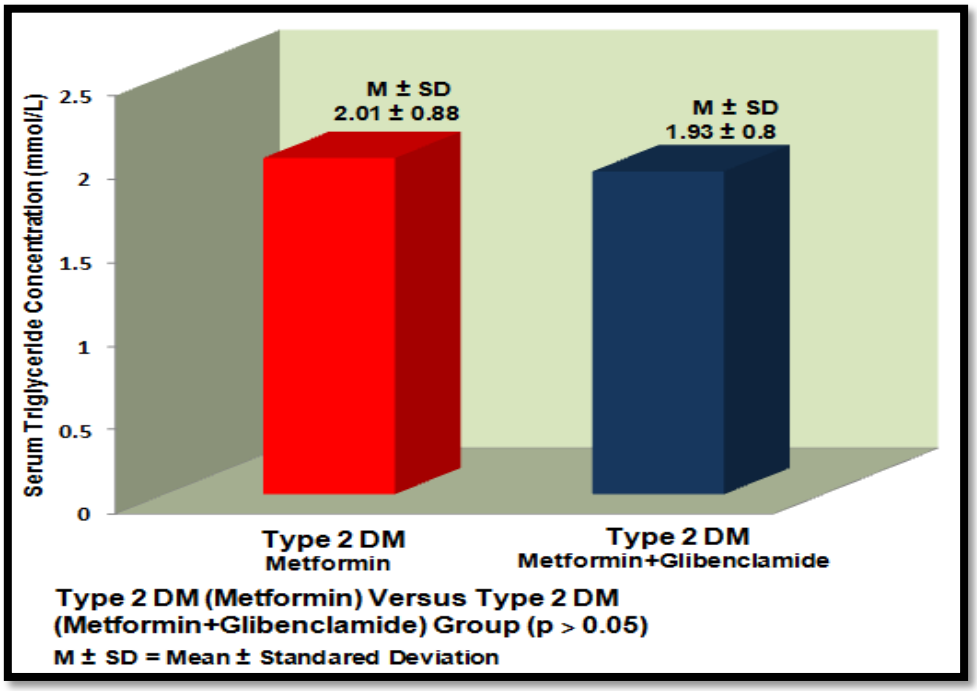

Figure 3-4: Mean Fasting Triglyceride Concentration in Sera of Type 2 Diabetic Patients (Metformin group and Metformin + Glibenclamide group)

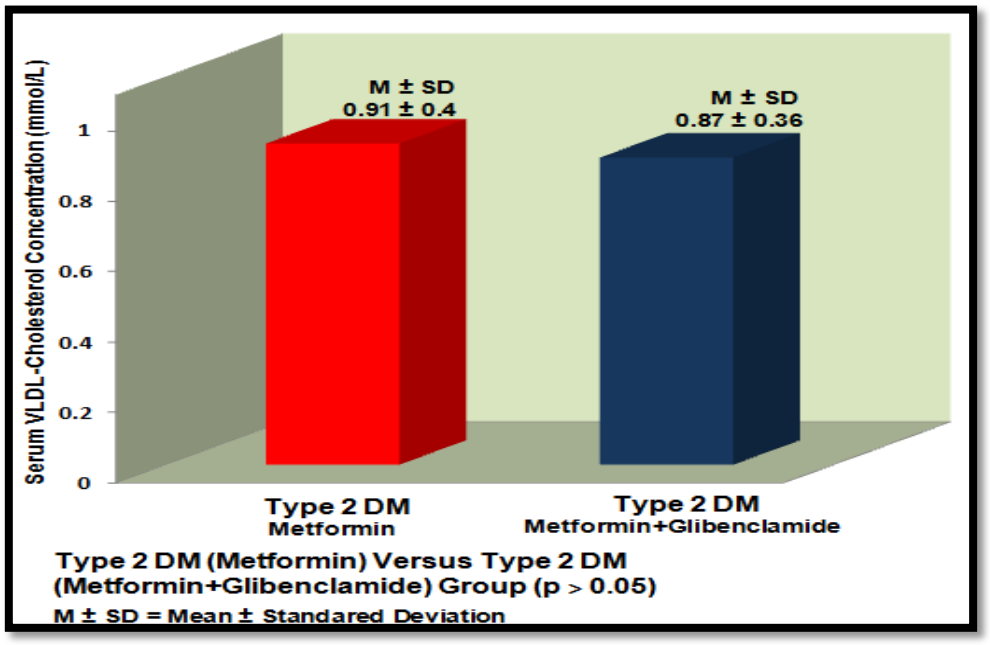

Figure 3-5: Mean Fasting VLDL-Cholesterol Concentration in Sera of Type 2 Diabetic Patients (Metformin group and Metformin + Glibenclamide group)

