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The Potential Cardio-Protective Effects of Canagliflozin alone and in combination with metformin on Fructose induced Insulin resistance in Rats.

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ABSTRACT

Diabetic cardiovascular complications are a leading cause for increased morbidity and mortality. Diabetic cardiomyopathy is one of these complications which develop independently of coronary artery disease or hypertension. Previous studies stated the potential role of a state of inflammation via upregulation of NF-kB. The extra pancreatic, diuretic effect of canagliflozin, a SGLT2 inhibitor, appears promising in treatment of diabetic cardiomyopathy or reversing cardiovascular side effects of insulin sensitizers. This study was designed to investigate the potential cardio-protective effects of canagliflozin alone and in combination with metformin on fructose induced rat model of insulin resistance. Forty male albino rats were divided into 5 groups: normal control group, non-treated fructose induced diabetic group, canagliflozin treated group (28 mg/kg/day), metformin treated group (250 mg/ kg /day) and canagliflozin+ metformin treated group. All drugs were given p.o. for 4 weeks. Insulin resistance parameters, lipid profile, BP, LV wall thickness, Lead II ECG, histopathological study of the cardiac tissue and GSH and NF-kB concentrations in LV tissue homogenate were investigated. Canagliflozin+ metformin combination showed more significant improvement of electrophysiological and biochemical parameters which were confirmed with histopathological examination than other groups, suggesting a very promising role of this combination in treatment of insulin resistance.

Keywords: Diabetes, Canagliflozin, Metformin, Cardiomyopathy, NF-kB.

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INTODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders manifested by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Diabetes is complicated by long-term damage, dysfunction and failure of different organs, especially the kidneys, eyes, heart, blood vessels and nerves, **(1).** Egypt has been identified by IDF has as the ninth leading country in the world for the number of patients with type 2 diabetes (T2DM). This major rise could be due to either the prevalence of traditional risk factors for the disease such as obesity or other risk factors unique to Egypt including pesticides and chronic hepatitis C. Those risk factors have been recently shown to play role in insulin resistance but the precise mechanisms are not entirely clear **(2).**

The complications of DM are responsible for increased morbidity, disability and mortality **(3).** The chronic complications of diabetes involve micro vascular complications namely diabetic retinopathy, diabetic nephropathy and diabetic neuropathy, and macrovascular disease that are atherosclerosis and diabetic cardiomyopathy **(4).**

Diabetic cardiomyopathy is a specific complication which occurs independently of coronary artery disease or hypertension and may lead to increased morbidity and mortality. It is defined by presence of diastolic or systolic dysfunction in the presence of diabetes without known hypertension or coronary artery disease **(5, 6).** It includes left ventricular hypertrophy, myocardial fibrosis and myocardial energy dysregulation which associated with myocardial biochemical, mechanical or structural dysfunction **(7).** Diabetic cardiomyopathy is developed by a combination of several metabolic disorders including prediabetes, hyperglycemia, insulin resistance (in T2DM), hypertension and obesity. It results from increased glucose uptake and metabolism and increased production of advanced glycation end products (AGEs) **(8).** A state of inflammation is also mediated via upregulation of multiple signaling pathways, such as nuclear factor kappa light chain enhancer of activated B cells (NF-kB), c-Jun NH2-terminal kinase or p38-mitogen activated protein kinase (p38-MAPK) associated with insulin resistance **(9).** NF-kB is a primary regulator of inflammatory responses, is activated in the heart upon exposure to fatty acids or glucose **(10).** It induces the expression of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα), interleukin 6 (IL6), pro-IL1β, and pro-IL18 **(11).**

Canagliflozin (Invokana®, Janssen Pharmaceuticals) has been approved by the Food and Drug Administration (FDA) for the treatment of T2DM since 2013 **(12).** The drug is a competitive inhibitor of type 2 sodium glucose transporter (SGLT2) and so reduces both renal threshold for glucose and glucose reabsorption, with subsequent increases in urinary glucose excretion with low risk of hypoglycemia **(13)**. So it provides calorie and thus bodyweight loss and has an osmotic diuretic effect that may reduce blood pressure. The transient increases in urine output with canagliflozin may also reflect increased natriuresis **(14).**

Metformin is an oral biguanide norm glycemic drug; it is widely prescribed to treat type 2 diabetic patients. It suppresses intestinal glucose absorption and reduces its liver production. Also it suppresses insulin resistance and increases insulin sensitivity **(15).**

The main aim of this work is to investigate the efficacy of canagliflozin, with a unique mechanism of action on FBG and lipid profile in fructose induced rat model of insulin resistance which, in many aspects, resembles T2DM. In addition, the tested drug was compared and combined with metformin the main oral antidiabetic agent. This may help to find the exact role of this new drug on the recommended ideal protocol of treatment T2DM.

