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RESEARCH ARTICLE

Effects of Garlic, Repaglinide, and Amlodipine on Myocardial Injury in Diabetic Rats F ollowing Ischemia-Reperfusion

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ABSTRACT

Ischaemia reperfusion-induced myocardial injury is a major issue, especially in diabetes, where oxidative stress enhances myocardial dysfunction. The therapeutic applications of garlic homogenate, repaglinide, and amlodipine have not been well studied. This study aims to examine the impact of Garlic homogenate, Repaglinide, and Amlodipine on Myocardial Injury in Diabetic Rats post Ischemia-Reperfusion. Diabetes was induced in Sprague-Dawley rats using a single intraperitoneal administration of alloxan. A 10-day treatment with garlic homogenate, repaglinide, and amlodipine followed by I/R-induced myocardial injury was performed. Cardiac function recovery was evaluated by contractile force, and biochemical studies were carried out using lactate dehydrogenase and creatine kinase-MB (CK-MB). Antioxidant status was studied by estimating superoxide dismutase, catalase activity, and TBARS levels. Histopathological analysis of myocardial and pancreatic tissues was performed. Triple co-treatment of diabetic rats with garlic extract, repaglinide, and amlodipine resulted in maximum antioxidant activity, lowered myocardial enzyme activity (LDH and CK-MB), and improved recovery of heart function after I/R injury. The combination of garlic extract, repaglinide, and amlodipine treatment resulted in the highest levels of SOD and catalase and the lowest amount of TBARS compared to dual or single therapies. Histopathological findings demonstrated that the triple therapy maintained myocardial integrity better than the other therapies did. This study offers new evidence that the combination of amlodipine, repaglinide, and garlic homogenate provides better cardioprotective effects against ischaemia-reperfusion injury in diabetic rats.

Keywords: Diabetes; Garlic; Repaglinide; Amlodipine; Myocardial Injury

INTRODUCTION

Cardiovascular disease (CVD) is one of the most common causes of morbidity and mortality in patients with type 2 diabetes[1]. Individuals with type 2 diabetes are more likely to develop cardiovascular disease[2]. Diabetes mellitus is a multifactorial disorder characterized by hyperglycaemia, polydipsia, polyuria, and glycosuria[3]. CVD is affected by conditions such as elevated cholesterol, fibrinogen, clotting factors, platelet function, glucose metabolic disturbances, and smoking[4]. Therapeutic interventions are necessary to reverse diabetes-induced cardiovascular damage, and cardioprotective drugs are particularly important during hypoglycaemic management. Ischaemia-reperfusion (I-R) injury results in functional and structural heart defects, primarily due to uncontrolled excess cytosolic calcium (Ca^{2+}) and oxidative stress[5–7]. Reperfusion augments reactive oxygen and nitrogen species,[4,8] and aging hearts can exhibit increased sensitivity to IR injury[9]. Herbal consumption has increased worldwide. Although herbs are traditionally safe, they can interact with drugs. Approximately 15–20% of patients with prescriptions take herbal supplements, with less than 40% reporting to physicians, often out of fear of being judged[10]. Most physicians are not aware of herb-drug interaction hazards,[11] making it imperative to conduct reliable research[12].

Garlic (Allium sativum) is an ancient culinary spice and folk medicine with antidiabetic and cardioprotective properties[13]. It prevents cardiovascular and metabolic diseases owing to its high bioactive compound content[14]. Repaglinide (REP) enhances cardiovascular function and glucose metabolism by inducing insulin secretion, although its rapid absorption and metabolism are different from sulfonylureas[15]. Therefore, complementary herbs must be used to support their efficacy. Short-acting hypoglycemics

assist in correcting the initial insulin secretion deficiencies in type 2 diabetes[16]. Calcium channel blockers, such as amlodipine (AML), block calcium entrance, which is advantageous for heart and vessel function[17]. AML is used to treat hypertension and angina with few cardiac effects and assists in healing after ischaemia[18,19]. The current study was conducted to evaluate whether garlic could enhance the cardioprotective effects of AML and REP during ischaemia-reperfusion-induced myocardial injury in diabetic rats.

