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ORIGINAL ARTICLE

BENEFICIAL EFFECTS OF SITAGLIPTIN ,METFORMIN AND THEIR COMBINATION ON MYOCARDIAL ISCHEMIC AND VASCULAR CHANGES IN TYPE-TWO DIABETIC RATS

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ABSTRACT

Background: Myocardial infarction is a critical complication frequently occurs with type 2 diabetes mellitus (T2DM). Sitagliptin is antidiabetic drug inhibits DPP-4,which augments endogenous level of glucagon like peptide 1(GLP-1).Metformin is an FDA-approved antidiabetic drug, which is commonly prescribed for management of T2DM. **Objectives:** Is to investigate the possible beneficial effects of sitagliptin, metformin, and their combination on myocardial ischemic and vascular changes in T2D rats and possible mechanisms underlying these effects. **Methods:** Adult male albino rats were used in this study and were randomly divided into control normal group, control diabetic group , sham diabetic group and diabetic with induction of MI group. Diabetic rats with myocardial infarction(MI) were divided into the following treated subgroups: Oral Sitagliptin (300 mg/kg/day), Metformin(120mg/kg/day) and Combined metformin sitagliptin treated subgroups for 6 weeks. blood glucose **(**bl gl) level, serum Triglycerides (TG) and Low-density lipoprotein (LDL) levels, markers of oxidative stress(vascular Malondialdehyde (MDA) and cardiac Superoxide dismutase(SOD)levels),inflammation marker(plasma Interleukin-6(IL6),and plasma Creatin kinase-MB (CK-MB) were measured. Hematoxylin and Eosin stained sections of cardiac tissue were examined. vascular reactivity of thoracic aortas were measured. **Results:**DMT2 with induction of MI evoked oxidative stress, inflammation, as well as histopathological derangements in cardiac tissue and decreased vascular reactivity. Treatment with sitagliptin , metformin and their combination improved the cardiac histopathological changes and vascular reactivity as well as attenuating the oxidative stress and inflammatory processes**. Conclusion**: Sitagliptin has beneficial protective effects against myocardial ischemic changes induced in T2D rats but combined administration of metformin sitagliptin was superior to each drug alone in cardiovascular protection effects**.** This protective effect of sitagliptin may be due to its anti-inflammatory and antioxidant potentials.

Key words: metformin, sitagliptin , diabetes mellitus.

INTRODUCTION

Myocardial infarction is critical complication frequently occurs with T2DM. Progression of cardiovascular disease is accelerated, and outcomes are worse, if DM is present, and there is strong evidence that T2DM independently increases risk of atherosclerosis and pervasiveness of atherosclerosis[1].large number of factors contribute to progression of atherosclerosis with DM including Oxidative stress and inflammation. Intracellular hyperglycemia promotes production of mitochondrial reactive oxygen species(ROS)**.**ROS increase expression of inflammatory and adhesion factors, formation of oxidized-low density lipoprotein, and insulin resistance[2].They inhibit the activation of AMP-protein kinase

and adiponectin, decrease endothelial nitric oxide synthase activity, all of which accelerate atherosclerosis[3].

Sitagliptin, lowers bl.g by inhibiting DPP-4,which augments endogenous levels of GLP-1. Cardioprotective effect of sitagliptin may be due to reduction in inflammatory markers by down-regulation of COX-2expression and iNOS expression, decreasing free radicals and nitro oxidative stress parameters e.g.(MDA)by increasing SOD activity[4].Stromal cell-derived factor1α(SDF-1α)is degraded by DPP-4,so, Sitagliptin by inhibition of DPP-4can increase(SDF-1α)that promote vascular repair and neoangiogenesis. Also, DPP-4inhibition exerts antiatherosclerotic effects and reduces inflammation via inhibition of toll-like receptor4-mediated up regulation of IL-6and other proinflammatory cytokines[5].

Metformin is FDA-approved antidiabetic drug, commonly prescribed for management of T2DM. Metformin through its activation of AMPK reduces the generation of ROS and increases endothelial nitric oxide synthase phosphorylation which prevents opening of mitochondrial permeability pores[6].Metformin increases circulating levels of adiponectin. Adiponectin is adipokine synthesized in adipose tissue, exerts vasodilator, anti-apoptotic, anti-inflammatory and anti-oxidative activities in both cardiac and vascular cells[7].Prevention of activation of caspase-3may attribute in metformin induced cardio protection. Metformin can ameliorate oxidative stress through increasing cardiac(SOD)activity, increasing glutathione activity in heart tissue and enhancement of endothelium-dependent relaxation factor protection[8]**.**

Current work aimed to study the beneficial effects of sitagliptin ,metformin and combined metformin sitagliptin on myocardial ischemic and vascular changes in T2D rats

MATERIALS AND METHODS

Animals

Adult male albino rats weighing 200-250gm used in current study Animals were purchased from Faculty of Veterinary Medicine, Zagazig University, Egypt. Rats received standard diet and water add labitum. They were kept under standard humidity cycle with controlled temperature(28°C)and12hours light/dark cycle in plastic cages with wood shave bedding, each cage containing 6 rats. The study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. Experiments complied with the ARRIVE guidelines and was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) .

Experimental design with induction of T2D and induction of MI:

T2DM was induced by single intraperitoneal injection of nicotinamide at (270/mg/kg)dissolved in saline then after15minI.P injection of Streptozotocin (60/mg/kg) dissolved in saline. Bl.g measured every other day. After one week from injection moderate hyperglycemia occurred (PP bl.g>137mg/dl) were recorded[8].For induction of MI rats were anesthetized with1.5–2%isoflurane with100%O_2Tracheostomy was done through incision in trachea and insertion of endotracheal tube connected to artificial ventilator. Thorax was opened to exteriorize the heart and ligation of the proximal left anterior descending coronary artery. Heart was returned to its normal position then thorax was closed[9].

Determination of blood glucose level:

Blood glucose was measured using One Touch Brand strips , bl.g meter and one drop of blood obtained by tail vein puncture . Blood glucose level was recorded before and after induction of T2D. Fasting,1and2hour postprandial bl.g levels recorded after2,4and6weeks, Rats deprived from food for12hours at night before measuring fasting bl.g[9].

Blood pressure measurement: Prior to sacrifice, animals undergo blood pressure measurement by using invasive method. By Pressure catheter(2F, single,straight,140cm,PU)for the Quad Bridge Amplifier coupled to Power Lab(4/35)data

acquisition system .

Blood, heart and vascular tissue samples: Blood samples were taken after measuring bl.p. Tail blood was collected in test tubes and left to coagulate at room temperature and centrifuged at3000rpm for30min. Clear, non hemolysed, supernatant sera were quickly removed and kept at-20C till used for biochemical investigations[10]Animals then sacrificed and hearts harvested. Rats hearts were excised and divided into2portion .One portion was rapidly preserved in10%formalin for histopathological analysis. Other portion was frozen in liquid nitrogen and stored at-80C[11]Thoracic aortas obtained for evaluation of vascular reactivity.

Biochemical assays: For quantitative determination of bl.g using the One Touch Brand, blood glucose meter made in USA. Lifescan canda Ltd BumayB.CV5.C6.C6Jonson &Jonson company. TG and LDL estimated by Quantitation Kit(sigma Aldrich ,Egypt).MDA estimated by(MDA)assay Kit (Bio-diagnostic company, Egypt). DPP4 estimated by DPP4 Activity assay Kit(sigma Aldrich ,USA)**.**SOD estimated by SOD assay Kit(Bio-diagnostic company, Egypt).CK-MB estimated by Serum CK-MB: Bioassay technology laboratory (Shanghai Crystal Day Biotech CO.,LTD).

Histopathological analysis: Heart tissues are fixed in10%formalin, embedded in paraffin, sectioned at5-mm thick placed on glass slide, stained with H&E in standard histological manner and observed under light microscope to assess morphological changes in cardiac tissue[12]

Statistical analysis: Values of the obtained results were expressed and tabulated as mean ± standard error of mean. The data were Statistically analyzed by one-way analysis of variance(one-way ANOVA)followed by post hoctest LSD for determination of significance of difference between means by using spss statistical program version12window XP. Values of $p<0.05$ were considered significant[13].