The extra pancreatic, diuretic effect of canagliflozin appears promising in treatment of diabetic cardiomyopathy or reversing cardiovascular side effects of insulin sensitizers. Therefore, combination of canagliflozin with metformin were tested to determine the validity of such combination on glycemic control, development of diabetic cardiomyopathy as well as reversing weight gain and cardiac hypertrophy induced by insulin sensitizers. This may help to point out suggestions as regards to the use of combined oral hypoglycemic agents in treatment of T2DM if a single one failed.

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MATERIALS AND METHODS

Animals

Forty adult male albino rats [from the experimental animal breeding farm Helwan, Cairo], each weighting 120- 150 g were used. They have acclimatized for one week and were caged (4 /cage) in a fully ventilated room. They were allowed to free access to water and diet containing cereals and bread. The study was approved by the ethical committee of Benha faculty of medicine, Benha University who adopts the guidelines for ethical conduct in the care and use of laboratory animals provided by National Research Center, Cairo, Egypt.

Drugs and chemicals

Canagliflozin hemihydrate (Janssen, USA), Metformin (Novartis, USA), Urethane (Ethyl carbamate): (prolabo, Paris), D-Fructose (El-Nasr pharmaceuticals Chemicals Co. ADWIC, Egypt), Cholesterol, HDL and Triglycerides liquicolor kits (Human, Egypt)**,** Glucose test stripes (Roche Diagnostic, Germany), Glucose and HbA1c commercial diagnostic kits (biodiagnostic, Egypt). Reduced glutathione kits (biodiagnostic, Egypt), NF-kB ELIZA kits (biodiagnostic, Egypt), Rat Insulin ELIZA kits (SunLong Biotech Co., LTD).

Experimental design

At the start of the experiment animals were divided into 5 groups (8 rats each). All animals except group 1 received high fructose diet (60%) for 8 weeks as a method for induction of type 2 DM [(15).] For such animals, fasting blood glucose level was measured by glucometer (ACCU-CHEK Active apparatus, Germany) using a blood drop from rat tail puncture. Animals with fasting blood glucose more than 250 mg/dl were considered diabetic, enrolled in the experiment and drugs were administered. Other animals were excluded and replaced by others in which type 2 DM was induced by fructose in other settings.

Animal groups included: Group 1: Normal control rats. They were allowed standard normal diet and water and received saline by gastric gavage in comparable volume to administered drugs. **Group 2: Non treated fructose induced diabetic rats.** Diabetes was induced by fructose as previously mentioned. No drugs were administered. **Group 3: Canagliflozin treated diabetic rats (CAN).** Canagliflozin was administered by gastric gavage in a dose 28 mg/ kg /day for 4 weeks **(16). Group 4: Metformin treated diabetic rats (MIT).** Metformin was administered by gastric gavage in a dose 250 mg/ kg /day for 4 weeks **(17). Group 5: Canagliflozin and Metformin treated diabetic rats (CAN+MIT).** Canagliflozin and metformin were administered as groups 3 and 4 respectively.

Total body weight was serially measured every 2 days throughout the experiment. At the end of the experiment, all animals were anesthetized by intraperitoneal (I.P.) injection of urethane a dose of 0.6 ml of 0.25% solution/100 gram body weight **(18),** then Lead II ECG and measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were performed. 4 ml blood sample will be obtained from the heart puncture. All blood samples were incubated at 37oC for 2 hours then serum was collected and kept at - 20oC till used for determination of biochemical parameters. 2 ml whole blood was kept for HbA1c % measurement. All animals were dissected for the heart. 0.2 g of myocardial tissue was extracted from upper part of left ventricular wall, homogenized using Tress buffer and used for determination of tissue concentration of NF-kB and reduced glutathione (GSH). The rest of the heart was kept in 10% formaldehyde to be used for assessment of the left ventricular wall thickness at the apex and cardiac histopathology. After the end of the overall experiment, rats were eliminated by incineration.