MATERIALS AND METHODS

Instruments and Chemicals: The study used an analytical balance (Shimadzu, Japan), autoanalyser (Qualigens, Mumbai), centrifuge, colorimeter, perfusion pump, Polyrite (RMS, Chandigarh), Student's Physiograph (INCO, India), spectrophotometer, and reagents including calcium chloride, CK-MB kits, LDH kits, sucrose, D-glucose, EDTA, ethanol, heparin, ketamine, magnesium sulfate, NBT, propranolol, and others.

Experimental Animals: Male or female rats with body weights of 175–250 g were maintained in a well-ventilated animal house at a temperature of 25 ± 5°C under a light-dark cycle of 12:12 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (KCP/IAEC-27/2009-10), and animals were handled according to the regulations of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Preparation of Garlic Homogenate: Locally brought garlic bulbs were peeled, chopped, and ground into paste. A garlic homogenate (GH) concentration of 0.1 gm/ml, equivalent to 250 mg/kg body weight, was prepared in suspension in distilled water and administered to the rats within 30 min of preparation.

Induction of Diabetes: Rats were fasted for 24 h prior to the induction of diabetes. Diabetes was induced by a single intraperitoneal injection of alloxan (150 mg/kg body weight). Three days after alloxan administration, blood glucose levels were measured, and rats with serum glucose levels ≥300 mg/dl were selected for further experimentation.

Experimental protocol: Sprague-Dawley rats of both sexes were separated into two groups (normal and diabetic), and each group was further separated into eight groups, with six animals in each group. The groups were distributed as follows: Group I (Control), Group II (GH, 30 days oral treatment), Group III (REP, 10 days oral), Group IV (AML, 10 days oral), Group V (GH+REP), Group VI (GH+AML), Group VII (REP+AML), and Group VIII (GH+REP+AML).

Ischaemia reperfusion-induced myocardial dysfunction: The rats were anaesthetised with sodium pentobarbital (35 mg/kg, i.p.) at the end of the treatment schedule. The heart and pancreas were collected. Hearts were perfused using a modified Langendorff apparatus with Krebs-Henseleit solution, carbogen-gassed, at 37°C, and a constant flow rate of 5 ml/min. The contractile force was recorded using a grass electromechanical recorder with a displacement transducer. Baseline readings were recorded for 15 minutes after a 15-minute equilibration time, followed by 15 min of global ischaemia and reperfusion. The recovery of inotropic and chronotropic functions was measured, and cardioprotection was checked by assessing the developed tension. Perfusates were harvested during both the pre- and post-ischaemic phases and used for biochemical estimation of LDH and CK-MB. Three excised hearts per group were homogenized in 0.25 M sucrose to prepare heart tissue homogenate (HTH) for subsequent biochemical analyses. Histopathological sections of pancreatic tissue were stained with haematoxylin and eosin (H&E) and observed under a microscope to assess myocardial changes in diabetic rats following different treatments.

Measurement of Antioxidant Status: Total protein was estimated on the basis of coloured complex formation with Folin-Ciocalteu reagent, monitored at 610 nm, using bovine albumin as a standard. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of NBT reduction at 560 nm after hydroxylamine hydrochloride oxidation. Catalase activity was measured by measuring hydrogen peroxide decomposition at 240 nm. Thiobarbituric acid-reactive substances (TBARS) were measured as an indicator of lipid peroxidation, with malondialdehyde (MDA) as a marker, measured at 532 nm.

Cardiac Markers Estimation: CK-MB activity was determined by using enzyme reagent and starter reagent, incubated and read by an autoanalyzer at 340 nm. LDH activity was determined by the oxidation of NADH to NAD^+ in the presence of pyruvate, and the decrease in absorbance was measured, which was equivalent to the LDH activity in the sample.

RESULTS

Ischemia-reperfusion (ISC) injury in normal rats reduced superoxide dismutase (SOD) and catalase activities (0.97±0.06 and 3.49±0.35 units/mg protein, respectively) and increased TBARS levels (50.01±1.70). GH treatment significantly improved SOD (3.98±0.08), catalase (10.4±0.8), and reduced TBARS (26.66±1.26). REP showed moderate improvement, whereas AML treatment provided greater protection (SOD: 4.7±0.2; catalase: 11.5±0.7; TBARS: 28.89±1.7). Co-treatment with REP+GH and AML+GH further enhanced antioxidant levels, and the combined GH+REP+AML treatment resulted in the highest SOD (12.89±1.2), catalase (43±2.67), and lowest TBARS (13.39±1.84) (Table 1).

