

The Role of Glimepiride and Metformin on Biomarker Galectin-3 in Patients with Type 2 Diabetes and Left Ventricular Diastolic Dysfunction

Zahraa R. Mohammed¹, Hussein A Saheb, Ph. D²

¹ Department of Pharmacology and Therapeutics, College of Medicine, University of Al-Qadisiyah, Iraq.

² Pharmacology and Therapeutic. Al Qadesiyah College of pharmacy, Iraq

ABSTRACT

Background: Schiff bases and their derivatives are widely recognized for their diverse biological activities, including antimicrobial properties. The synthesis and structural characterization of these compounds contribute to the development of novel antimicrobial agents with potential clinical applications. **Objective:** This study aimed to synthesize and characterize a series of novel Schiff base derivatives and evaluate their antibacterial and antifungal activities using standard laboratory microbiological assays. **Methods:** A series of substituted thiazole derivatives were synthesized, starting from 3,5-dinitrobenzoic acid, which was converted into its acid chloride derivative. The acid chloride was then treated with p-hydroxybenzoic acid in pyridine as a solvent and a base, with a catalytic amount of DMF, to obtain 4-(3,5-dinitrobenzoate) benzoic acid [1]. Further reaction of compound [1] with thiosemicarbazide and phosphorus oxychloride (POCl₃) yielded 4-(5-amino-1,3,4-thiadiazol-2-yl) phenyl-3,5-dinitrobenzoate [2]. Schiff bases [3a-h] were synthesized by refluxing compound [2] with various substituted benzaldehydes in absolute ethanol with glacial acetic acid (GAA) as a catalyst. The newly synthesized compounds were structurally characterized using Fourier Transform Infrared Spectroscopy (FT-IR) and Proton Nuclear Magnetic Resonance Spectroscopy (¹H-NMR). Their purity was confirmed by melting point determination and Thin Layer Chromatography (TLC). The antibacterial and antifungal activities of the compounds were assessed using disk diffusion and minimum inhibitory concentration (MIC) methods against a panel of Gram-positive and Gram-negative bacterial strains, as well as fungal pathogens. **Results:** The synthesized Schiff base derivatives exhibited characteristic absorption bands in the FT-IR spectra, confirming the presence of azomethine (-C=N-) functional groups. The ¹H-NMR spectra provided further confirmation of structural integrity. Preliminary microbiological evaluation demonstrated that several Schiff base derivatives displayed promising antibacterial and antifungal activities, with notable inhibition zones against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*. **Conclusion:** The synthesized Schiff base derivatives showed potential as antimicrobial agents, suggesting their possible application in pharmaceutical and clinical microbiology. Further studies, including in-depth cytotoxicity and mechanistic evaluations, are recommended to explore their full therapeutic potential.

Keywords: Glimepiride, Metformin, Left Ventricular Diastolic Dysfunction, Diabetes Mellitus Type 2, Galectin-3.

INTRODUCTION

Diabetes Mellitus (T2DM) is a widespread metabolic condition that occurs worldwide. The development of this condition is mainly caused by two primary factors: reduced insulin secretion by pancreatic β -cells and the inadequate response of insulin-sensitive tissues to insulin [1]. Globally, around 462 million individuals suffer with type 2 diabetes, representing 6.28% of the total world population. In 2017, this disease resulted in more than 1 million deaths, ranking it as the tenth leading cause of mortality [2]. Type 2 diabetes (T2D) is a major public health issue since it has a considerable effect on death rates, illness rates, and the financial strain it puts on the healthcare system. The rising incidence of chronic diseases, such as cardiovascular disease, hypertension, and obesity, in both developed and emerging countries underscores the growing worldwide burden, with Type 2 Diabetes (T2D) being a significant contributing factor as a common comorbidity [3]. Diastolic dysfunction refers to an anomaly in the relaxation and/or flexibility of the left ventricle (LV) that affects the timing, speed, and degree of the decrease in LV pressure and filling during the diastolic phase. These modifications lead to an unusual relationship between the pressure and volume of the left ventricle, requiring greater pressures to maintain a normal volume of blood in the left ventricle at the end of diastole and the amount of blood pumped by the heart every minute. LVDD is a notable precursor to