Measurement of SBP and DBP and Recording for Lead II ECG

The blood pressure was determined following the method of **Cangiano et al. (1978).** After being anesthetized, rats were dissected for the carotid artery. The arterial cannula filled with glucose and heparin solution was inserted towards the heart through the carotid artery**.** The cannula was connected to the previously calibrated blood pressure transducer (Power lab of research, AD instruments, Australia) and the pressure was recorded as tracing by Labchart 8 **(18)**. ECG limb leads were attached to the rat for lead II ECG recording at the same power lab setting.

Biochemical Assays

Blood samples were incubated at 37^{0}C until blood clotted and then centrifuged at 3000 revolution per minute (rpm) for 15 min for separation of serum and stored at – 20^{o}C for biochemical analysis.

a- Determination of FBG after enzymatic oxidation in the presence of glucose oxidase (GOD). **(19).** Serum insulin level was assayed by rat-specific enzyme immunoassay **(19).** Insulin resistance was measured by the homeostasis model assessment of insulin resistance (HOMA-IR index) **(19)**. Blood glycated hemoglobin (HbA1c) was estimated according to the chemical separation and colorimetric method **(20).**

b- Serum total cholesterol was measured by enzymatic colorimetric (CHOD-PAP) method **(21).** Serum triglycerides (TGs) were carried out by enzymatic colorimetric test according to (GPO-PAP) method **(22).** Serum HDL and LDL cholesterol was carried out by a method that depends on separation of HDL and LDL and détermination of cholesterol bound to these fractions **(23).** GSH in the left ventricular tissue was measured by optimized enzymatic recycling method using glutathione reductase and Ellman's reagent (DTNB) **(24).** NF-kB was assayed by its corresponding ELISA kits.

Histopathology of the heart

Hearts were removed and put into a buffered 10% formalin fixation solution and processed with paraffin wax for histopathological examination. Sections (5 µm) were stained with H & E **(25).**

Statistical analysis:

The results were experienced as mean ± standard error of the mean (S.E.). The overall significance was measured by One Way Analysis of Variance (ANOVA). The significance between individual groups was detected by t test. P value less than 0.05% was considered significant **(26).**

RESULTS

Experimental induction of type 2 diabetes resulted in significant increase in all tested parameters related to insulin resistance namely body weight, FBG, serum insulin, HOMA-IR index and HbA1c%, total cholesterol, LDL cholesterol and TGs compared with normal control group **(table 1,2).** On the contrary serum HDL cholesterol was reduced**.** Hemodynamic parameters showed significant elevation in SBP, DBP, MABP, ECG- R wave height and average wall thickness of the left ventricle, when all compared to control group **(table 3, fig. 2)**. Cardiac tissue homogenates of such group showed significant reduction of GSH and elevation of NF-kB **(Table 4).** Histopathological study showed hypertrophy and distortion in the myocardial cells, which were irregularly arranged, with increased intercellular gap and extracellular matrix **(fig. 1).**

Treatment with oral canagliflozin for 4 weeks after starting diabetes induction reduced significantly body weight, insulin resistance related parameters and elevated HDL cholesterol, compared with non-treated rats but their values were significantly different from respective normal rats **(Table 1, 2)**. SBP, DBP, MABP, R wave height and left ventricular wall thickness were significantly reduced compared to non-treated group but still above normal which was statistically significant **(Table 3 & fig. 2).** The above mentioned changes were associated with significant elevation of GSH level in cardiac tissue homogenates compared with non-treated group. Such value was still significantly below normal. NF-kB was still significant above normal group (**Table 4).**

Metformin treatment reduced body weight, FBG, serum insulin, HOMA-IR, HbA1c% and lipid profile value but their values were above normal with statistical significance **(Table 1, 2).** SBP, DBP, MABP, R wave height and left ventricular wall thickness were significantly reduced compared with non-treated group **(Table 3 & fig. 2).** In addition, the tested drug increased significantly GSH and NF-kB was significantly decreased compared with non-treated group **(Table 4)**

Concomitant administration of canagliflozin and metformin resulted in significant reduction of all tested parameters related to insulin resistance compared with non-treated diabetic group. FBG, serum insulin and HOMA-IR index were significantly reduced when compared with metformin treated group and canagliflozin treated group. However, the above mentioned parameters were significantly above normal