In diabetic rats, reperfusion-ischemia injury caused oxidative stress, shown by decreased SOD (0.56±0.09), catalase (6.8±0.98), and increased TBARS (56.98±1.9). GH treatment improved SOD (5.58±0.3) and catalase (16.33±2.88) levels and reduced TBARS (34.98±2.9) levels. REP raised SOD to 4.77±0.8, catalase to 17.18±1.34,

Table 1: Effect of treatments on SOD, Catalase and TBARS in ischemia-induced myocardial injury in diabetic in comparison with normal groups

Normal group with ischemia-induced damage in myocardial injury			
Treatments	**SOD (Unit/mg protein)**	**Catalase (Unit/mg protein)**	**TBARS (Unit/mg protein)**
ISC	0.97±0.06	3.49±0.35	50.01±1.70
GH	3.98±0.08	10.4±0.8$^{\#}$	26.66±1.26$^{\#}$
REP	1.08+0.088%	7.87±0.98$^{\$}$	44.45±3.54$^{\#\&}$
AML	4.7±0.2$^{\$\infty}$	11.5±0.70$^{\#\infty}$	28.89±1.7$^{\#Ø}$
REP+GH	3.78±0.34$^{@\Delta}$	10.8±0.78$^{\#\Delta}$	29.98±1.55$^{\#\%Ø}$
AML+GH	9.73±0.49$^{\#\&\mu}$	37.61±0.70$^{\#\&\mu}$	16.93±0.9$^{\#\&\mu}$
REP+AML	5.1±0.65$^{\$\infty}$	12.98±1.34$^{\#Ø}$	22±1.54$^{\#\&Ø\mu}$
GH+REP+AML	12.89±1.2$^{\#\&Ø\mu\S}$	43±2.67$^{\#\&Ø\mu\S}$	13.39±1.84$^{\#\&Ø\mu\S}$
All values are expressed as mean ± SEM (n=6); significance levels are @ p<0.05, $ p<0.01, # p<0.001 vs NC; % p<0.05, β p<0.01, & p<0.001 vs GH; Δ p<0.05, ∞ p<0.01, Ø p<0.001 vs REP; ≠ p<0.05, £p<0.01, μ p<0.001 vs AML; Þ p<0.05, ¥ p<0.01, §p<0.001 vs REP+AML; NC = Normal control, DC = Diabetic control, GH = Garlic homogenate (250 mg/kg), REP = Repaglinide (1 mg/kg), AML = Amlodipine (5 mg/kg).			
Diabetic group with ischemia-induced damage in myocardial injury			
Treatments	**SOD (Unit/mg protein)**	**Catalase (Unit/mg protein)**	**TBARS (Unit/mg protein)**
DC	0.56±0.09$^{@}$	6.8±0.98	56.98±1.9$^{\$}$
GH	5.58±0.3$^{€\#}$	16.33±2.88$^{€\#}$	34.98±2.9$^{€\#}$
REP	4.77±0.8$^{@€}$	17.18±1.34$^{€\#}$	42.78±3.1$^{€\#\&}$
AML	8.95±0.50$^{€\#\&Ø}$	23.84±2.88$^{€\#\&Ø}$	29.88±2.3$^{€\#\&Ø}$
REP+GH	8.6±0.5$^{€\#\&Ø}$	23.21±3.08$^{€\#\&\infty}$	30.54±3.33$^{€\#\%Ø}$
AML+GH	11.39±0.3$^{€\#\&Ø£}$	36.14±2.34$^{€\#\&Ø\mu}$	20.01±1.37$^{€\#\&\mu}$
REP+AML	11.22±0.67$^{€Ø\#\&}$	29.31±1.23$^{€\#\&Ø}$	23.66±2.89$^{€\#\&Ø}$
GH+REP+AML	13.8±0.61$^{€\#\&Ø\neq¥}$	48.4±3.75$^{€\#\&Ø\mu\S}$	17.06±0.99$^{€\#\&Ø\mu\S}$
All values are expressed as mean ± SEM (n=6); @ p<0.05, $ p<0.01, # p<0.001 vs NC; * p<0.05, ¢¢P<0.01, €€P<0.001 vs DC; %P<0.05, β P<0.01, & p<0.001 vs GH; ΔP<0.05, ∞P<0.01, ØP<0.001 vs REP; ≠ p<0.05, £p<0.01, μ p<0.001 vs AML; Þ p<0.05, ¥ p<0.01, §p<0.001 vs REP+AML combination; with NC = Normal control, DC = Diabetic control, GH = Garlic homogenate (250 mg/kg), REP = Repaglinide (1 mg/kg), and AML = Amlodipine (5 mg/kg).			