several cardiovascular diseases. The primary mechanism responsible for the development of heart failure (HF) with preserved ejection fraction (HFpEF) is represented by this, accounting for two-thirds of patients. HFpEF is increasingly prevalent in the older population, with an estimated 8% of people over 65 expected to have HFpEF by 2020. This condition is linked to a poor prognosis [4]. In order to postpone or avoid the occurrence of heart failure in people with type 2 diabetes, it is imperative to determine the elements that elevate the likelihood of LV diastolic dysfunction, which serves as a sign of the initial phase of diabetic cardiomyopathy. This is extremely significant due to the high prevalence and severe morbidity and mortality associated with heart failure in these individuals. Age, hypertension, and ischemic heart disease (IHD) are acknowledged as significant risk factors for diastolic dysfunction in both individuals with diabetes and those without diabetes [5-7]. Galectin-3 is a specialized type of lectin that binds specifically to beta-galactosidase and performs several crucial roles in processes such as inflammation, immune response, and cancer. Recently, there has been a proposal that it could serve as a new biomarker for LVDD [8]. This molecule plays a role in the processes of cell adhesion, proliferation, and differentiation. In addition, it plays a role in stimulating fibroblasts and has been shown to act as a chemoattractant and proapoptotic agent [9]. Galectin-3 has lately

emerged as a potent indicator of heart failure and mortality, therefore establishing its importance as a substantial prognostic marker in clinical settings. Galectin-3 is recognized as a biomarker for disease and a catalyst for the advancement and growth of heart failure [10, 11]. Without a doubt, fibrosis is the primary mechanism accountable for the increased stiffness in the ventricles and the compromised ability to relax during diastole. The pro-fibrotic and immune-modulatory capabilities of galectin-3 have been suggested as a potential explanation for the correlation between the levels of this lectin in the bloodstream and the progression and severity of heart failure. Multiple autonomous clinical trials have demonstrated a positive correlation between elevated galectin-3 levels (>17.8 ng/mL) and an increased probability of hospitalization or mortality in the immediate or distant future. Multiple clinical research have confirmed that individuals with Type 2 Diabetes (T2D) have notably elevated levels of Galectin-3 in their blood [12-15]. Weigert et al. [12] reported a substantial increase in serum Galectin-3 levels among persons diagnosed with Type 2 Diabetes (T2D). The researchers noted a substantial decrease in Galectin-3 levels after treatment with metformin. The reduction in Galectin-3 expression can be ascribed to metformin's ability to lower oxidative stress and the production of advanced glycation end products (AGEs). In 2019, Atalar et al. [13] did a study which found that individuals with Type 2 Diabetes (T2D) have higher levels of serum Galectin-3 compared to individuals with prediabetes and healthy controls. The study also discovered a clear correlation between Galectin-3 levels and fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), and high-sensitivity C-reactive protein (hs-CRP). The level of hs-CRP can serve as a useful biomarker for promptly detecting the chronic inflammatory process in diabetes. The study's findings indicate that Galectin-3 may play a role in the transition from the prediabetes stage to the diabetes stage. To summarize, all existing human research have presented compelling evidence that Galectin-3 functions as a marker, and potentially even a contributing factor, for Type 2 Diabetes (T2D). Glimepiride, a second-generation sulfonylurea, was granted FDA approval in 1995 for the purpose of enhancing glycemic control in individuals diagnosed with type 2 diabetes mellitus. The user's input is "[16]". Moreover, glimepiride does not impact the ischemic preconditioning of cardiac myocytes. Ischemic preconditioning is a physiological mechanism in the body that serves to postpone heart attack and reduce harm to the heart muscle after an episode of reduced blood flow (ischemia). Glimepiride selectively blocks ATP-dependent potassium channels located on the cell membrane of cardiac muscle cells, without affecting mitochondrial potassium channels. This activity contributes to the maintenance of cardiac preconditioning. The number 18 is encapsulated between square brackets. The differentiation from other drugs in the same class suggests that the use of glimepiride may be safer and more advantageous for those with cardiovascular issues [17].