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values **(Table 1).** This combination normalized body weight**,** HbA1c %, serum total Cholesterol**,** serum HDL cholesterol and significantly reduced serum TGs compared with non-treated diabetic rats **(Table 2).** The combination was far more potent than metformin or canagliflozin alone in this concern**.** Moreover, the combination normalized SBP, DBP, MABP and significantly reduced R wave height and left ventricular wall thickness compared to non-treated diabetic **(Table 3 & fig. 2).** In addition, this drug combination exerted significant increase of GSH in cardiac tissue homogenate compared with non-treated, metformin treated and canagliflozin treated groups. Nevertheless, it was less than normal. Concentration of NF-kB was markedly decreased compared with non-treated diabetic group and canagliflozin treated group but it was still above normal values **(table 4).** Histopathological study showed less hypertrophy with the intercellular gaps and the extracellular matrix dramatically reduced, if compared to non-treated diabetic group **(Fig. 1).**

Table (1): BW, FBG, Insulin, HOMA-IR and HbA1c in different studied groups (Mean ± SEM):

Groups	BW g	FBG mg/dl	Insulin µIU/ml	HOMA-IR	HbA1c
Control	260 ± 6.42	89.63 ± 2.32	7.82 ± 0.43	1.60 ± 0.19	5.54 ± 0.14
Diabetic	350 ± 11.43	210 ± 6.71	28.9± 1.67	14.54 ± 1.31	8.53 ± 0.33
% change vs Control	+32.6 a	+134 a	+270 a	+798 a	+54 a
CAN	310 ± 11.41	170.65 ± 5.32	20.43 ± 1.44	9.50 ± 0.85	7.45 ± 0.25
% change vs Control	+19.2 a	+73 a	+161 a	+493 a	+25.6 a
% change vs Diabetic	-11.4 b	-18.7 b	-29.3 b	-34 b	-12.7 b
MIT	295 ± 10.65	160.52 ± 5.65	16.42 ± 1.34	6.1 ± 0.65	7.15 ± 0.21
% change vs Control	+13.5 a	+79.1 a	+110 a	+653 a	+29 a
% change vs Diabetic	-15.7 b	- 23.6 b	-43.2 b	-16 b	-16.2 b
CAN+MIT	262 ± 9.32	140.53 ± 3.86	12.1 ± 1.32	4.24 ± 0.43	5.62 ± 0.16
% change vs Control	+ 0.8	+46.7 a	+54.7 a	+165 a	+1.4
% change vs Diabetic	- 25.1 b	-33 b	- 58.1 b	-70.9 b	-34.1 b
% change vs CAN	- 15.5 c	-17.6 c	- 40.8 c	-55.4 c	-24.6 c
% change vs MIT	- 11.2 d	-12.5 d	- 26.3 d	-30.5 d	-21.4 d

a: Significant versus control at p<0.05; **b:** Significant versus non treated diabetic group at p<0.05;
c: Significant versus CAN at p<0.05; **d:** Significant versus MIT at p<0.05.

Table (2): Total Cholesterol, LDL, HDL, TGs in different studied groups (Mean ± SEM):

Groups	Total Cholesterol mg/dl	LDL mg/dl	HDL mg/dl	TGs mg/dl
Control	**99.5 ± 3.74**	**49 ± 2.34**	**32.3 ± 1.76**	**119.5 ± 4.21**
Diabetic	**210.3 ± 6.23**	**146.6± 4.54**	**21.6 ± 1.43**	**250.6 ± 7.11**
% change vs Control	**+111 a**	**+199 a**	**-33.1 a**	**+109.7 a**
CAN	**175.6 ± 5.46**	**125.4 ± 4.21**	**26.6 ± 1.45**	**210.7 ± 5.97**
% change vs Control	**+76.4 a**	**+156 a**	**-17.6 a**	**+76.3 a**
% change vs Diabetic	**- 16.5 b**	**- 14.5 b**	**+23.1 b**	**-15.6 b**
MIT	**150.5 ± 4.78**	**108.5 ± 4.11**	**28.1 ± 1.67**	**175.6 ± 5.23**
% change vs Control	**+51.3 a**	**+121.4 a**	**-13 a**	**+47 a**
% change vs Diabetic	**- 28.4 b**	**-26 b**	**+30 b**	**-30 b**
CAN+MIT	**109 ± 4.12**	**76.2 ± 3.02**	**33.5 ± 1.98**	**134.2 ± 4.72**
% change vs Control	**+ 9.5**	**+55.5 a**	**+3.7**	**+12.3 a**
% change vs Diabetic	**- 48.2 b**	**- 48 b**	**+55.1 b**	**-46.4 b**
% change vs CAN	**- 37.9 c**	**- 39.2 c**	**+30 c**	**-36.3 c**
% change vs MIT	**-27.6 d**	**- 29.8 d**	**+19.2 d**	**-23.6 d**