and lowered TBARS to 42.78±3.1. AML treatment resulted in SOD (8.95±0.50), catalase (23.84±2.88), and TBARS (29.88±2.3). Combined treatments (REP+GH, AML+GH, REP+AML) further improved results, with the GH+REP+AML combination showing the highest protection: SOD (13.8±0.61), catalase (48.4±3.75), and TBARS (17.06±0.99) (Table 1). ISC significantly increased CK-MB and LDH activities in both the perfusate and HTH of control rats, with CK-MB (82±4.8 unit/lit, 33±0.7 unit/gm) and LDH (perfusate: 141±10.8 unit/lit, HTH: 32±3.1 unit/gm). GH treatment reduced CK-MB (perfusate: 39±5.4, HTH: 67±3.3) and LDH (perfusate: 65±4.1, HTH: 114±5.2) levels. REP and AML alone partially restored levels, while the GH+REP+AML combination provided maximum protection, with CK-MB (perfusate: 16±1.56, HTH: 124±3.78) and LDH (perfusate: 13.99±1.09, HTH: 187±4.77) at their lowest levels (Table 2).

In STZ-induced diabetic rats, the DC group of rats exhibited highly significant elevation in CK-MB (112.3±3.7) and LDH (296.6±7.6) in perfusate and lowered HTH CK-MB (18.43) and LDH (16.36). GH increased these values to CK-MB perfusate 52±3.4, HTH 70.77, LDH perfusate 195±5.0, and HTH 49.15. REP and AML treatments improved, with the AML+GH and REP+AML combinations having more recovery. The GH+REP+AML group had maximum improvement with CK-MB perfusate at 12.43±0.6, HTH at 134.78, LDH perfusate at 128±3.9, and HTH at 81.39 (Table 2).

Recovery of heart function, as measured by the developed tension and heart rate, was weak in ischaemia-reperfusion rats. GH treatment significantly improved recovery, whereas REP and AML individually improved recovery moderately. Co-treatment with GH and either REP, AML, or a combination of the two drugs resulted in improved recovery, and the GH+REP+AML group showed the best improvement. In diabetic rats, the diabetic control group showed very poor recovery. GH alone significantly enhanced heart function, while REP and AML treatments improved recovery compared with the control. The GH+REP+AML group showed the highest recovery (Table 3).

Oral glucose test: Fasting blood glucose level (FBS) was 60 ± 3.45 mg/dl in the normal control (NC) group and

Table 2: Effects of ischemia-reperfusion induced damage on LDH and CKMB levels in perfusate and heart tissue homogenate of normal and diabetic groups

Normal group with ischemia-reperfusion induced damage				
Treatments	**CK-MB ACTIVITY**		**LDH ACTIVITY**	
	Perfusate (unit/lit)	**HTH (unit/gm)**	**Perfusate (unit/lit)**	**HTH (unit/gm)**
ISC	82±4.8	33±0.7	141±10.8	32±3.1
GH	39±5.4$^{\#}$	67±3.3$^{\#}$	65±4.1$^{\#}$	114±5.2$^{\#}$
REP	71 ±3.9$^{\#\&}$	44±2.68$^{\$\&}$	79 ± 3.66$^{\#\%}$	85.8 ± 3.67$^{\#\&}$
AML	32±5.7$^{\#\&\varnothing}$	73±2.7$^{\#\varnothing}$	43±3.3$^{\#\beta\varnothing}$	125±9.0$^{\#\varnothing}$
REP+GH	42± 3.81$^{\#\beta\varnothing}$	70.98±3.33$^{\#\varnothing}$	59.98 ± 2.65$^{\#\infty}$	118 ± 6.34$^{\#\&\varnothing}$
AML+ GH	20±2.0$^{\#\&\mu}$	94±2.4$^{\#\&\mu}$	22±4.8$^{\#\beta\neq}$	164±5.4$^{\#\&\mu}$
REP+AML	24±2.87$^{\#\&\Delta\mu\infty}$	82±4.6$^{\#\&\varnothing\neq}$	32 ± 2.44$^{\#\&\varnothing}$	153.78±7.88$^{\#\&\varnothing\mu}$
GH+REP+AML	16±1.56$^{\#\&\varnothing\mu\S}$	124±3.78$^{\#\&\varnothing\mu\S}$	13.99±1.09$^{\#\&\varnothing\mu\S}$	187 ± 4.77$^{\#\&\varnothing\mu\S}$