Methods and Materials

Study Design

A cross-sectional study was conducted, involving 60 patients aged between 45 and 65 years. These patients' information was collected from the file sheet in the inpatients ward, these patients were previously treated for type 2 diabetes mellitus. Each patient fulfils the inclusion criteria, and the patients were contacted and written informed consent obtained from them. Patients divided into two groups group G1 (received standard treatment for T2DM with

metformin $1\text{g} \times 2$ max daily dose) and group G2 (received standard treatment for T2DM with metformin+Glimepiride 4mg daily). All patients' variables collected includes. These variables include age, gender, body mass index (BMI), glycated hemoglobin (HbA1c), creatinine, urea, cholesterol, CRP, Triglycerides.

Study Settings

The patient's data collected from the inpatients ward from Al-Diwaniyah Teaching hospital in Al-Diwaniyah Province, Iraq. All information taken from the datafiles between the period from 1st of September 2023 and till 1st of March 2024.

Inclusion Criteria

T2DM, HbA1C (6.5-9), Age 45-65 years, Onset of Diseases >3 years, Onset of current Therapy > 6 months.

Exclusion Criteria

HbA1C $> (9.5)$, Renal (eGFR < 30 ml/min/1.73m² of body surface area) or end stage renal failure or dialysis, hepatic impairment, Pregnancy, Heart failure, Arrhythmia AF.

Laboratory Analysis

Information was taken from the patients: Name, age, gender, weight, height, BMI, and comorbidities smoker, LLVD. Biochemical parameters were being measured for all patients with Glimepiride which were: HbA1C, FBS urea, creatinine, C-Reactive protein, Cholesterol, Triglyceride, Magnesium. Blood samples of 4 ml were collected from the patients that were aspirated from an antecubital vein and divided into two portions: A volume of one milliliter (ml) of the patient's whole blood was obtained and placed in a tube containing EDTA for the purpose of DNA extraction. The sample was then refrigerated at a temperature of -20 degrees Celsius until the DNA extraction procedure.

❖ Three milliliters (ml) of the patient's whole blood were collected in a gel tube, spun at 5,000 revolutions per minute for five minutes, and the serum was collected to be used in biochemical tests.

Demographics Assessment

Evaluation of Body Mass Index (BMI)

The study measures the weight of the patients using an electronic scale, expressed in kilograms. The height is measured using a height scale in square meters (m²). The Body Mass Index (BMI) is computed by dividing the weight by the square of the height (kg/m²).

RNA Extraction

Quantitative Reverse Transcription Real-Time PCR (RT-qPCR)

RNA Extraction

200 μ l of each whole blood sample was lysed by adding 600 μ l of Triazol in a 1.5 ml micro-centrifuge tube and left for 5 minutes at room temperature. This followed by centrifugation at 13000 rpm for 2 minutes meanwhile the supernatant was transferred into new tube and 600 μ l of ethanol was added and vortexed. The lysate was carefully put into the upper reservoir of the spin column for RNA binding. Subsequently, it was centrifuged at a speed of 13,000 revolutions per minute for a duration of 1 minute, while the flow-through was discarded. The spin column was rinsed with washing buffer and subsequently treated with DNase by adding 45 μ l of DNase

buffer and 5 µl of DNase. The mixture was then allowed to incubate for 15 minutes. Next, the spin column was rinsed three times with washing buffer. This was done by adding 500 µl of washing buffer and then centrifuging at 13000 rpm for 1 minute. To elute the RNA, the column was spun to remove any remaining liquid from the silica gel. Then, 50 µl of elution buffer was added and the column was centrifuged at a speed of 13000 rpm for a duration of 2 minutes.

RNA concentration measurement by Quantus™ Fluorometer (Promega, USA):

A 1.1-fold dilution of 20X TE Buffer (pH 7.5) was prepared by adding nuclease-free water to obtain a 1.1X TE buffer. 2. The

QuantiFluor® Dye working solution was prepared by combining 10 µl of QuantiFluor® Dye with 3,990 µl of 1X TE buffer, resulting in a dilution ratio of 1:400.