RESULTS

Effect of oral administration of sitagliptin(300mg/kg),metformin (120mg/kg)and combined treatment on fasting and PP bl.g of T2Drats with MI for6weeks:

Fasting,1hour.pp and 2hour.pp bl.g levels in diabetic group with MI insignificantly changed compared with sham diabetic group**(table1)**.

Sitagliptin group showed significant(p<0.05)decrease in fasting bl.g compared with diabetic rats with MI from167.14±1.75to87.57±1.81, 75.86±1.79,70.86±1.18mg/dl when measured2,4,6weeks respectively . 1h.postprandial bl.g showed significant(p<0.05)decrease compared with diabetic rats with MI from 252±5.13to115.86±2.98,97.86±2.18,85.43± 1.89mg/dl when measured2,4,6weeks respectively. 2h.postprandial bl.g showed significant (p<0.05)decrease compared with diabetic rats with MI from243.29±13.8to107.57±2.84,84.8±1.74,73 .86±0.77mg/dl. when measured 2,4,6weeks respectively**(table1)**.

Metformin group showed significant(p<0.05)decrease in fasting bl.g compared with diabetic group with MI from167.14±1.75to67±1.31, 66±0.79,63.71±0.64mg/dl when measured2,4,6weeks respectively.1h.post prandial bl.g showed significant(p<0.05)decrease compared with diabetic group with MI from252±5.13to89.86±0.91,82.43±1.07,78.29 ± 0.68mg/dl when measured2,4,6weeks respectively. 2h.postprandial bl.g showed significant(p<0,05)decrease compared with diabetic group with MI from243.29±13.8to80.57±1.53,72.86±0.86,68 .29±0.57mg/dl when measured 2,4,6weeks respectively**(table1).**

Combined group showed significant (p<0.05)decrease in fasting bl.g compared with diabetic rats with MI from167.14±1.75to60.86±1.18, 57.14±0.74,53±0.98mg/dl when measured 2,4,6 weeks respectively. Combined group showed significant(p<0.05)decrease in fasting bl.g compared with sitagliptin group when measured 2,4,6weeks.

1h.postprandial bl.g showed significant(p<0.05)decrease compared with diabetic group with MI from252±5.13to75.29±1.15,68.86±0.77,64.43 ±0.43 mg/dl when measured2,4,6weeks respectively**.**
1h.postprandial bl.g showed significant(p<0.05)decrease compared with sitagliptin group from115.86±2.98to83±0.98,69±0.90,65.57±0. 57mg/dl when measured2,4,6weeks.
2h.postprandial bl.g showed significant (p<0.05)decrease compared with diabetic rats with MIfromin from 243.29±13.8to70.29±0.94,63.29±0.87,60±0.0 9 mg/dl when measured 2,4,6 weeks respectively. 2hpostprandial bl.g showed significant(p<0.05) decrease compared with sitagliptin group from 107±2.84 to70.29±0.94,63.29±0.87,60±0.09mg/dl when measured 2,4,6 weeks respectively**.**
Fasting,1hour.pp and 2hour.pp bl.g levels in combined group significantly(p<0.05)decrease compared with metformin group when measured after 6weeks**(table1)**.

Effect of sitagliptin(300mg/kg),metformin(120mg/kg)and combined treatment on systolic ,diastolic and mean arterial bl.p of type 2 diabetic rats with MI:

Systolic ,diastolic and mean arterial bl.p were significantly (p<0.05)decreased in diabetic group with MI compared with sham diabetic group. Systolic Bl.P was significantly decreased from 144.29±4.29to107.14±2.86mmHg and diastolic bl.p was significantly(P<0.05)decreased from103.57±3.03to74.29±1.7mmHg.Mean arterial Bl.P significantly decreased from123.93±3.57to90.71±2.09mmHg.

Arterial blood pressure in sitagliptin ,metformin and combined groups showed insignificant changes in systolic ,diastolic and mean arterial Bl.P compared with diabetic group with MI**(Fig1).**

Effect of oral administration of sitagliptin(300mg/kg),metformin (120mg/kg)and combined treatment on plasma triglycerides and LDL, Vascular MDA, Cardiac SOD, plasma CK-MB, plasma and vascular DPP4 of T2D rats with MI:

Diabetic group with MI showed insignificant changes in triglycerides and LDL compared with sham diabetic group.

Sitagliptin group showed significant(p<0.05)decrease in triglycerides and LDL compared with diabetic group with MI from44.90±0.24,34.79± 0.46to36.8±0.32,25.79±0.41mg/dl.

Metformin group showed significant(p<0.05)decrease in triglycerides and LDL compared with diabetic rats with MI from 44.90±0.24,34.79± 0.46to32.5±0.31,19.14 ±0.36mg/dl respectively.

Combined group showed significant(p<0.05)decrease in triglycerides and LDL levels compared with diabetic group with MI in triglycerides and LDL from44.90±0.24,34.79±0.46to24.29±0.32,17. 21±0.49mg/dl respectively.
Combined group showed insignificant change in the levels of LDL compared with metformin group. Triglycerides combined group significantly decreased from 32.5±0.31to24.29±0.32mg/dl compared with metformin group**.**
combined group showed significant(p<0.05)decrease in triglycerides and LDL compared with sitagliptin group from 36.8±0.32,25.79±0.4 to24.29±0.32,17.07±0.49mg/dl respectively**(table2)**.

Diabetic group with MI showed insignificant changes in vascular MDA compared with sham diabetic group.

Sitagliptin group showed insignificant decrease in vascular MDA compared with the diabetic group with MI**.** Sitagliptin group showed significant(p<0.05)increase in vascular MDA compared with Metformin treated group from2.03±0.2to2.51±0.21nmol/mg.

Metformin group showed significant(p<0.05)decrease in vascular MDA compared with diabetic group with MI from3.26±0.44 to2.03±0.12nmol/mg**.**

Combined group showed significant(p<0.05)decrease in vascular MDA

compared with diabetic group with MI from3.26± 0.44to1.4±0.18nmol/mg**.** Combined group showed significant(p<0.05)decrease in vascular MDA compared with the sitagliptin group from2.51±0.2to1.4±0.18nmol/mg respectively**. (table2)**.

Diabetic group with MI showed insignificant changes in cardiac SOD compared with sham diabetic group.

Sitagliptin group showed significant(p<0.05)increase in cardiac SOD compared with diabetic group with MI from5.1±0.24to 6.93±0.12u/mg**.**

Metformin group showed significant(p<0.05)increase in cardiac SOD compared with diabetic group with MI from5.1±0.24to8.86±0.28u/mg**.**

Combined group showed significant(p<0.05)increase in cardiac SOD compared with diabetic group with MI from5.1±0.24to10.74±0.50u/mg. Combined group showed significant(p<0.05)increase in cardiac SOD compared with metformin group from8.86±0.28to10.74±0.50u/mg**.** Combined group showed significant(p<0.05)increase in cardiac SOD compared with sitagliptin group from6.93±0.12to10.74±0.50u/mg**(table2).**

Diabetic group with MI showed significant(p<0.05)increase in plasma CK-MB and IL6 compared with sham diabetic group from 646±5.07to728.43±7.81U/Landfrom105±1.43 to191±3.85pg/ml respectively.

Sitagliptin group showed significant(p<0.05)decrease in plasma CK-MB and IL6 compared with diabetic group with MI from728.43±7.81to541.43±4.72U/L and from191±3.85to 133.57±4.44pg/ml respectively.