All values are mean ± SEM, n=6, with statistical significance denoted as @ P<0.05, $ P<0.01, # p<0.001 when compared to NC; % p<0.05, β P<0.01, & p<0.001 when compared to GH; Δ P<0.05, ∞ P<0.01, Ø p<0.001 when compared to REP; $\neq$ p<0.05, £p<0.01, μ p<0.001 when compared to AML; Þ p<0.05, ¥ p<0.01, §p<0.001 when compared to REP + AML combination; NC - Normal control, DC - Diabetic control, GH - Garlic homogenate (250mg/kg), REP - Repaglinide (1mg/kg), AML - Amlodipine (5mg/kg).

Diabetic group ischemia-reperfusion induced damage				
Treatments	**CK-MB ACTIVITY**		**LDH ACTIVITY**	
	Perfusate (unit/lit)	**HTH (unit/gm)**	**Perfusate (unit/lit)**	**HTH (unit/gm)**
NC	82±4.8	33±0.7	141+10.8	32+3.1
DC	112.3+3.7$^{\#}$	18.43$^{\#}$	296.6+7.6$^{€\#}$	16.36+1.7$^{@}$
GH	52+3.4$^{€\#}$	70.77$^{\#€}$	195+5.0$^{€\#}$	49.15+1.3$^{\$€}$
REP	38.4+1.6$^{€\#\beta}$	53.2$^{\#€}$aaa	245+5.4$^{€\#\&}$	37.6+3.4$^{€}$
AML	26.29+2.3$^{€\#\&\Delta}$	63.45$^{\#€\Delta}$	148+3.0$^{€\&\varnothing}$	64+2.6$^{€\#\%\varnothing}$
REP+GH	27.5+1.5$^{€\#\&}$	89.32$^{\#€}$a$^{\varnothing\beta}$	185+4.7$^{€\#\varnothing}$	63.29+1.8$^{€\#\%\varnothing}$
AML+GH	14.6+0.4$^{€\#\&\neq}$	94.98$^{\#€\beta}$a$^{\mu}$	132+3.5$^{@€\&\varnothing£}$	78.33+5.7$^{€\#\&\neq}$
REP+AML	23.88+2.69$^{€\#\&\infty}$	74.17$^{\#€\varnothing}$c	140+2.5$^{€\&\varnothing}$	62.3+1.6$^{€\#\varnothing}$
GH +REP +AML	12.43+0.6$^{€\#\&\varnothing£Þ}$	134.78$^{\#€\beta}$a$^{\varnothing\mu\S}$	128+3.9$^{\$€\&\varnothing\mu Þ}$	81.39+1.40$^{€\#\&\varnothing\mu¥}$

All values are mean ± SEM, n=6, with statistical significance indicated as @ p<0.05, $ p<0.01, # p<0.001 when compared to NC; * p<0.05, ¢p<0.01, € p<0.001 when compared to DC; % p<0.05, β p<0.01, & p<0.001 when compared to GH; Δ p<0.05, ∞ p<0.01, Ø p<0.001 when compared to REP; $\neq$ p<0.05, £p<0.01, μ p<0.001 when compared to AML; Þ p<0.05, ¥ p<0.01, §p<0.001 when compared to REP + AML COMBINATION. NC - Normal control, DC - Diabetic control, GH - Garlic homogenate (250mg/kg), REP - Repaglinide (1mg/kg), AML - Amlodipine (5mg/kg).