The blending was performed in a solution of 1X TE buffer. 3. The blank sample for the QuantiFluor® ONE RNA System was prepared by combining 200 µl of QuantiFluor® RNA Dye with a 0.5ml PCR tube. 4. The prescribed quantity of the nucleic acid standard (RNA) was introduced into a 0.5ml PCR tube as per the instructions provided by the manufacturer (2 µl of standard to 200 µl of working solution). 5. Set up standard measurement by the Quantus Fluorometer reading then measure the RNA samples according to the recommended procedures by the Quantus machine.

cDNA Synthesis

Table 1: The Given Kit by ADDBio (Korea) was used to Reverse Transcribe a Total Amount of RNA into cDNA, Following the Protocols Specified.

Substance	Amount
H ₂ O	6 µl
Reverse Transcriptase (RT) 2X Add Script Cdna	20 µl
dNTPs	4 µl
Random Oligos Hexamer	2 µl
RNA	8 µl
Total Volume	40 µl

Table 2: B-The Thermal Conditions Were as Following in Table 2:

Temperature	Time	Purpose
25 C	10 min	Priming
50 C	60 min	Reverse Transcriptase (RT)
80 C	5 min	RT Inactivation
4 C	On hold	Store cDNA

Gene Expression of Galectin-3:

This study utilized the comparative Ct technique ($\Delta\Delta C_t$) to Compare the amounts of transcripts to those of GAPDH mRNA. The normalization was done based on the level of the control group. This was accomplished based on the suggestion provided by Schmittgen and Livak in 2008. To achieve this objective, the Galectin-3 gene was amplified using the primers.

Quantitative Reverse Transcriptase PCR (RT-qPCR) Preparation

RT-qPCR Amplification:

Initially, the process of amplification was carried out using the AddScript RT-qPCR Syber master kit manufactured by AddBio in Korea. Table 5 contains the specific information regarding the reaction.

Table 3: Gene of Interest (Galectin-3).

Sequence (5'→3')	Template strand (5'.....3')
Gal3-Forward	TGCAGTGAATGATGCTCATTG
Gal3-Reverse	CAGAAATTCOCAGTTTGCTGATT

Table 4: Housekeeping Gene (HKG) or Internal Reference Gene; Human Glyceraldehyde 3-Phosphate Dehydrogenase

Sequence (5'→3')	Template strand (5'.....3')
GAPDH-F	CAGAACATCATCCCTGCCTCTA
GAPDH-R	CCAGTGAGCTTCCCGTTCA

Table 5: RT-qPCR Amplification

Substance	Amount
H ₂ O	4 µl
AddScript RT-qPCR	10 µl
Forward Primer (0.05 pmol/20 µl)	2 µl
Reverse Primer (0.05 pmol/20 µl)	2 µl
cDNA	2 µl
Total	20 µl

Table 6: B/The Thermal Conditions were Implemented using BioRAD (USA) in Table 6 as Indicated:

Temperature	Time	Repeat
Initial Denaturation	95 C	5 min
Denaturation	95 C	20 sec
Annealing	55 C	30 sec
Extension	72 C	30 sec
Melting Analysis	95 C	15 sec
Melting Analysis	60 C	60 sec
Melting Analysis	+0.3 C of 95C	15 sec

RT-qPCR Data Normalisation

The transcript levels were normalized using the delta-delta Ct approach, as described by Schmittgen and Livak (2008). This method involved employing the following calculation and comparing the results to the levels of GAPDH mRNA. The formula for $2^{-\Delta\Delta Ct}$ is calculation involves subtracting the CT value of the internal control from the CT value of the gene of interest for sample A., and then subtracting the CT value of the internal control from the CT value of the gene of interest for sample B. The result is then multiplied by 2. Note: Sample A refers to a specific group. Sample B refers to a distinct and specific group.

Glycated Hemoglobin (HbA1c), (Linear, Sapin)

The enzymatic approach of the hemoglobin A1c assay selectively measures the N-terminal fructosyl dipeptides of the HbA1c α -chain.