Metformin group showed significant(p<0.05)decrease in plasma CK-MB and IL6 compared with diabetic group with MI from728.43±7.81to452.86±8.08U/L and from19±3.85to98.29±3.09pg/ml respectively**.**

Combined group showed significant(p<0.05) decreases in CK-MB and IL6 compared with diabetic group with MI from728.43±7.81to377.86±7.54U/L and from191±3.85to84.29±1.6pg/ml Combined group showed significant(p<0.05)decrease in plasma CK-MB and IL6 compared with metformin group from 452.86±8.08to377.86±7.54U/L and from98.29±3.09to84.29±1.63pg**.**Combined group showed significant (p<0.05)decrease in plasma CK-MB and IL6 compared with sitagliptin group from541.43±4.72to377.86±7.54U/L and from133.57±4.44to 84.29±1.63pg/ml respectively. **(table2).**

Diabetic group with MI showed insignificant changes in plasma and vascularDPP4compared with sham diabetic group.

Sitagliptin group showed significant(p<0.05)decrease in plasma and vascularDPP4 compared with diabetic group with MI from4.07±0.21,12±0.31to1.34±0.15,6.99±0.21mu/ml respectively.

Metformin group showed significant(p<0.05)decrease in plasma ascularDPP4compared with diabetic group with MI values decrease from4.07±0.21,12±0.31to3.26±0.16,10.31±0.32mu/ml respectively

Combined group showed significant(p<0.05) decrease in plasma and vascularDPP4compared with diabetic group with MI the from4.56±0.13,12±0.31to0.91±0.06, 6.06±0.27mu/ml respectively**.** Combined group showed significant(p<0.05)decrease in plasma and vascularDPP4 compared with metformin group from 3.26±0.16,10.31±0.32to 0.91±0.06,6.06±0.27mu/ml respectively**.** Combined group showed significant(p<0.05)decrease in plasma DPP4 compared with sitagliptin group from1.34±0.15to0.9±0.06mu/ml**(table2).**

Effect of oral administration of sitagliptin(300mg/kg),metformin (120mg/kg)and their combination on vascular reactivity of aortic ring of T2D rats with MI:

Diabetic rats with MI showed insignificant change in reactivity of rat aortic strip to Norepinephrine (NE) and

Acetylcholine (ACH) when compared with sham diabetic group.

Sitagliptin group showed insignificant change in reactivity of aortic strip in comparison with diabetic rats with MI group**.**

Metformin group showed significant($p<0.05$)increase in reactivity of the rat aortic strip to NE and ACH compared with diabetic rats with MI from12.29±0.18to18.79±0.18mm and from-11±0.3to-16.43±0.2mm respectively**.**

Combined group showed significant($p<0.05$)increase in reactivity of the rat aortic strip to NE and ACH compared with diabetic rats with MI from12.29±0.18to20.21±0.15 mm and from-11±0.31to-19.57±0.2mm respectively.

Combined treated group showed significant($p<0.05$)increase in reactivity of rat aortic strip to NE and ACH compared with sitagliptin treated rats with MI from14.21±0.01to20.07±0.13mm and from-11.71±0.29to-19.57±0.2mm respectively **(Fig2,3)**.

Effect of oral administration of sitagliptin(300mg/kg),metformin (120mg/kg)and combined treatment on cardiac histology of T2D rats with MI:

Histological observations fundamentally confirmed our results obtained from serum and tissue analysis. In control group, normal cardiac tissue, cardiac muscle fiber separated by thin connective stroma**(Fig4.A)**. In sham diabetic group cardiac tissue showed wide stroma containing thick walled vascular spaces surrounded by aggregates of inflammatory cells and cardiac muscle bundles**(Fig4.C).** observing the H&E stained slides of cardiac tissue of diabetic rat with MI showed markedly degenerated atrophied cardiac muscle bundle separated by wide of dense collagenous stroma**(Fig2.D)**.Observed cardiac injuries were improved in metformin , sitagliptin and combined treatment**(Fig4.E.F&G)**compared with diabetic rat with MI. These improvements are best observed in combined treated group relative to each drug alone.

Table(1): Effect of oral administration of sitagliptin(300mg/kg) , metformin (120mg/kg) and combined treatment on fasting and postprandial blood glucose level of type 2 diabetic rats with MI:

Parameter	Group	Control normal	Diabetic control	Sham diabetic	Diabetic with cardiac ischemia	Sitagliptin treated	Metformin treated	Combined treated
After 2 weeks	FBS: (mg/dl)	A 62.71 ± 0.62	B 161.3 3±1.49	B 161.43±1.49	B 167.14±1.75	C 87.57 ± 1.8	A 67 ± 1.31	A 60.86 ± 1.18
	1h PP:(mg/dl)	A 90±1.88	B 274.14±2.26	B 275.14±2.26	B 252 ± 5.13	C 115.86± 2.98	A 89.86 ± 0.91	A 75.29 ± 1.15
	2h PP:(mg/dl)	A 74.29±1.75	B 236.71±1.97	B 238.71±1.97	B 243.29±13.8	C 107.57 ±2.84	A 80.57 ± 1.53	A 70.29 ± 0.94
After 4 weeks	FBS: (mg/dl)	A 62.14 ± 1.20	B 165.0 ±1.19	B 165.86±1.19	B 174.29 ±4.1	C 75.86 ± 1.79	A 66 ± 0.79	A 57.14 ± 0.74
	1h PP:(mg/dl	A 78.43 ±1.76	B 334.15±8.27	B 334.14±8.27	B 351.43±7.14	C 97.86 ± 2.18	A 82.43 ± 1.07	A 68.86 ± 0.77
	2h PP: mg/dl)	A 68.86 ± 0.71	B 280.75±0.99	B 281.71±0.99	B 296.71± 4.7	C 84.86 ± 1.74	A 72.86 ± 0.86	A 63.29 ± 0.87
After 6 weeks	FBS: (mg/dl)	A 62.29 ±1.16	B 177.7 6±1.06	B 179.86±1.06	B 181.29±4.05	A 70.86 ± 1.18	A 63.71 ± 0.64	C 53 ± 0.98
	1h PP:(mg/dl)	A 86.71 ± 1.65	B 323.29±1.61	B 324.29±1.61	B 346.29±4.2	A 85.43 ± 1.89	A 78.29 ± 0.68	C 64.43 ± 0.43
	2h PP:(mg/dl)	A 70.14 ± 0.76	B 310.33 ±1.81	B 312.43±1.81	B 308.71±2.84	A 73.86 ± 0.77	A 68.29 ± 0.57	C 60 ± 0.90

FBS :fasting blood sugar **pp** :post prandial

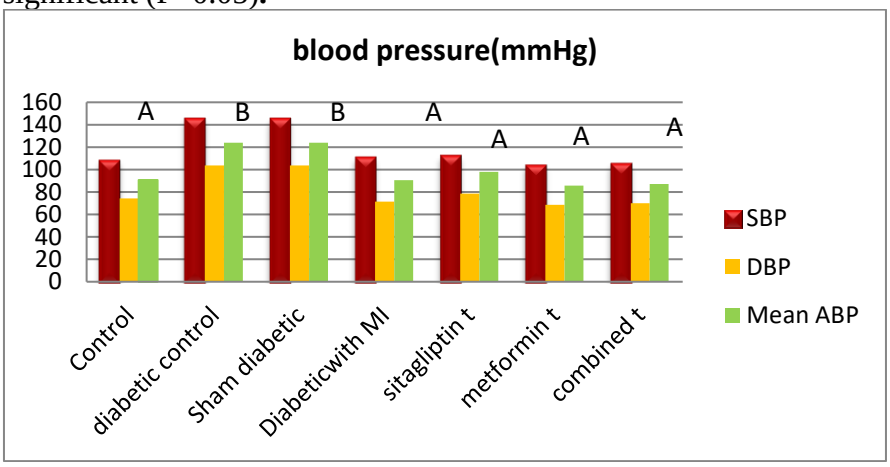
values represents :Mean ± standard error

Groups with different letters are statistically significant ($P<0.05$)

Table(2):Effect of oral administration of sitagliptin(300mg/kg), metformin (120mg/kg) and combined treatment on plasma triglycerides and LDL level, Vascular MDA ,Cardiac SOD and plasma CK-MB levels of type 2 diabetic rats with MI

