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The potential protective effect of metformin on selective cardiac biomarkers in diabetic male rats

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Abstract:

Metformin, a biguanide, first line antidiabetic agent, undergoes extensive debate to explain its possible cardioprotective effect. This study represents an attempt to help clarifying this cardioprotective effect. 24rats divided into three diabetic group (8 rats for each) (diabetes induced by Streptozocin 60 mg/kgi.p.). Diabetic groups treated with (saline, 75 mg/kg metformin and 150 mg/kg metformin, i.p.) for 6 weeks, then cardiac stress was induced by isoproterenol (ISO) (150 mg/kg i.p.) for two successive days. Selective biomarkers were assessed; brain natriuretic peptide (BNP), matrix metalloproteinase - 1 (MMP-1) and histopathology examination. The results of this study showed that metformin produce a dose dependent cardioprotection effect in diabetic rats.

الخلاصة:

الميتفورمين هو أحد أفراد مجموعة البايكوناید التي تسخدم كعوامل مضادة لداء السكري، والذي يخضع لدراسات مستفيضة و واسعة حول دوره في وقاية وحماية عضلة القلب من الإعتلال في المرضى المصابين بداء السكر وتمثل هذه الدراسة محاولة لتفسير هذه التأثيرات الواقية للقلب. تم استخدام 24 الفئران مقسمة إلى ثلاثة مجموعة السكري (8 جرذ لكل منهما) (تم إصابة الجرذان بالسكري عن طريق اعطائهم الستريبتوزوسين 60 ملغ / كغ عن طريق الحقن في التجويف البريتون). تم إعطاء المتفورمين عن طريق الحقن في التجويف البرتوني وبجرعات (75 ملغ / كغ ميتفورمين و 150 ملغ / كغ ميتفورمين عن طريق الحقن في التجويف البريتون) لمدة 6 أسابيع، ثم تم استحداث الاجهاد لعضلة القلب عن طريق إعطاء الايزوبروتيرينول وبرجعة (150 ملغ / كغعن طريق الحقن في التجويف البريتون) لمدة يومين متتاليين. تم تقييم المؤشرات الحيوية الانتقائية. الببتيد (هضميد) الناتريوتريك الدماغي، مصفوفة الميتالوبرتينيز (الفلزي) والتشريح النسيجي. وأظهرت نتائج هذه الدراسة أن الميتفورمين ينتج تاثيرا معنويا في كل المجاميع و يكون هذا التاثير معتمد على الجرع المعطاة.

Introduction:

Ischemic heart disease is still the main cause of death in patients with type 2 diabetes mellitus (T2DM) [1]. Metformin is antidiabetic drug, one of biguanide class, and prescribed widely as antihyperglycemic medication for treatment of type 2 diabetes mellitus. in addition to that, it has been reported that metformin reduce the mortality rate and produced a direct cardioprotection effect in addition to the glycaemic control effect[2][3][4]. Metformin has been reported to reduce the diabetes related death by 42% and all causes of death by 36%[5]. The mechanisms are not clearly understood, but reports accumulated concerning some of the potential mechanisms of action of metformin in the heart include the decrease in cardiomyocyte apoptosis during ischaemia, the elevation of myocardial preconditioning, the adaptation of cardiomyocyte metabolism during ischaemia or the protection against the development of heart failure[5]. This pleotropic effect of metformin was reported to be mediated via the activation of 5' AMP-activated protein kinas (AMPK) by metformin[6]. Metformin enhances the endothelium-dependent microvascular blood flow and improves symptoms of myocardial infarction (MI), including a 38% decrease in maximal ST-segment depression and a 30% decrease in the occurrence of chest pain[7].

Additionally,metformin treatment improves the lipoprotein profile in insulin resistance patient. Metformin suppresses plasma levels of free fatty acids and very low-density lipoprotein, decreases low density lipoprotein (LDL) cholesterol and enhances high density lipoprotein (HDL) cholesterol[8].When metformin is given on daily bases, it is effectively reducing the scar and left ventricular dilation, and protect myocardial function 12 weeks after constant coronary artery ligation, demonstrating that reperfusion is not necessary for the effects of metformin on myocardial remodeling. Metformin activates AMPK, eNOS, and peroxisome proliferator-activated receptor-Ɣ co-activation which is a regulator of cellular energy metabolism.Finally, metformin reduced the collagen expression that occurred after coronary artery ligation(9).The purpose of this study is to evaluate the possible cardioprotective effect of metformin in diabetic rats in different doses against ISO-induced cardiac stress.