Ethical Consideration

The study was authorized by the Ethics Committee of the College of Medicine, University of Al-Qadisiyah. Prior to any procedures, all patients were provided with a comprehensive description of the therapies and their consent was acquired. All patients will be informed both verbally and in writing about the procedure, and the purpose of the study, Before enrolling in the study, all participants will provide their written informed consent.

Statistical Analysis

Graph pad prizm software (version 8.4.3) was used for data analysis. All data were shown as mean $\pm$ standard deviation (SD) and a difference with a P value <0.05 was considered statistically significant.

Results

Efficiency of the Assay's Amplification in Tested Groups for (Galectin-3).

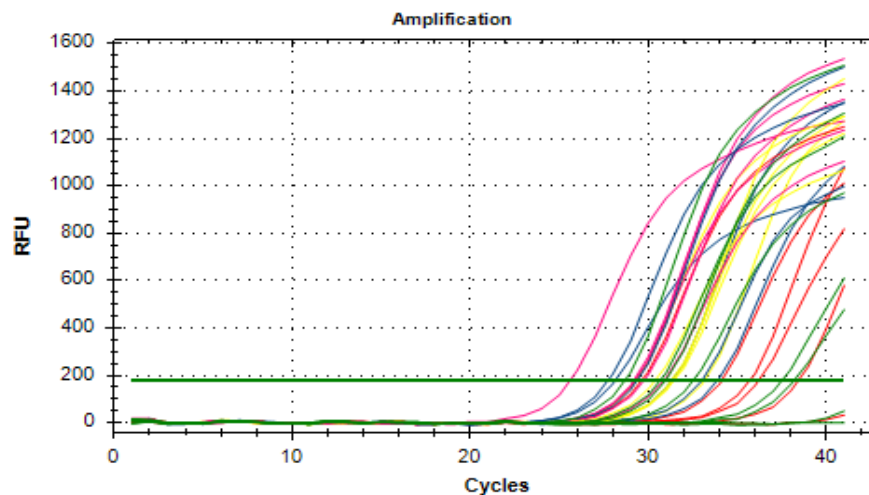


FIGURE 1: amplification curve of the tested samples for expression of gene of interest (Galectin-3) in the Metformine group. As shown the successful amplification curves with corresponding crossing threshold (CT) as the number of cycles with the round forming unit (RFU).

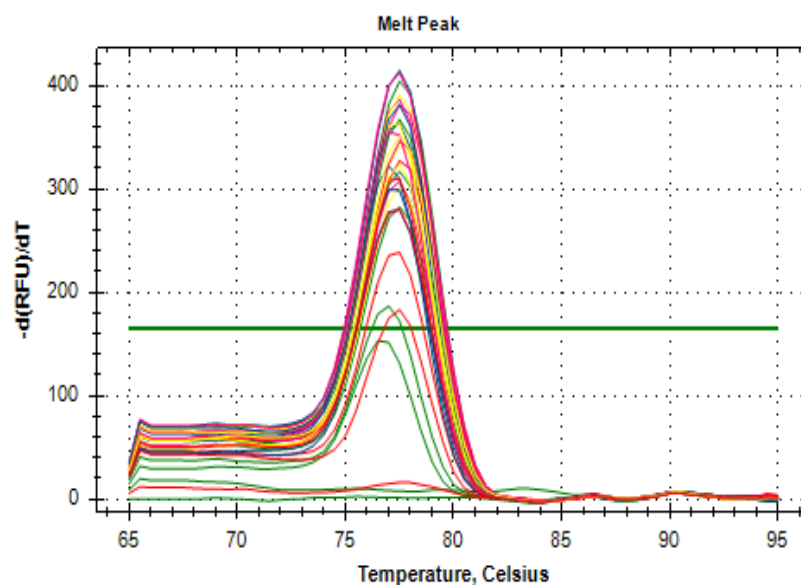


FIGURE 2: Disassociation melting curve analysis of the tested samples for the expression of (Galectin-3) in the Metformine group. As shown the successful disassociation curves with single peak