Group Parameter	Control	Diabetic control	Sham diabetic	Diabetic with cardiac ischemia	Sitagliptin Treated	Metformin treated	Combined treated
TG: (mg/dl)	A 22.3 ± 0.35	B 41.96 ± 0.36	B 41.96 ± 0.36	B 44.90 ± 0.24	D 36.8 ± 0.32	C 32.5 ± 0.31	A 24.29 ± 0.32
LDL:(mg /dl)	A 15.14 ± 0.40	B 41.96 ± 0.37	B 41.96 ± 0.37	B 34.79 ± 0.46	C 25.79 ± 0.41	A 19.14 ± 0.36	A 17.21 ± 0.49
Vascular MDA	A 0.73 ± 0.02	B 2.43 ± 0.20	B 2.43 ± 0.20	B 3.26 ± 0.47	B 2.51 ± 0.21	C 2.03 ± 0.21	C 1.4 ± 0.18
Cardiac SOD	A 12.96 ± 0.22	B 7.09 ± 0.26	B 7.09 ± 0.26	B 5.1 ± 0.24	D 6.93 ± 0.12	C 8.86 ± 0.28	A 10.74 ± 0.50
CK.MB	A 309.29±8. 20	B 646±5.07	B 646±5.07	C 728.43±7.81	E 541.43±4.72	D 452.86±8.08	F 377.86±7.54
IL-6	A 75.71 ±1.02	B 104.71±1.43	B 105±1.43	C 191 ± 3.85	E 133.57± 4.44	D 98.29 ± 3.09	A 84.29 ±1.63
Plasma DPP4	A 2.99 ±0.10	B 4.56 ± 0.13	B 4.52 ± 0.13	B 4.07 ± 0.21	C 1.34 ± 0.15	A 3.26 ± 0.16	D 0.91 ± 0.06
Vascular DPP4	A 8.34 ±0.24	B 12.39 ±0.26	B 12.37 ±0.26	B 12 ± 0.31	D 6.99 ± 0.21	C 10.31 ± 0.32	D 6.06 ± 0.27

TG = triglycerides **LDL =** low density lipoprotein
vascular MDA=vascular malondialdehyde
Cardiac SOD =cardiac superoxide dismutase **IL-6**=interleukin6
DPP4=dipeptidyle peptidas 4 enzyme **CK.MB**= Creatine kinases
values represent :Means ± standard error Groups with different letters are statistically significant (P<0.05).



Fig(1:):Effect of sitagliptin(300mg/kg) , metformin (120mg/kg) and combined treatment on systolic ,diastolic and mean arterial blood pressure of type 2 diabetic rats with MI.
values represents :Mean ± standard error
Groups with different letters are statistically significant (P<0.05)

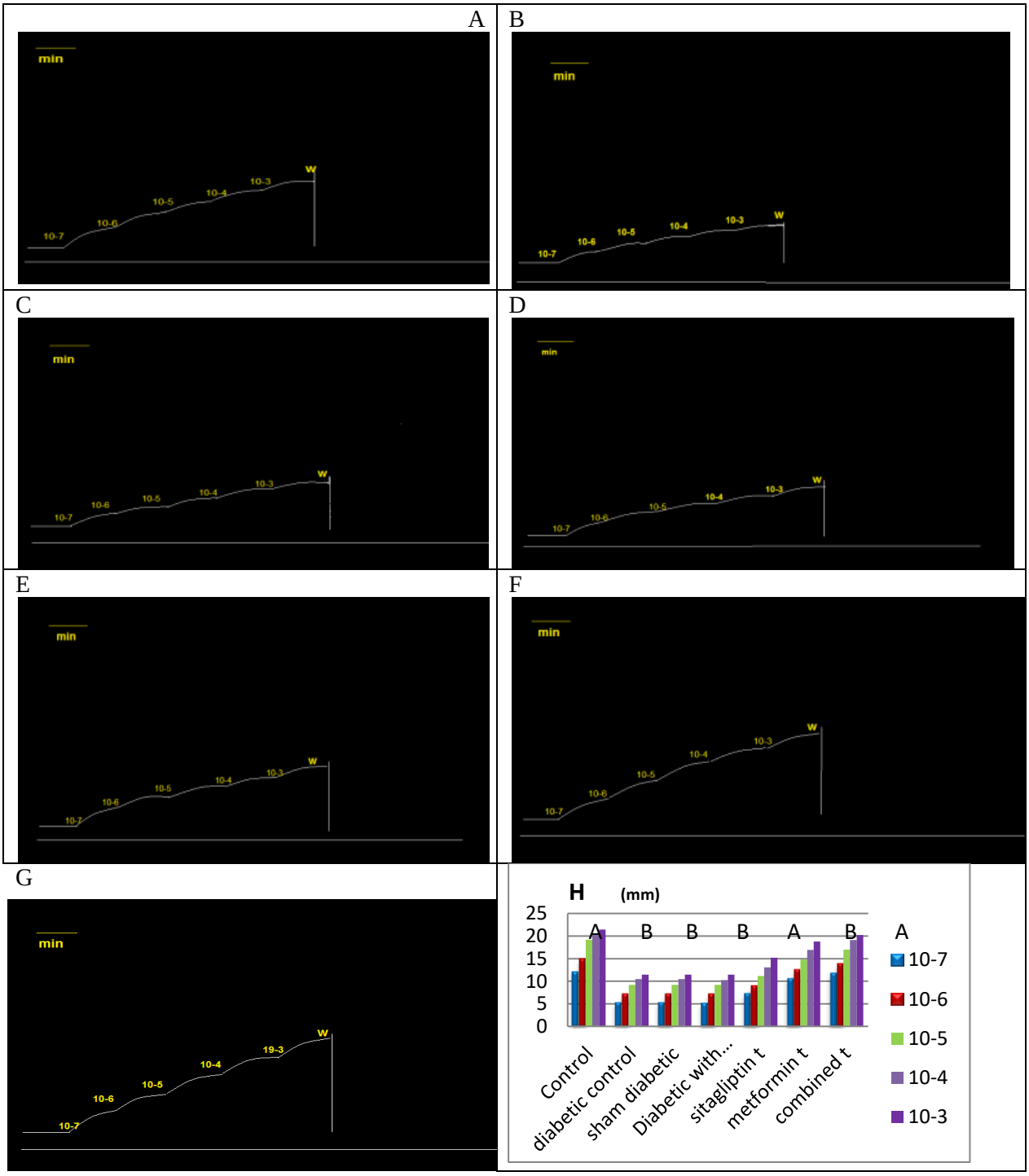


Fig2:
A: The effect of noradrnaline (10^{-7} M -10^{-3} M) on isolated rat aortic spiral strips of control group.
B: The effect of noradrnaline (10^{-7} M -10^{-3} M) on isolated rat aortic spiral strips of diabetic control rats.
C: The effect of noradrnaline (10^{-7} M -10^{-3} M) on isolated rat aortic spiral strips of sham diabetic rats.
D: The effect of noradrnaline (10^{-7} M -10^{-3} M) on isolated rat aortic spiral strips of diabetic rats with MI
E: The effect of noradrenalin(10^{-7} M -10^{-3} M) on isolated rat aortic spiral strip sitagliptin treated group
F: The effect of noradrenalin (10^{-7} M -10^{-3} M) on isolated rat aortic spiral strip of metformin treated group
G: The effect of noradrenalin (10^{-7} M -10^{-3} M) on isolated rat aortic spiral strip of combined treated group
H:Effect of oral administration of sitagliptin(300mg/kg), metformin (120mg/kg) and their combination on cumulative response of the rat aortic spiral strip to noradrenalin in type 2 diabetic rats
values represents :Mean ± standard error

Groups with different letters are statistically significant (P<0.05)