Materials and Method:

1.1. Animals

Twenty-four male Wister rats weighted 120 – 180 gm were used in this study. The rats were housed in cages (8 rats per cage) and 10 days' acclimatization period (12hr/ light-dark place) with availability of food and water and kept at suitable conditions that meet the guidelines of the ethics committee, faculty of pharmacy, Al-Mustanserya University.

1.2. Induction of cardiac stress

Isoproterenol (ISO) solution prepared freshly with saline before injection. Rats received ISO (150 mg/kg) i.p.for two days to induce cardiac stress.

1.3. Induction of diabetes:

Streptozocin is administered (60 mg/kg) i.p. as a single dose, after 10 days, rats that showed a stagnant high FBS (>250 g/dl) considered as a diabetic rat.

1.4. Experimental design

Twenty-four diabetic rats randomly divided into three groups: control group treated with saline i.p. for 6 weeks, STZ –

1.5. Materials and chemicals

Streptozocin and isoproterenol are purchased from Sigma-Aldrich Gillingham, UK, metformin purchased from Pioneer pharmaceutical company, Al-Sulaymaniya, Iraq. ELISA kits for (BNP, MMP-1) purchased from YEHUA biological, shanghai, china.

1.6. Histopathology

Hearts were collected from the rats and fixed with 10% formalin for 24 hr and then embedded into paraffin, then sectioning into 5um by slide microtome and finally stained with haematoxylin and eosin (H&E). the sections were examined under light microscope and photomicrograph were obtained.

1.7. Statistical analysis

Data were displayed as mean ± SEM. Groups were compared by one-way ANOVA and subsequent Newman-Keulspost-hoc test. At p value < 0.05, differences considered as significant.

2. Results

2.1. Effect of metformin on serum BNP level

Metformin with doses (75 and 150 mg/kg) significantly reduced the elevated serum BNP level to (316.7 ± 13.95 ng/L and 278.1 ± 19 ng/L respectively) compared with the control non-treated rats(STZ – control group) that showed a serum BNP level (375.4 ± 13.07 ng/L), with a $p < 0.05$. (figure 1).

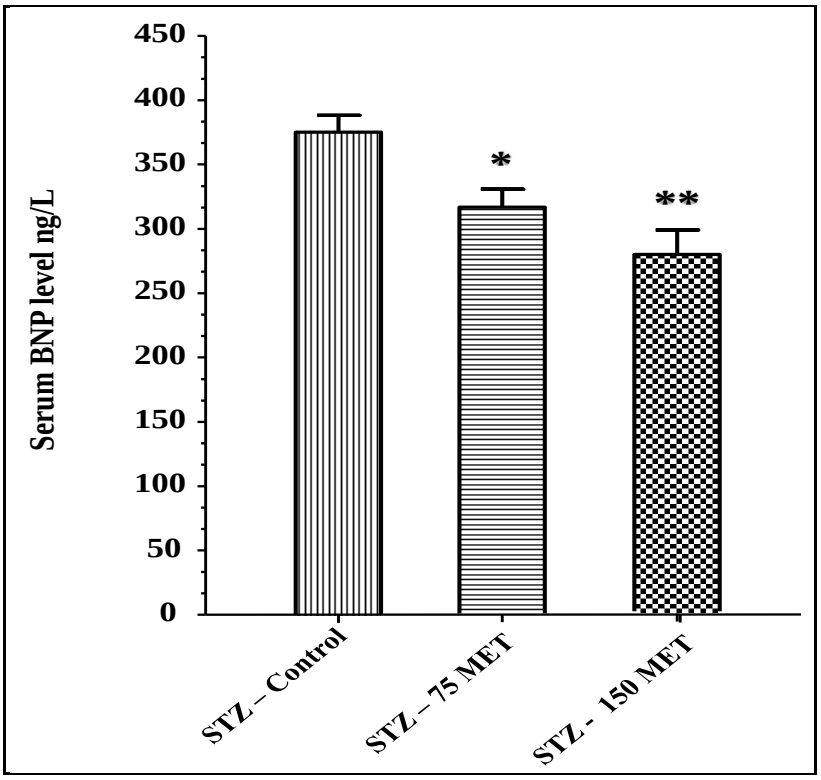

Figure-1:Serum brain natriuretic peptide in groups of diabetic rats; STZ - control grouptreated with saline, (STZ – 75 MET) treated with (75 mg/kg/24hr of metformin), (STZ – 150 MET) treated with (150 mg/kg/24hr of metformin). All four groups receiving isoproterenol (150 mg/kg) for two days. The results represent the mean ± SEM from 3 independent experiments. *; P<0.05, **; P<0.01, by using one-way-ANOVA, *Post hoc*: Newman-Keuls multiple comparison test.