Glimepiride Group

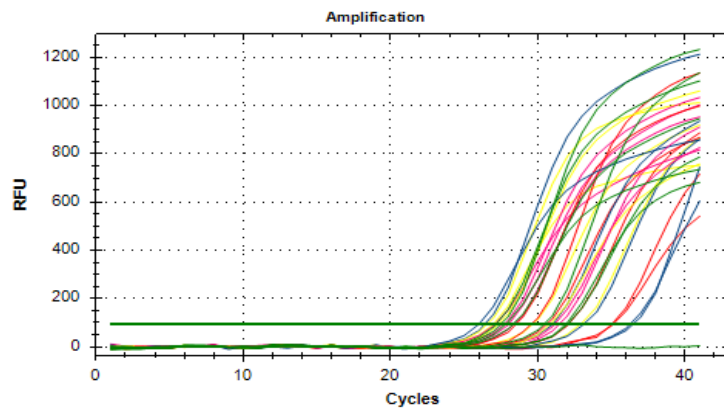


FIGURE 3: amplification curve of the tested samples for expression of gene of interest (Galectin-3) in the Glimepiride group. As shown the successful amplification curves with corresponding crossing threshold (CT) as the number of cycles with the round forming unit (RFU).

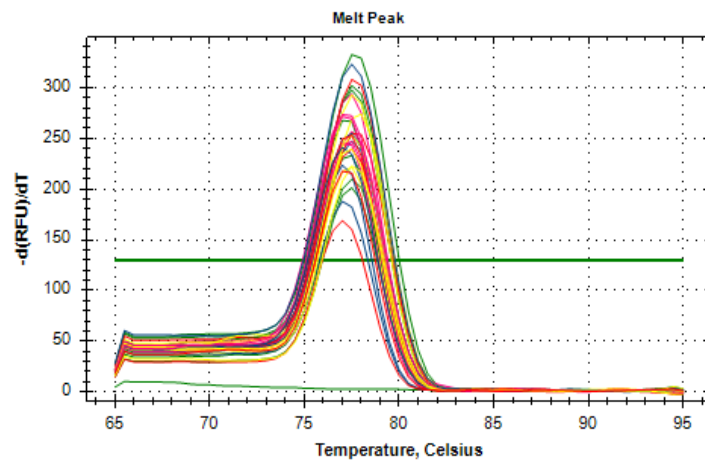


FIGURE 4: Disassociation melting curve analysis of the tested samples for the expression of (Galectin-3) in the Glimepiride group. As shown the successful disassociation curves with single peak.

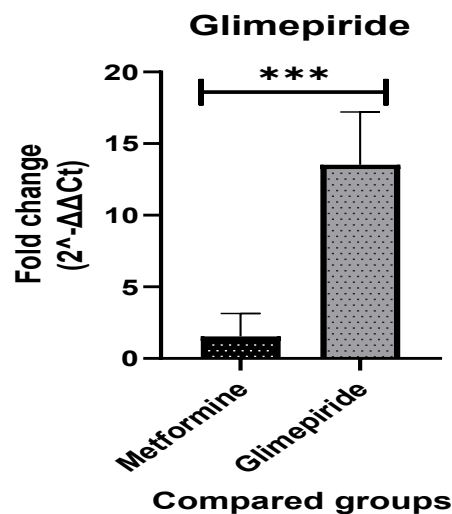


FIGURE 5: Gene expression of Galectin-3 in the Glimepiride treatment group. This shows a significant upregulation of Galectin-3 in the Glimepiride treated group ($P < 0.0001$) in comparison with metformine treatment. This was analyzed by Graph pad prism software (version 8.4.3).

Table 7: Descriptive Statistics in the Galectin-3 Expression in Glimepiride and Metformine Treated Groups. This was Analysed by t Test.