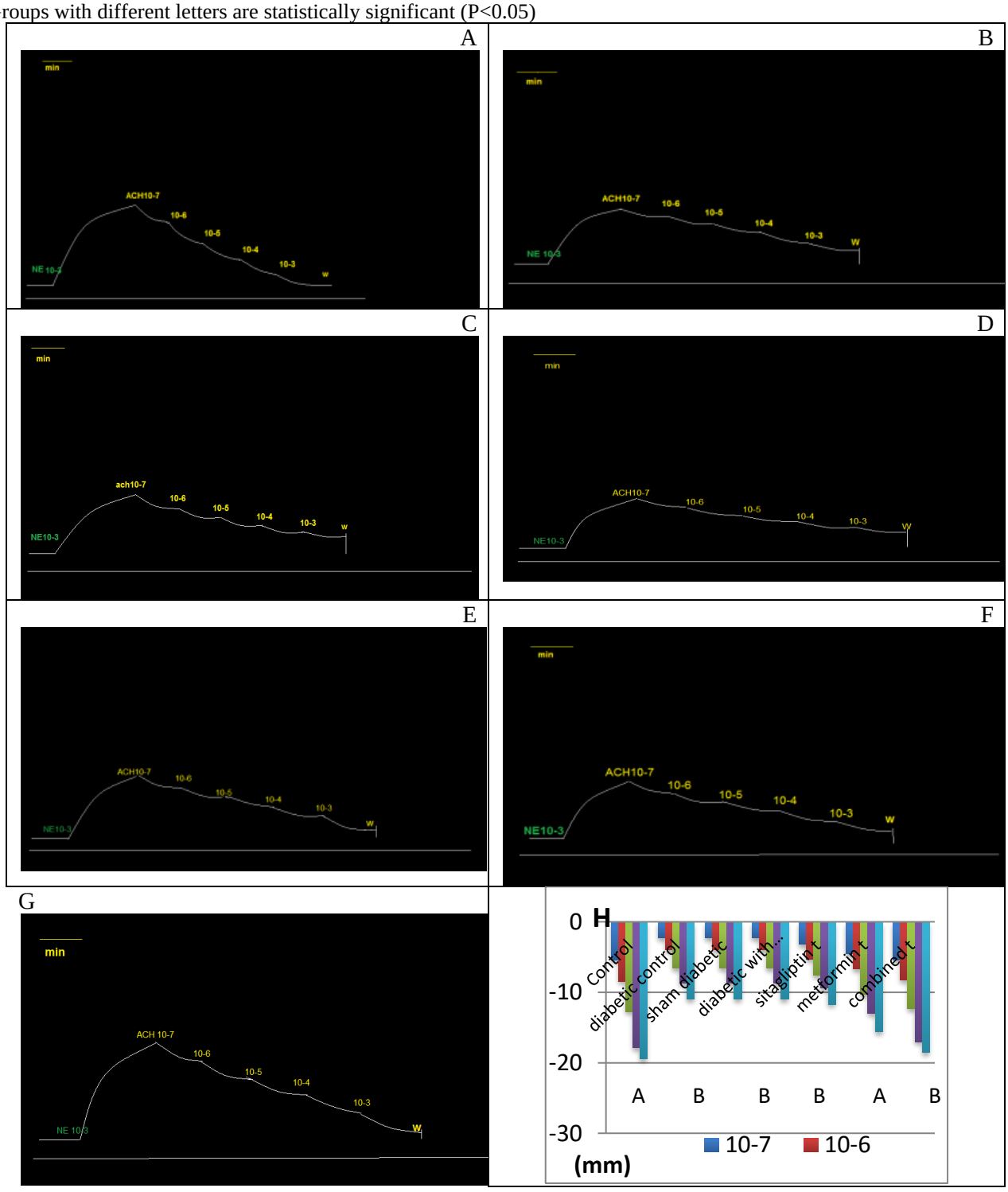


Fig3: A: The effect of ACH (10^{-7} M -10^{-3} M) upon contracted isolated rat aortic spiral strips of control group.
B: The effect of ACH (10^{-7} M -10^{-3} M) upon contracted isolated rat aortic spiral strips of diabetic control rats.
C: The effect of ACH (10^{-7} M -10^{-3} M) upon contracted isolated rat aortic spiral strips of sham diabetic rats.
D: The effect of ACH (10^{-7} M -10^{-3} M) upon contracted isolated rat aortic spiral strips of diabetic rats with MI
E: The effect of ACH (10^{-7} M -10^{-3} M) upon contracted isolated rat aortic spiral strip of sitagliptin treated group
F: The effect of ACH (10^{-7} M -10^{-3} M) upon contracted isolated rat aortic spiral strip of metformin treated group
G: The effect of ACH (10^{-7} M -10^{-3} M) upon contracted isolated rat aortic spiral strip of combined treated group
H:Effect of oral administration of sitagliptin (300mg/kg), metformin (120mg/kg) and their combination on cumulative response of the rat aortic spiral strip to acetylcholine in type 2 diabetic rats with MI.
values represents :Mean ± standard error
Groups with different letters are statistically significant (P<0.05)