significantly increased to 130 ± 4.78 mg/dl in the diabetic control (DC) group. The GH-, REP-, and AML-treated groups showed FBS levels of 124 ± 2.92, 106 ± 4.11, and 112 ± 5.67 mg/dl, respectively. The combination groups (GH+REP, GH+AML, REP+AML, GH+AML+REP) showed better control, with FBS values between 79 ± 3.74 and 103 ± 3.77 mg/dl. At 30 min post-glucose load, blood glucose peaked in the DC group (330 ± 7.10 mg/dl) compared to that in the NC group (160 ± 3.45 mg/dl), while the GH, REP, and AML groups recorded 310 ± 6.83, 429 ± 8.55, and 230 ± 2.45 mg/dl respectively. Combination therapies, especially GH+AML+REP (214 ± 5.24 mg/dl), showed better glucose control. At 120 min, glucose in the NC group decreased to 110 ± 1.33 mg/dl, while the DC remained high at 375 ± 2.56 mg/dl. REP+AML (292 ± 7.59 mg/dl) and GH+AML+REP (139 ± 2.64 mg/dl) groups showed significant improvement compared to monotherapies. After 240 min, the NC returned to 90 ± 2.65 mg/dl, while GH+AML+REP achieved near-normal levels (89 ± 3.88 mg/dl), indicating the strongest glucose-lowering effect among the treated groups.

Histopathological evaluation: Histopathological analysis revealed that ISC induced severe myocardial damage in both diabetic and non-diabetic rats. GH maintained the myocardial structure, whereas AML and REP alone induced mild-to-moderate damage. GH, in combination with AML or REP, further enhances the architecture. The GH+REP+AML group had an almost normal myocardial structure, with minimal infiltration. In diabetic rats, treatment with GH ensured heart integrity, and triple therapy with GH, REP, and AML showed the maximum cardioprotective effect. (Figure 1)

Histopathological evaluation revealed that the normal group exhibited no damage to pancreatic β cells. However, the diabetic control group showed clear signs of pancreatic

Table 3: Percentage recovery in terms of heart rate and developed tension in the normal and diabetic groups against ischaemia-reperfusion-induced damage.

Normal group against Ischemia induced damage		
Treatment	**% Recovery of heart rate**	**% Recovery of Developed tension**
NC	47.50±3.46	45.88 ±2.52
GH	76.36±2.2$^{\#}$	76.32±1.03$^{\#}$
REP	54.7± 2.6$^{\$\&}$	57.05±2.33$^{\#\&}$
AML	69.55±1.2$^{\#\beta\emptyset}$	74.57±3.4$^{\#\emptyset}$
REP + GH	81± 2.6$^{\#\emptyset}$	72.55 ± 4.6$^{\#\emptyset}$
AML+ GH	89.36±1.7$^{\#\&\mu}$	90.88±2.30$^{\#\&\mu}$
REP + AML	74.78 ± 1.9$^{\#\%\emptyset\mu}$	87.07 ± 3.97$^{\#\beta\emptyset\mu}$
GH + REP + AML	93.82 ± 2.75$^{\#\&\emptyset\mu¥}$	92.01±2.1$^{\#\&\emptyset\mu}$
Diabetic group against Ischemia damage		
Treatment	**% Recovery of heart rate**	**% Recovery of developed tension**
NC	47.50 + 3.46	45.88 + 2.52
DC	24.47 + 1.1$^{\#}$	37.72 + 1.09 $^{\#}$
GH	65.47 + 1.64$^{€\#}$	52.50 + 1.30 $^{\$€}$
REP	62.37 + 2.56 $^{€\#}$	51.29 + 0.88 $^{@€}$
AML	75.39 + 1.7 $^{€\#\beta\emptyset}$	63.71 + 1.37 $^{€\#\&\emptyset}$
REP + GH	72.42 + 2.65 $^{€\#\infty}$	56.31 + 3.30 $^{€\#\Delta}$
AML + GH	84.25 + 1.23 $^{€\#\&\emptyset\neq}$	74.29 + 2.89 $^{€\#\%\emptyset\beta\mu}$
REP + AML	77.91 +1.87 $^{€\#\&\emptyset}$	70.53 + 2.34 $^{€\#\&\emptyset}$

All values are mean ± SEM, n=6; $^{@}$ P<0.05, $^{\$}$ P <0.01, $^{\#}$ P <0.001 compared to NC; $^{\%}$P<0.05, $^{\beta}$P <0.01,$^{\&}$P <0.001 compared to GH; $^{\Delta}$P<0.05, $^{\infty}$P <0.01, $^{\emptyset}$P <0.001 compared to REP; $^{\neq}$P<0.05,$^{£}$ P <0.01,$^{\mu}$ P <0.001 when compared to AML; ^{Þ}P<0.05,$^{¥}$ P <0.01,§ P <0.001 compared to RPL + AML

necrosis, along with a marked reduction in β and α cells. REP treatment increased the β cell population within the islets of Langerhans, and when combined with GH, this effect was further enhanced compared to REP alone. Notably, GH alone did not exhibit any significant protective effects. (Figure 2)