2.2.Effect of metformin on serum MMP-1 level

Metformin with doses (75 and 150 mg/kg) significantly reduced the elevated serum MMP-1 level to (19.45 ± 0.919 ng/L and 16.44 ± 1.072 ng/L respectively) compared with the control non-treated rats (STZ – control group) that showed a serum MMP-1 level (23.28 ± 0.941 ng/L), with a p <0.05. figure 2.

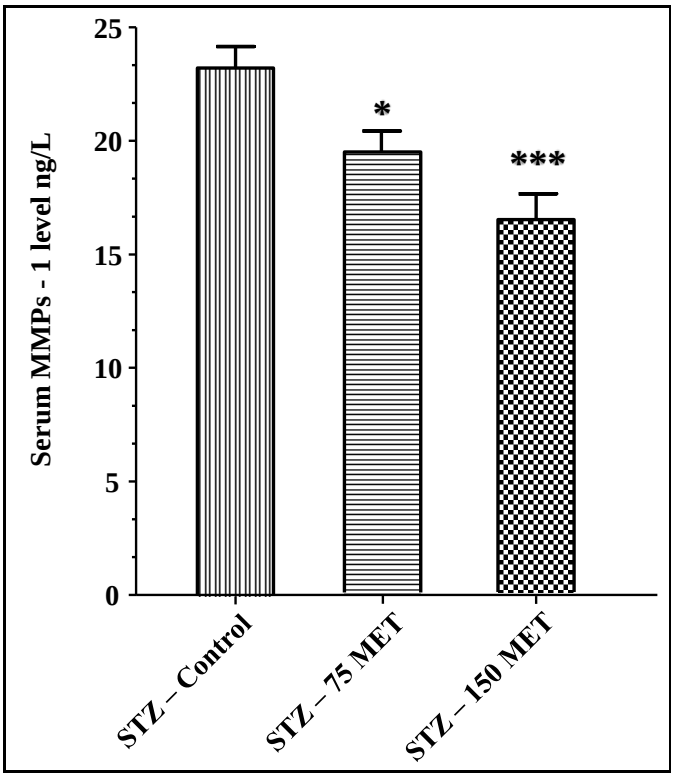

Figure-1:Serum MMP-1 in groups of diabetic rats; STZ - control grouptreated with saline, (STZ – 75 MET) treated with (75 mg/kg/24hr of metformin), (STZ – 150 MET) treated with (150 mg/kg/24hr of metformin). All four groups receiving isoproterenol (150 mg/kg) for two days. The results represent the mean ± SEM from 3 independent experiments. *; P<0.05, ***; P<0.001, by using one-way-ANOVA, Post hoc: Newman-Keuls multiple comparison test.

2.3. Effect of metformin on myocardial architecture

On histopathology examination, the control group showed extensive severe myocytes necrosis with deeply eosinophilic, loss of cross striations and absent nuclei, and granulation tissue which is loose oedematous tissue with fibroblasts. in (STZ – 75 MET group) showed a little decrease in myocyte tissue necrosis and granulation tissue. While in (STZ – 150 MET group) showed a moderate decrease in myocyte tissue necrosis and granulation tissue (figure 3).