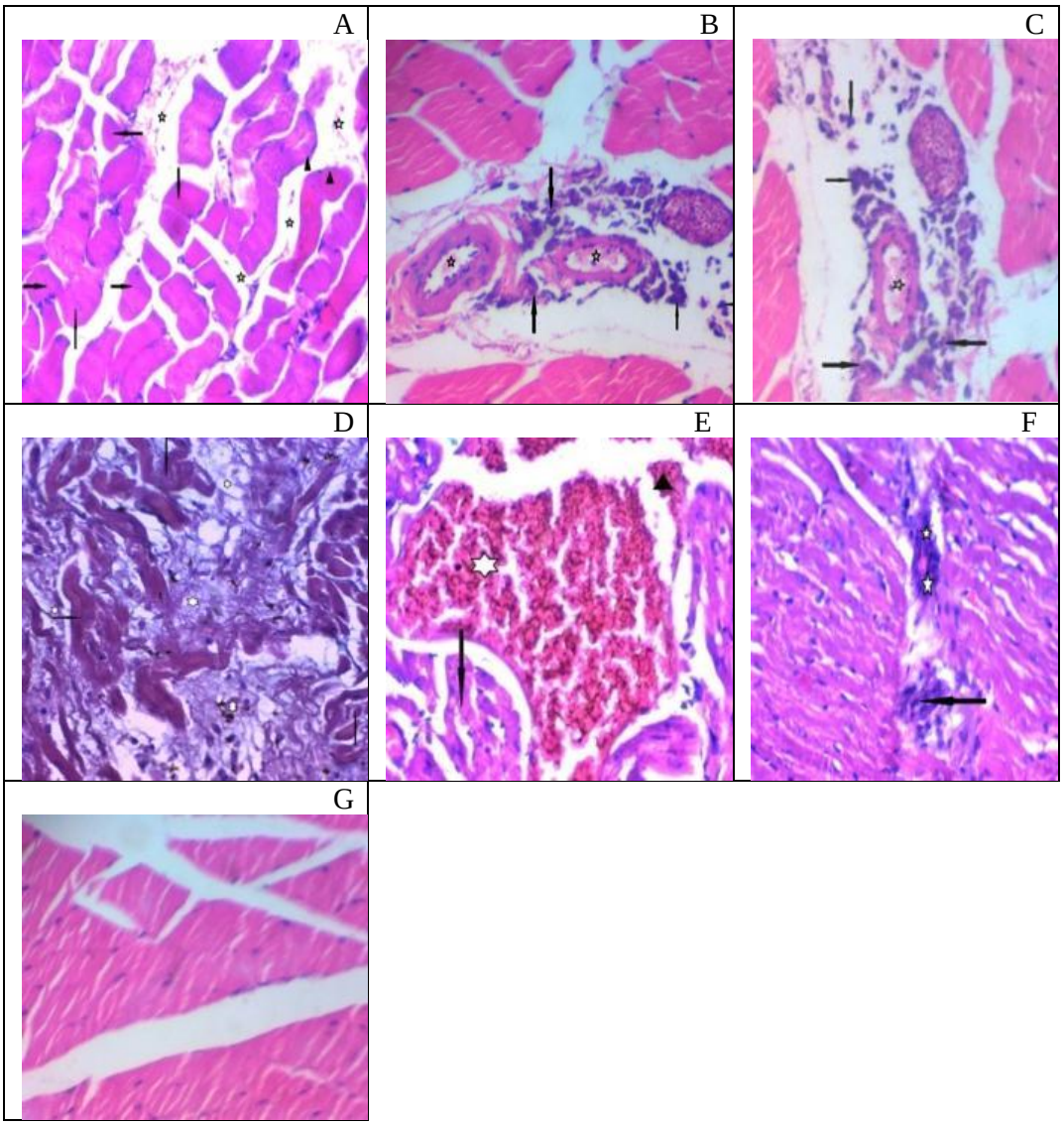


Fig4: Representative HE stained sections photomicrographs (magnification 400x) (A) control group showed normal cardiac tissue of cross(black arrow) and longitudinal (triangle)sectional cardiac muscle fiber separated by thin connective stroma (white star).
(B) Diabetic control group showed wide stroma containing thick walled vascular spaces(white star) surrounded by aggregates of inflammatory cells (black arrow) between cardiac muscle bundles.
(C)Sham diabetic group showed wide stroma containing thick walled vascular spaces(white star) surrounded by aggregates of inflammatory cells (black arrow) between cardiac muscle bundles.
(D) diabetic group with MI showed markedly degenerated atrophied cardiac muscle bundle (black arrow) seperated by wide of dense collagenous stroma (white star).
(E) diabetic group with coronary ligation treated with sitagliptin showed areas of hemorrhage (white star) surrounded by atrophied cardiac muscle fibers (black arrow) and scattered inflammatory cells(triangle)
(F) diabetic group with coronary ligation treated with metformin showed oblique cardiac muscles separated by scanty inflammatory cells(black arrow) and mild fibrosis(white star)
(G) diabetic group with coronary ligation treated with combined treatment showed return of the cardiac muscle to its state and absence of inflammatory cells

DISCUSSION

Current study represented that sitagliptin lowered fasting and postprandial bl.g levels of type2diabetic rats with MI. This result is in agreement with ***Carolina et al*****[14]**who studied the effect of sitagliptin on T2D patient .Sitagliptin works to competitively inhibit DPP4. This enzyme breaks down GLP-

1andGIP, gastrointestinal hormones released in response to meal. By preventing GLP-1and GIP inactivation, they are able to increase secretion of insulin and suppress release of glucagon. This drives bl.g levels towards normal[15].

Results of present study demonstrated that metformin lowered fasting postprandial bl.g levels of type2diabetic rats with MI. This result is in agreement with ***Madiraju et al*****[16]**It was explained by AMPK Activation**.** Patient withT2DM has three times the normal rate of gluconeogenesis. Inability of insulin to suppress hepatic glucose output is major etiological factor in hyperglycemia of T2DM. AMPK was required for metformin's inhibitory effect on liver glucose production**.** AMPK is an enzyme that plays an important role in insulin signaling and metabolism of glucose and fats[17]They found that metformin increases insulin sensitivity, enhances peripheral glucose uptake by inducing phosphorylation of GLUT4enhancer factor, decreases insulin-induced suppression of fatty acid oxidation and decreases absorption of glucose from GIT. Increased peripheral use of glucose may be due to improved insulin binding to insulin receptors[18].

In current study, combined metformin sitagliptin lowered fasting and postprandial bl.g levels of type2diabetic rats with MI more than sitagliptin alone. These results are in agreement with ***Bo-Ahrén*****[19].**He found that diabetes is disease with at least three main defects, which need to be corrected: impaired insulin secretion, insulin resistance and hypersecretion of glucagon. Rationale for combining metformin with DPP-4inhibitors is complimentary mechanism of action of the two strategies. Thus, metformin acts primarily by reducing hepatic glucose output and improving insulin sensitivity in liver and muscle**.** whereas DPP-4inhibitors act by increasing GLP-1level and thereby stimulating insulin secretion and inhibiting glucagon secretion[20].

Results of our study showed that sitagliptin decreased arterial bl.p of type2diabetic rats. These results are in agreement with ***Ferreira et al***[21]They explained that administration of Sitagliptin increased incretins increased insulin secretion. Insulin has vasodilator effect also, sitagliptin decreased sodium reabsorbtion due to decrease in bl.g .***Ogawa et al***[22] studied the effect of sitagliptin in hypertensive type2diabetic patients found that sitagliptin cause inhibition to NA+/H exchanger3 in proximal renal tubule by GLP1 receptor on renal tubule cells lead to increase sodium excretion and produce diuretic effect lead to decrease in mean arterial bl.p.

In our work it was found that metformin decreased arterial bl.p of T2Drats. These results are in agreement with ***Mahdi et al***[23]Increased NO bioactivity after metformin is also in agreement with the results of previous studies which suggested that metformin improves endothelial vascular function in T2DM by increasing AMPK-dependent hsp90-mediated eNOS activation. Metformin has been shown to raise hydrogen sulfide tissue concentration that sulfhydrates potassium channels and functions as vasculoprotective factor[24].

Present work revealed that combined metformin sitagliptin decreased arterial bl.p in type2diabetic rats. These results are in agreement with ***Hussain etal*****[25]**They found that increase level of(GLP-1)in body by both drugs made them render to reduce bl.p due to the two mechanisms, one is stimulation of NO in vessels by(GLP-1),it is indirect effect, the other one is direct and independent vasodilator effect of(GLP-1)**.** And finally urinary loss of sodium by renal tubules. In addition weight reductions by both drugs[26]**.**

In present work it was found that sitagliptin lowered LDL and TG levels of type2diabetic rats with MI. This result is in agreement with ***Minhua et al*****[27]**beneficial effect of sitagliptin on serum lipid parameters could be explained by an improvement in glycemic control and insulin resistance, anti-diabetic effects of sitagliptin related to mechanism of insulin in body like the inhibition effect of insulin on hormone-sensitive lipase enzyme in adipose tissue which leads to inhibition of the degradation of TG into FFA, thereby inhibiting cholesterol synthesis or the stimulation effect of insulin on ATP-citrate lyase, acetyl-COA carboxylase, fatty acid synthase and glucose6-phosphate dehydrogenase which lead to increase adipose tissue lipogenesis[28].

Result of current work reported that metformin improved lipid profile of type2diabetic rats with MI. This result is in agreement with ***Lucas et al*****[29].** Metformin can activate an upstream liver kinaseB then phosphorylates and activates(AMPK)which can then affect the transcription of several regulators of hepatic lipogenesis and gluconeogenesis. First regulation of lipogenesis is a reduction in expression and activity of sterol regulatory element binding protein-1 which leads to two beneficial effects on lipids. One effect is reduced expression of fatty acid synthase, which leads to reduction in fatty acid synthesis[30]. These are essential steps in formation TG. Another effect is phosphorylation of 3-hydroxy-3-methyl-glutary-CoA reductase

which reduces its cholesterol synthesis capabilities[30].

Our study reported that combined metformin sitagliptin improved lipid profile in type2diabetic rats with MI more than each one alone. This result is in agreement with ***Hussain et al***[25]. They reported that dyslipidemic effect of both drugs may be related through(GLP-1)mediated effect of decrease in intestinal lymph flow, reduced absorption of TG from intestinal cells, reduction in synthesis of intestinal and hepatic derived apoB-48 and apoB-100 containing lipoprotein[31].