	Metformine	Glimepiride
Mean	1.238	13.52
Std. Deviation	0.6908	3.680

Discussion

In the present study metformin treatment showed significantly higher reduction in Gal-3 concentration and improvements in echocardiographic parameters associated with LVDD indicate its potential as an adjunct therapy targeting LVDD compared to glimepiride therapy in addition to the standard of care group [18]. Additional studies investigating different treatments in a mouse model of coxsackievirus B3 (CVB3)-induced myocarditis produced similar results. The results demonstrated that mice infected with CVB3 and treated with liposome-encapsulated clodronate to deplete macrophages experienced a decrease in both acute myocarditis and chronic fibrosis, in contrast to CVB3-infected mice that did not receive any treatment [19]. The interaction between Gal-3 and aldosterone led to the stimulation of macrophage infiltration and the development of cardiac fibrosis in a mouse model of heart failure caused by pressure overload. Pharmacologically inhibiting Gal-3 reduced the activation of genes associated with fibrogenesis, specifically collagen type 1 and collagen type 3, as well as the infiltration of macrophages and cardiac remodeling [20]. Gal-3 expression was found to increase three days after the transverse aortic constriction in a mouse model experiencing high pressure. The investigation found an increase in particular groups of macrophages that had infiltrated the interstitial tissue [21]. However, significant quantities of Gal-3 were found in a specific group of heart muscle cells located next to regions of fibrosis following 7-28 days of transverse aortic constriction [22]. The findings suggest that the cells responsible for producing Gal-3 undergo distinct modifications depending on the stage of the disease, which can vary from early to late. Moreover, findings from animal experiments suggest that macrophages that infiltrate the heart and generate Gal-3 in the initial phase could serve as promising targets for addressing cardiac fibrosis and remodeling. Hence, it is crucial to promptly identify macrophages that produce Gal-3 by employing a diagnostic indicator. The correlation between blood levels of Gal-3 and the efficacy of specific medicines for HFrEF has been minimally investigated. Aldosterone can potentially have detrimental effects, primarily as a result of its interaction with Gal-3. Hence, it is recommended that inhibiting aldosterone could be advantageous for those exhibiting elevated levels of Gal-3 in their bloodstream. The Aldo-DHF study included individuals with symptomatic heart failure (HF) who were categorized as NYHA class II or III. These individuals had a maximum oxygen consumption (VO₂) of 25 mL/kg/min or lower on a cardiopulmonary

test. In addition, they maintained their left ventricular (LV) systolic function, as evidenced by a resting LV ejection fraction (LVEF) of 50% or above. Nevertheless, they did have clear LV diastolic dysfunction, as indicated by a grade of I or higher on echocardiography or the presence of atrial fibrillation (AF). The participants were randomly allocated to receive either a daily dosage of 25 mg of spironolactone or a placebo. Throughout the 12-month follow-up period, there were no notable disparities in Serum Gal-3 concentrations among the groups. The HOMAGE randomized research included participants who were at an elevated risk of developing heart failure (HF). The study comprised of 527 participants who either had coronary disease or were at a high risk of getting it. Additionally, these patients had elevated levels of B-type natriuretic peptides in their blood plasma. Gal-3's study did not see any notable reductions in the levels of collagen biomarkers in the blood when a daily dose of 50 mg of spironolactone was administered [23]. The TOPCAT study revealed that spironolactone has the ability to alter the variables that impact the remodeling of the extracellular matrix, resulting in a decrease in fibrosis among individuals with diabetes. It was reported that the levels of hs-TnT and TIMP-1 increased with time, whereas no changes were detected in Gal-3 levels [24]. A study conducted on a cohort of individuals with heart failure and reduced ejection fraction (HFrEF) yielded contradictory findings. A cross-sectional descriptive analysis was carried out on a sample of 122 patients who were not using spironolactone. Following a 12-week course of spironolactone medication, the concentration of Gal-3 fell from 54.82 ± 26.06 to 44.20 ± 24.36 ($p < 0.05$). The treatment of spironolactone at a daily dosage of 50 mg significantly increased the concentration of Gal-3, compared to a dosage of 25 mg. The concentration decreased by 29.05% to 17.11 ± 20.81 ng/mL ($p < 0.05$) in the group that was administered 50 mg, and by 6.87% to 3.46 ± 6.81 ng/mL ($p < 0.05$) in the group that was administered 25 mg [25].

Study Limitations

There are several limitations identified in this study. The trial was limited by its short duration, and no significant morbidity or death outcomes were observed due to this short period.

Conclusions

Metformin play important role in improving cardiac events and cardiac biomarkers like: Gal-3, the addition of metformin to usual diabetes type 2 treatment may benefit in patient with cardiac complication.

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