DISCUSSION

This study investigated the therapeutic potential of GH, REP, and AML in reducing ischaemia-reperfusion-induced myocardial damage in diabetic rats. ISC damage in normal rats caused a significant reduction in antioxidant enzyme activities, namely SOD and catalase, and a significant increase in TBARS, an indicator of oxidative stress. These results align with previous observations by Banerjee et al., who found a reduction in antioxidant activity and an increase in oxidative markers following ischaemia-reperfusion injury in rat hearts[20]. GH treatment dramatically enhanced antioxidant defense by increasing SOD and catalase activity and decreasing TBARS levels. These findings are similar to those of Bhatti et al., who established the cardioprotective properties of garlic through modulation of oxidative stress in ischaemia-reperfusion injury[21] .

The individual effects of REP and AML were mild, with partially improved oxidative stress parameters in the present study. The combination treatments, especially the GH+REP+AML group, displayed the strongest protection, with considerably increased SOD and catalase activities, and the lowest TBARS values. This synergy between garlic and REP and AML is consistent with the findings of Asdaq and Inamdar, who also reported a pharmacodynamic interaction between garlic and captopril, which also showed increased myocardial protection in ischaemia-reperfusion injury[22].

In the present study, oxidative stress caused by ischaemia-reperfusion was enhanced in diabetic rats, as indicated by decreased SOD and catalase levels and increased TBARS content compared with normal controls. GH treatment in diabetic rats indicated a significant enhancement in oxidative stress parameters, which aligns with the findings of Sener et al., who showed the antioxidant properties of garlic to prevent ischaemia-reperfusion injury[23]. In addition, REP and AML treatments enhanced these parameters, with the combination of GH+REP+AML having the most complete cardioprotection. The synergistic effects of this combination treatment suggest a multi-target strategy to reduce oxidative damage, as previously reported by Iwalokun et al., who showed that Allium sativum (garlic) extracts regulated oxidative stress and diminished tissue damage in animal models[24] .

With regard to myocardial injury biomarkers, CK-MB and LDH activities were greatly increased in the perfusate and heart tissue homogenates (HTH) of control and diabetic rats with ischaemia-reperfusion injury in the

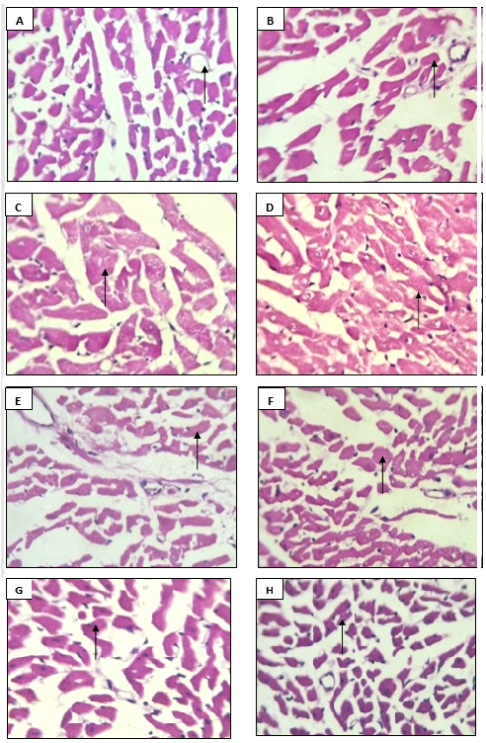

Fig. 1: Histopathological sections of heart tissue stained with hematoxylin and eosin showing myocardial changes in diabetic rats under different treatments. In the diabetic control group (A), heart sections showed scattered mononuclear inflammatory cells and edema.GH treatment (B) Improved the myocardial structure with fibrovascular septae. AML treatment (C) showed mild focal necrosis with infiltration, whereas REP treatment (D) showed areas of coagulative necrosis. The combination of GH with AML (E) and GH with REP (F) reduced necrosis and infiltration. REP with AML (G) shows a near-normal cardiac structure with some vascular congestion. The triple combination of REP, AML, and GH (H) preserved an almost normal cardiac architecture with minimal mononuclear infiltration