In our study it was found that sitagliptin increased cardiac SOD inT2DM with MI. These results are in agreement with ***Al-Rasheed et al***[32]They reported that hyperglycemia in diabetes is associated with increased mitochondrial production of ROS and elevated FFA in diabetic heart modulates mitochondrial electron chain and activates nicotinamide adenine dinucleotide phosphate oxidases to generate superoxide. Superoxide can combine with NO forming highly reactive and damaging peroxynitrite species. Increased lipid peroxidation and NO in the heart of diabetic rats, demonstrating oxidative stress condition. SOD catalyzes dismutation of superoxide radicals to oxygen and hydrogen peroxide. The latter is converted to molecular oxygen and water .this antioxidant represents vital line of defense against diabetes-induced oxidative stress. sitagliptin showed significant decrease in cardiac lipid peroxidation and NO levels, with concomitant enhancement of the antioxidant defenses[33]

From results of the present work it was observed that metformin decreased vascular MDA level and increased cardiac SOD of type2diabetic rats with MI. These results are in agreement with ***Bonaventure Chukwunonso et al***[34]. They reported that metformin through its activation of AMPK reduces the generation of ROS and increases endothelial NOS phosphorylation which prevents the opening of mitochondrial permeability pores. and increase cardiac SOD level which provides cardioprotective effect aganist ROS.

Present study reported that combined metformin sitagliptin decreased the level of vascular MDA and increased cardiac SOD levels of type2diabetic rats with MI more than occurred with each one alone. These results are in agreement with ***Ali et al***[35]They revealed that combination attenuated(MDA) and SOD levels in T2DM..

Our study showed that sitagliptin decreased levels of plasma CK-MB and IL6 of type2diabetic rats with MI. This is in agreement with *Mohamed et al*[36] in their study They reported that Cardioprotective effect of sitagliptin may be due to reduction in inflammatory markers by down-regulation of COX-2 expression and iNOS expression, decreasing free radicals and nitro oxidative stress parameters e.g. MDA by increase SOD activity[4].(SDF-1α)is degraded byDPP-4,so,Sitagliptin by inhibition of DPP-4increases(SDF-1α) concentration that promote vascular repair. DPP-4inhibition exerts anti atherosclerotic effects and reduces inflammation via inhibition of toll-like receptor4-mediated up-regulation of IL-6and other proinflammatory cytokines[5].

From results of the present study, it was observed that metformin decreased plasma CK-MB and IL6of type2diabetic rats with MI. This result is in agreement with ***Bonaventure Chukwunonso et al***[34]Metformin through its activation of(AMPK)reduces generation of ROS and increases eNOS phosphorylation which prevents opening of the mitochondrial permeability pores . Also, AMPK up regulates activity of peroxisome proliferator activated receptor gamma coactivator-1αwhich plays role in regulating energy metabolism and mitochondrial bioenergetics. Metformin increases circulating levels of adiponectin. Adiponectin is an adipokine synthesized in adipose tissue, exerts vasodilator, anti-apoptotic, anti-inflammatory and anti-oxidative activities in both cardiovascular cells. Prevention of activation of caspase-3attribute in metformin induced cardio protection. Metformin decreased serum IL6 level of T2D patients due to a reduction in activity of(NF-κB)and increase in activity of protein Akt.

Results of present study showed that combined metformin sitagliptin decreased the levels of plasma CK-MB and IL6 of type2diabetic rats with MI more than the administration of each one alone. This is in agreement with ***Ali et al*****[35]**who studied the effects of metformin Alone and in combination with Sitagliptin on oxidative Stress and proinflammatory Markers in patients with T2DM.They revealed that metformin alone and in combination with sitagliptin attenuated plasma CK-MB and IL6levels. Metformin in combination with sitagliptin had greater effect than Metformin alone.

Present study showed that sitaglipin lowered plasma and vascularDPP4 activity of type2diabetic rats with MI . These results are in agreement with ***Herman et al*****[37]**Treatment of

diabetic patient with sitagliptin lead to sustained inhibition of DPP-4 over 24-hour dosing interval, which was associated with an approximately 2to3folds increase in activeGLP-1levels relative to placebo

Present study revealed that metformin lowered vascular DPP4 activity of type2diabetic rats with MI. These results are in agreement with ***Laura et al*[38].** they measured DPP-4activity and mRNA expression in cultured human aortic endothelial cells and human microvascular dermal endothelial cells exposed to high glucose and Metformin. Their study showed that hyperglycemia is capable of increasing the DPP-4 activity in microvascular endothelial cells.

In current study it was found that combined metformin sitagliptin lowered plasma and vascular DPP4activity of type2diabetic rats with MI more than the effect of each one alone.

Current work demonstrated that metformin improved vascular reactivity of type2diabetic rats with MI**.** This result is in agreement with ***Cristina et al[39]***They reported that metformin results in increased production of NO by increasing AMPK-dependent activation of(eNOS).Metformin decreases circulating endothelin-1level in insulin-resistant. It inhibits oxidative stress production in mitochondria. Reduction of oxidative stress by metformin may be due to inhibition of glycation process that directly causes free-radical production. Intracellular oxidant properties of metformin result in inhibition of both receptor for AGEs and lectin-like oxidized receptor1. Long-term benefit from metformin due to anti-glycating properties[40].

In our study combined metformin sitagliptin improved vascular reactivity of type2diabetic rats with MI. combined metformin sitagliptin improved vascular reactivity of diabetic rats more than sitagliptin alone.

COCLUSION

Sitagliptin has beneficial protective effects against MI changes in T2D rats but combined administration of metformin sitagliptin was superior to each drug alone in cardiovascular protection effects**.** This protective effect of sitagliptin may be due to its anti-inflammatory and antioxidant potentials.

RECOMMENDATION

We recommend to use sitagliptin with metformin in treatment of diabetes mellitus with MI due to its beneficial cardiovascular protective effects with its anti-inflammatory and antioxidant effects.

Conflict interest : No

financial disclosure :No

REFERENCE

1. **Ross S., Gerstein H., Paré G**. The genetic link between diabetes and atherosclerosis. Can. J. Cardiol. 2018;34:565–574.
2. **Forrester Steven J., Kikuchi Daniel S., Hernandes Marina S., Xu Qian, Griendling Kathy K**. Reactive oxygen species in metabolic and inflammatory signaling. Circ. Res. 2018;122:877–902.
3. **Förstermann U., Xia N., Li H**. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ. Res. 2017;120:713735.
4. **Chang G, Zhang P, Ye L, Lu K, Wang Y, Duan Q et al. (2013)** Protective effects of sitagliptin on myocardial injury and cardiac function in an ischemia/reperfusion rat model. Eur J Pharmacol 718: 105-113. 50.
5. **Ussher JR, Drucker DJ (2012)** Cardiovascular biology of the incretin system. Endocr Rev 33: 187-215. 52.
6. **Noeman SA, Hamooda HA, Baalash AA (2011)** Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetol Metab Syndr 3: 17.
7. **Li P., Shibata R., Unno K.*(2010)*** Evidence for the importance of adiponectin in the cardioprotective effects pioglitazone of. *Hypertension* 55, 69_75 doi:10.1161/HYPERTENSIONAHA.109.141655
8. **Ashour AE, Sayed MM, Abd-Allah AR, Korashy HM, Maayah ZH, Hisham Alkhalidi et al. (2012)** Metformin rescues the myocardium from doxorubicin-induced energy starvation and mitochondrial damage in rats. Oxidat med cel long 43: 1-13.
9. **Boyle AJ, Kelly DJ, Zhang Y, Cox AJ, Gow RM, Way K et al.** Inhibition of protein kinase C reduces left ventricular fibrosis and dysfunction following myocardial infarction. J Mol Cell Cardiol 2005.39:213–221.
10. **Muhammad Aslam , Rahila Nijam (2013)**Hypolipidemic and Anti-atherogenic Activity of Aqueous Extract of Leaves of Lagenaria Siceraria in Wistar Rats. Journal of natural remedies Volume 14, Issue 1, January 2014.
11. **He Huang, Jiang Shan, Xiao-hong Pan, Hui-ping Wang, and Ling-bo Qian**. Carvedilol Protects Early Diabetic Rat Hearts through Reducing Oxidative Stress; Proceedings of the 2005 IEEE Engineering in Medicine and Biology 27th Annual Conference; Shanghai. 2005. p. 339.
12. **Connelly KA, Kelly DJ, Zhang Y, Prior DL, Advani A, Cox AJ et al.** Inhibition of protein kinase C-beta by ruboxistaurin preserves cardiac function and reduces extracellular matrix production in diabetic cardiomyopathy. Circ Heart Fail 2009.2:129–137.
13. **Dean J ,Dean AJ, Coloumbier D,** Epi–Info version 6. 02. soft ware computer package on microcomputer for epidemiology statistics and data processing 2004**.**

14. **Solis-Herrera C, Triplitt C, Garduno-Garcia Jde J, Adams J, DeFronzo RA, Cersosimo E.** Mechanisms of glucose lowering of dipeptidyl peptidase-4 inhibitor sitagliptin when used alone or with metformin in type 2 diabetes: a double-tracer study. Diabetes Care. 2013
15. **Gadsby, Roger (2009)**. "Efficacy and Safety of Sitagliptin in the Treatment of Type 2 Diabetes" (pdf). Clinical Medicine: Therapeutics (1): 53–62
16. **Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA et al(June 2014)**. " Metformin suppresses gluconeogenesis by inhibiting mitochondria lglycerophosphate dehydrogenase".