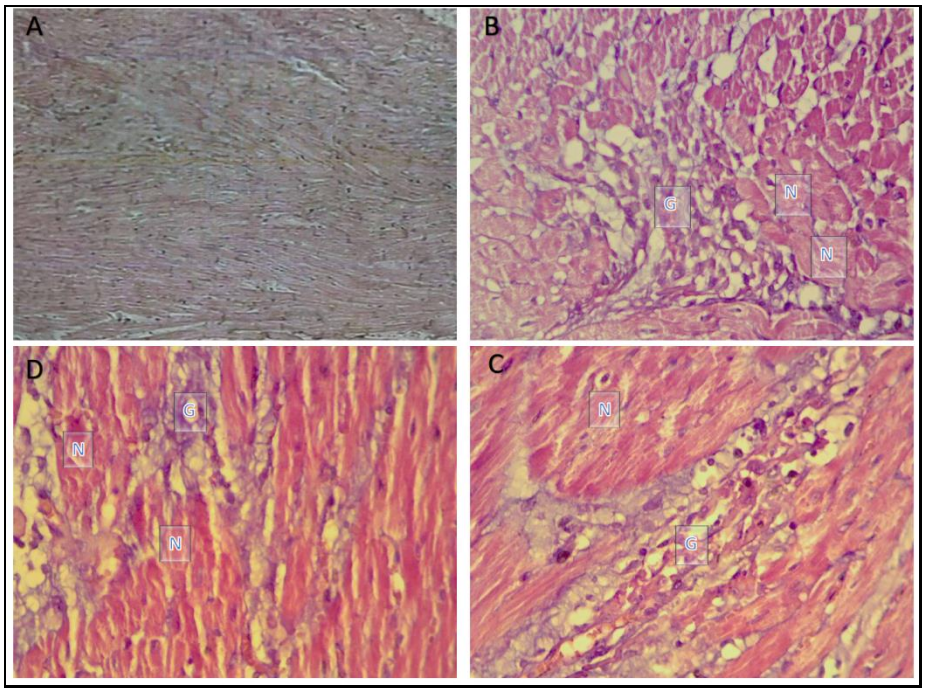

Figure-3:photomicrographs of cardiac tissue section. (A) normal heart tissue of rat, showing normal histology. (B) (STZ – Control group) showing extensive sever necrosis with massive granulation tissue. (C) (STZ – 75 MET group) showing a sever necrosis with granulation tissue. (D) (STZ – 150 MET group) showing a moderate myocytes necrosis with mild granulation tissue. (H&E, 400X).

3. Discussion:

Brain natriuretic peptide play an important role in the regulation of body fluids and blood pressure(10). BNP is an excellent indicator for acute and chronic heart failure, its level is increased when there is a stress or abnormality in the heart chamber or in volume overload. BNP is a neuro-hormone, released mainly from the left ventricle. BNP is an indicator for many cardiac diseases (HF, CHF, MI, LVH, Cardiac inflammation). Drugs that inhibit the level of BNP have a cardioprotective effect as its reflect the positive drug effect on the left ventricle, MI, HF. CHF, LVH, cardiac inflammation(11). It has been reported that an elevation in the BNP level occur in diabetic rats, as a result of volume expansion or fluid overload, while, glycaemic control lead to decrease in the BNP serum level(12). Isoproterenol caused an ER stress and elevation of BNP level through AMPK inhibition compared with normal non-treated rats(13). Metformin can produce a significant reduction in the BNP level, and this can explain one of its mechanisms of cardioprotection.

Metformin produces its' beneficial effect as a cardioprotective agent through activation AMPK and inhibit the ER stress and decrease the BNP level(13).Metformin with a dose of (100 mg/kg/24hr) for 4 weeks produced a significant reduction in the BNP level compared with non-treated rats. four months treatment with metformin reduced the BNP level in patientswith heart failure compared with non-treated patients(14). Also, its reported that treatment with metformin for 3 months lead to decrease the BNP level significantly when compared with non-treated rats(15).

Matrix metalloproteinase is a protease involved in the tissue remodelling in both pathological and physiological conditions. In case of CVD there is imbalance between the tissue inhibitor of metalloproteinase

(TIMP) and MMP, that lead to an excessive activation of MMP which in turn lead to derive abnormal cardiac extracellular matrix (cECM), leading to vascular and cardiac diseases and death(11). The first correlation between the MMP myocardial activity with cardiac function in human was reported in 2005. It has been showed that MMP play an important role in the myocardial dysfunction following I/R in rats. The early increase in MMP activity produces a proteolytic environment that may contribute to myocardial stunning injury in humans(16).Metformin exert a cardioprotective effect by decreasing the MMP level significantly when compared with the control non treated rats(17). In diabetic rats, the glycaemic control effect of metformin can protect the vascular structure and mechanics when compared with non-treated rats, so provide a protective effect against the vascular remodelling(18). Also, metformin treatment (150 mg/kg/24hr) significantly reduces the level of MMPs when compared with the control non-treated group(19).

Isoproterenol produced an intensive myocardial necrosis(20). It has been reported that there is a massive granulation tissue replaced the infarct tissue that induced by ISO(21)Acute treatment with metformin showed a marked attenuation in myocytes necrosis in a dose related suppression(20). Metformin has exerted a cardioprotective effect through attenuation of the myocyte necrosis against ISO-induced cardiac stress. Also, it has been documented that, metformin produced a dose dependant effect to produce a cardioprotection against ISO-induced cardiac stress(22). These results were shown that, metformin attenuate the necrosis in cardiomyocytes against ISO-induced cardiac stress in a dose related effect.

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