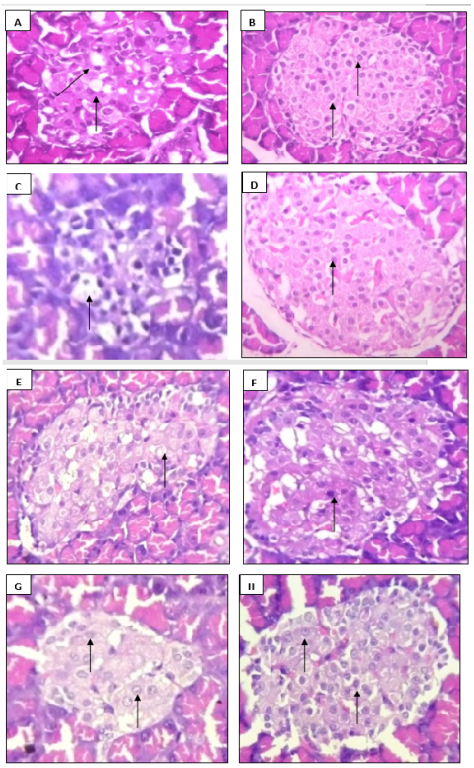

Fig. 2: Histopathological sections of the pancreas stained with hematoxylin and eosin showing myocardial changes in diabetic rats under different treatments. In the diabetic control group (A), diabetic control pancreas showed 60% β cells and 35% α cells, with some degenerated β cells. (B) The GH-treated group had 70% β cells and 25% α cells, with lobules separated by connective tissue. (C) AML-treated pancreas showed 50% β cells and 45% α cells, along with pancreatic necrosis. (D) REP-treated pancreas showed 75% β cells and 25% α cells. (E) GH- and AML-treated groups show less intact structures. (F) GH and REP-treated groups showed intact structures with 75% β cells and 20% α cells. (G) AML and REP-treated groups showed reduced β cells (65%) due to increased degeneration. (H) REP, AML, and GH combination showed intact structures with higher β and α cell populations in the islets

present study. GH treatment effectively decreased the levels of these markers, indicating a protective effect against myocardial injury. REP and AML treatments caused partial recovery, but the combination of GH+REP+AML resulted in the greatest reduction in CK-MB and LDH levels. These findings agree with those of Shackebaei et al., who similarly found decreased levels of myocardial injury markers when rats were treated with garlic in an ischaemia-reperfusion model[25].

In the present study, restoration of heart function, reflected in the developed tension and heart rate, was impaired in ischaemia-reperfusion rats. Recovery was markedly enhanced by GH, but not by REP or AML, to a modest extent. The GH+REP+AML combination showed optimal functional recovery, corroborating the findings of Patumraj and Jetapai, where the cardiovascular complications in diabetic rats were improved with the use of garlic extracts[26] . The diabetic control group of diabetic rats showed inferior recovery, which was significantly enhanced by GH alone and further augmented by combination therapies, especially GH+REP+AML.

In the present study, oral glucose tolerance tests showed that the combination treatments, particularly GH+REP+AML, maintained blood glucose levels. The glucose-lowering activity of the combination treatment was greater than that of the single treatments, supporting the synergistic potency of GH, REP, and AML, providing a combined metabolic advantage to diabetic rats. These findings complement those of Bhatti et al., who demonstrated improved glucose metabolism after garlic administration in diabetic animals[21].

In the present study, histopathological examination indicated extensive myocardial damage in diabetic and non-diabetic ischaemia-reperfusion injury-induced rats. GH therapy maintained myocardial architecture, and the GH+REP+AML combination provided maximum protection, with an almost normal myocardial structure. These findings agree with those of Banerjee et al., who showed that garlic treatment prevents ischaemia-induced myocardial damage and preserves myocardial integrity[20].

CONCLUSION

The current study demonstrated the cardioprotective effects of garlic combined with repaglinide and amlodipine against ischaemia-reperfusion injury in diabetic rats. These results suggest that combination treatment can be an effective way to counteract oxidative stress, enhance myocardial injury recovery, and offer better cardiovascular protection against diabetes. Further studies are needed to investigate the molecular basis of these synergistic actions and determine their potential therapeutic applications.

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