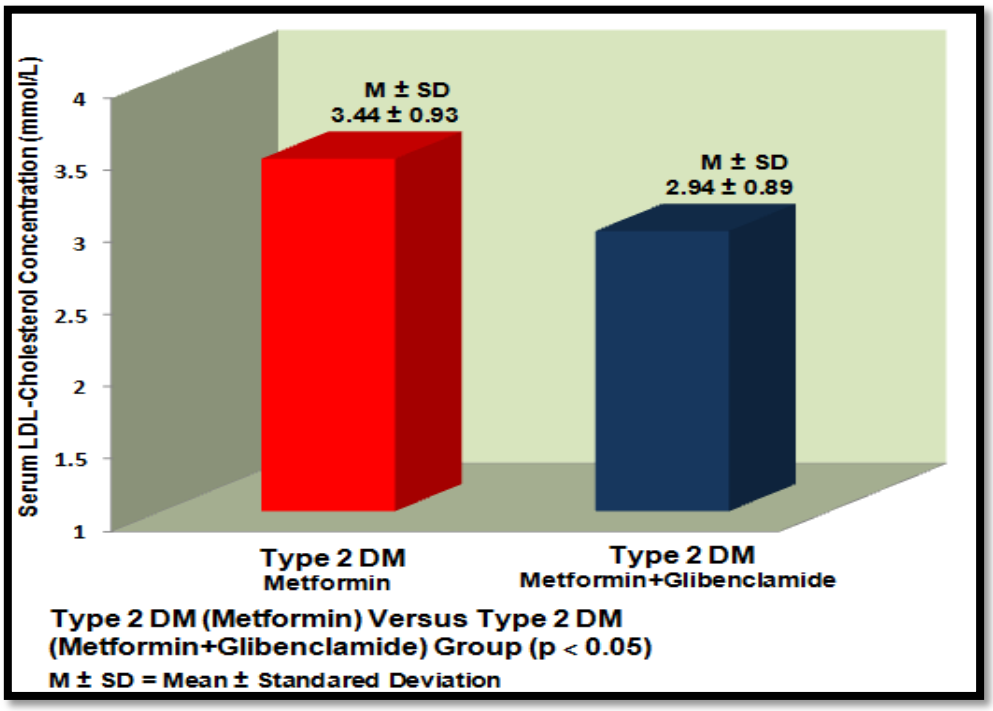

Figure 3-6: Mean Fasting LDL-Cholesterol Concentration in Sera of Type 2 Diabetic Patients (Metformin group and Metformin + Glibenclamide group)

Conclusions

1. In type 2 diabetic patients (Metformin group and Metformin + Glibenclamide group) there are a significant increase in serum total cholesterol, triglyceride, VLDL-cholesterol, LDL-cholesterol and significant decrease in HDL-cholesterol when compared to those of the control group. This indicate a high risk for heart diseases in 2 diabetic patients.

2.Type 2 diabetic patients (Metformin) group produces a non significant favorable effect on all lipid profile parameters.

3. Type 2 diabetic patients (Metformin +Glibenclamide) group showed a significant reduction (favorable effect) in LDL-cholesterol when compared with type 2 diabetic patients (Metformin) group.

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الخلاصة

تم تصميم هذه الدراسة لتقييم تركيز الدهون في مصل الدم في مرض السكري من النوع ٢ ومجموعة السيطرة وتقييم تأثير (الميتفورمين والميتفورمين + غليبينكلاميد) على تركيز الدهون في مصل الدم في النوع الثاني مرض السكري . ولتحقيق هذا الهدف، يتم أخذ ٦٠ مريضا مصابا بداء السكري من النوع الثاني تتراوح أعمارهم بين (٣٥-٦٥) سنة ، حيث تم تحديد تركيز الجلوكوز في الدم وتركيز الدهون بواسطة الطريقة (الأنزيمية الطيفية) أظهرت نتائج الدراسة الحالية وجود ارتفاع ملحوظ في مستويات الجلوكوز في الصيام والكولسترول الكلي والدهون الثلاثية ومستويات الكولسترول الواطئ الكثافة جدا والكولسترول في مرضى السكري من النوع الثاني (مجموعة ميتفورمين ومجموعة ميتفورمين + غليبينكلاميد) بالمقارنة مع مجموعة السيطرة. ومع ذلك، لوحظ انخفاض كبير في الكولسترول عالي الكثافة في مرضى السكري من النوع ٢ (مجموعة ميتفورمين ومجموعة ميتفورمين + غليبينكلاميد) بالمقارنة مع مجموعة السيطرة . كما أظهرت نتائج الدراسة الحالية وجود تغيرات غير ملحوظة في مستويات الجلوكوز في الصيام والكولسترول الكلي ومستويات الكوليسترول عالي الكثافة والدهون الثلاثية ومستويات الكولسترول في مرضى السكري من النوع الثاني (ميتفورمين) بالمقارنة مع مرضى السكري من النوع الثاني (ميتفورمين + غليبينكلاميد). ومع ذلك، لوحظ انخفاض الكولسترول واطئ الكثافة إلى حد كبير في مرضى السكري من النوع ٢ (ميتفورمين + غليبينكلاميد) مقارنة مع مرضى السكري من النوع ٢ في مجموعة (ميتفورمين) . في الختام، أظهرت المجموعة الثانية من مرضى السكري (ميتفورمين + غليبينكلاميد) انخفاض كبير (تأثير إيجابي) في الكولسترول واطئ الكثافة بالمقارنة مع مرضى السكري من النوع ٢ في مجموعة (ميتفورمين).

بِسْمِ اللهِ الرَّحْمنِ الرَّحِيم

(قالَ رَبِّ اشْرَحْ لي صَدْري (٢٥) وَيَسِرْ لي
أمْري (٢٦) وَاحْلُلْ عُقْدَةً مِنْ لِساني (٢٧)
يَفْقَهوا قَوْلي (٢٨))

صَدق اللهُ العَلي الْعَظِيمِ

سورة طه (٢٥-٢٨)

جامعة القادسية
كلية الصيدلة

تأثير بعض الأدوية الخافضة لمستوى السكر على نسبة الدهون لدى مرضى السكري من النوع الثاني في محافظة الديوانية

زينب رسول عبد

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إشراف

د.غفران محمد حسين

١٤٣٩هـ ٢٠١٧م