Nature. 510 (7506):542.6. Bibcode:2014Natur.510..54 2M

17. **Stepensky D, Friedman M, Srour W, Raz I, Hoffman A**. Preclinical evaluation of pharmacokinetic–pharmacodynamic rationale for oral CR metformin formulation. J Controlled Release. 2001;71:107–115.
18. **Cheng J, Huang C, Liu I, Tzeng TF, Chang CJ**.. Novel mechanism for plasma glucose lowering action of Metformin in streptozotocin induced diabetic rats. Diabetes. 2006;55:819–825.
19. **Bo Ahrén** (Novel combination treatment of type 2 diabetes DPP-4 inhibition+ metformin) Vasc Health Risk Manag. 2008 Apr; 4(2): 383–394.Published online 2008 Apr.
20. **Ahrén B.** Dipeptidyl peptidase-4 inhibitors – clinical data and clinical implications. Diabetes Care. 2007a;30:1344–50.
21. **Ferreira, L., Teixeira-de-Lemos, E., Pinto, F., Parada, B., Mega, C., Vala, H., Pinto, R. et al (2010)** Effects of sitagliptin treatment on dysmetabolism, inflammation, and oxidative stress in an animal model of type 2 diabetes (ZDF rat). *Mediators Inflamm*., 2010, 592760.
22. **Ogawa S, Ishiki M, Nako K, Okamura M, Senda M, Mori T,** Sitagliptin, a dipeptidyl peptidase-4 inhibitor, decreases systolic blood pressure in Japanese hypertensive patients with type 2 diabetes. Tohoku J Exp Med. 2011 Feb;223(2):133-5.
23. **Mahdi Hamidi Shishavan, Robert H, Henning, Azuwerus van Buiten, Maaike Goris, Leo E. et al** (Metformin Improves Endothelial Function and Reduces Blood Pressure in Diabetic Spontaneously Hypertensive Rats Independent from Glycemia Control: Comparison to Vildagliptin) Sci Rep. 2017
24. **Willinski B, Willinski J, Somogyi E, Piotrowska J, Opoka W.** Metformin raises hydrogen sulfide tissue concentrations in various mouse organs. Pharmacol Rep. 2013;65:737–742.
25. **Hussain M, Atif MA, Ghafoor MB.** (Beneficial effects of sitagliptin and metformin in non-diabetic hypertensive and dyslipidemic patients). Pak J Pharm Sci. 2016 Nov;29(6 Suppl):2385-2389.
26. **Gutzwiller JP, Tschopp S, Block A, Zehnder CE, HuberAR, Kreyenbuehl M, et al(2004**). Glucagon like peptide1 induces natriuresis in healthy subjects and ininsulin resistant obese men. *J. Clin. Endocrinol.Metab.*, 89: 3055-3061.
27. **Minhua Fan, MD, Yuelan Li, MD, and Shihong Zhang, MD**(Effects of Sitagliptin on Lipid Profiles in Patients With Type 2 Diabetes Mellitus A Meta-analysis of Randomized Clinical Trials) Medicine (Baltimore). 2016 Jan; 95(2): e2386. Published online 2016 Jan 15.
28. **Sakamoto Y, Oyama J, Ikeda H, et al**. Effects of sitagliptin beyond glycemic control: focus on quality of life. *Cardiovasc Diabetol* 2013; 12:35.
29. **Lucas Eduardo Campos de Oliveira , Luiz Augusto da Silva, Jéssica Wouk , Vinícius Müller Reis Weber , Camila da Luz Eltchechem , Pablo de Almeida, et al**(Role of AMPK and its possible interactions in metformin therapy and physical exercise) Journal of Applied Pharmaceutical Science Vol. 7 (10), pp. 196-199, October, 2017 Available online at http://www.japsonline.com .
30. **Leiberman M, Marks AD**, eds. *Mark's Basic Medical Biochemistry A Clinical Approach*. 3rd Ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2009:479-566
31. **Tremblay AJ, Lamarche B, Kelly I, Charest A, Lépine MC, Droit A, et al** (Effect of sitagliptin therapy on triglyceride-rich lipoprotein kinetics in patients with type 2 diabetes). Diabetes Obes Metab. 2014 Dec;16(12):1223-9.
32. **Al-RasheedNM, Al-Rasheed NM, Hasan IH, Al-Amin MA, Al-Ajmi HN, Mahmoud AM** Sitagliptin attenuates cardiomyopathy by modulating the JAK/STAT signaling pathway in experimental diabetic rats. Drug Des Devel Ther. 2016 Jun 28;10:2095-107.
33. **Silambarasan T, Raja B. Diosmin,** a bioflavonoid reverses alterations in blood pressure, nitric oxide, lipid peroxides and antioxidant status in DOCA-salt induced hypertensive rats. Eur J Pharmacol. 2012;679(1–3):81–89.
34. **Chukwunonso Obi B, Chinwuba Okoye T, Okpashi VE, Nonye Igwe C, Olisah, Alumanah E**.Comparative Study of the Antioxidant Effects of Metformin, Glibenclamide, and Repaglinide in Alloxan-Induced Diabetic Rats JDiabetes Res; 2016: 1635361.
35. **Ali M. Al Hussona, Ahmed R. Abu-Raghif , Methaq A. Al Ghazi** (Effects of Metformin Alone and in Combination with Sitagliptin on Oxidative Stress and Proinflammatory Markers in Patients with Diabetes Mellitus Type-2) Int. J. Pharm. Sci. Rev. Res., 42(1), January - February 2017; Article No. 31, Pages: 185-190
36. **Mohamed EL Shabrawy , Afaf Sayed , Omayma Anwar , Lubna Omar and Mahmoud M Kamel** Comparative Study of the Protective Effect of Metformin and Sitagliptin against Doxorubicin-Induced Cardiotoxicity in Rats Abdo et al., Clin Pharmacol Biopharm 2017.

37. **Herman GA, Bergman A, Stevens C, Kotey P, Yi B, Zhao P, Dietrich B, et al.**(Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes). J Clin Endocrinol Metab. 2006 Nov;91(11):4612-9. Epub 2006 Aug 15.
38. **Laura Pala ,Anna Pezzatini ,Ilaria Dicembrini ,Silvia Ciani ,Stefania Gelmini ,Barbara Gabriella Vannelli et al** Different modulation of dipeptidyl peptidase-4 activity between microvascular and macrovascular human endothelial cells Acta Diabetol. 2012.
39. **Cristina M Sena, Paulo Matafome, Teresa Louro,Elsa Nunes, Rosa Fernandes, and Raquel M Seiça**(Metformin restores endothelial function in aorta of diabetic rats) Br J Pharmacol. 2011 May; 163(2): 424–437.
40. **Davis BJ, Xie Z, Viollet B, Zou MH**. Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. Diabetes. 2006;55:496–505.

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