a: Significant versus control at p<0.05; **b:** Significant versus non treated diabetic group at p<0.05;
c: Significant versus CAN at p<0.05; **d:** Significant versus MIT at p<0.05.

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Table (3): SBP, DBP, MABP, R wave height, LV wall thickness in different studied groups (Mean ± SEM)

Groups	SBP mm/Hg	DBP mm/Hg	MABP mm/Hg	R wave height mV	LV wall thickness µm
Control	115± 4.22	73.2 ± 2.69	87 ± 3.32	0.16 ± 0.02	310± 6.47
Diabetic	154.34 ± 2.67	95.5 ± 3.46	115.1 ± 4.46	0.24 ± 0.06	540± 8.75
% change vs Control	+39.4 a	+30.5 a	+34.5 a	+50 a	+74.2 a
CAN	134.71 ± 4.54	85.8 ± 3.23	102.1 ± 3.47	0.18 ± 0.03	394 ± 7.56
% change vs Control	+17.1 a	+17.2 a	+17.4 a	+12.5 a	+27 a
% change vs Diabetic	-12.7 b	-10.2 b	-11.3 b	- 25 b	-27 b
MIT	137.21 ± 6.52	85.7 ± 3.29	102.9 ± 4.21	0.16 ± 0.02	417± 6.35
% change vs Control	+19.3 a	+17 a	+18.2 a	0	+34.5 a
% change vs Diabetic	-11.1 b	-10.3 b	- 10.6 b	-33.3 b	-22.8 b
CAN+MIT	125.51 ± 7.49	76.2 ± 2.56	92.64 ± 3.36	0.13 ± 0.05	360 ± 7.36
% change vs Control	+9.1	+ 4	+ 6.5	-18.6 a	+16.1 a
% change vs Diabetic	-18.7 b	- 20.2 b	- 19.5 b	-45.8 b	-33.4 b
% change vs CAN	-6.8	- 11.2 c	- 9.2	-27.8 c	-8.6
% change vs MIT	-8.5	- 11.1 d	- 9.6	-18.6 d	-13.7 d

a: Significant versus control at p<0.05; **b:** Significant versus non treated diabetic group at p<0.05;
c: Significant versus CAN at p<0.05; **d:** Significant versus MIT at p<0.05.

Table (4): Cardiac GSH and NF-kB in different studied groups (Mean ± SEM):

Groups	GSH µ mol/g	NF-kB µ mol/g
Control	**234.14 ± 6.85**	**0.210 ± 0.003**
Diabetic	**87.45 ± 3.12**	**1.081 ± 0.018**
% change vs Control	**-62.7 a**	**+415 a**
CAN	**137.48 ± 4.12**	**0.987 ± 0.015**
% change vs Control	**-41.3 a**	**+370 a**
% change vs Diabetic	**+57.2 b**	**-8.7**
MIT	**154.53 ± 4.63**	**0.644 ± 0.055**
% change vs Control	**-34 a**	**+207 a**
% change vs Diabetic	**+76.7 b**	**- 40.4 b**
CAN+MIT	**188.62 ± 5.65**	**0.560 ± 0.016**
% change vs Control	**-19.4 a**	**+166.7 a**
% change vs Diabetic	**+115.7 b**	**-48.2 b**
% change vs CAN	**+37.1 c**	**-43.2 c**
% change vs MIT	**+22 d**	**-13 d**

a: Significant versus control at p<0.05; **b:** Significant versus non treated diabetic group at p<0.05;
c: Significant versus CAN at p<0.05; **d:** Significant versus MIT at p<0.05.

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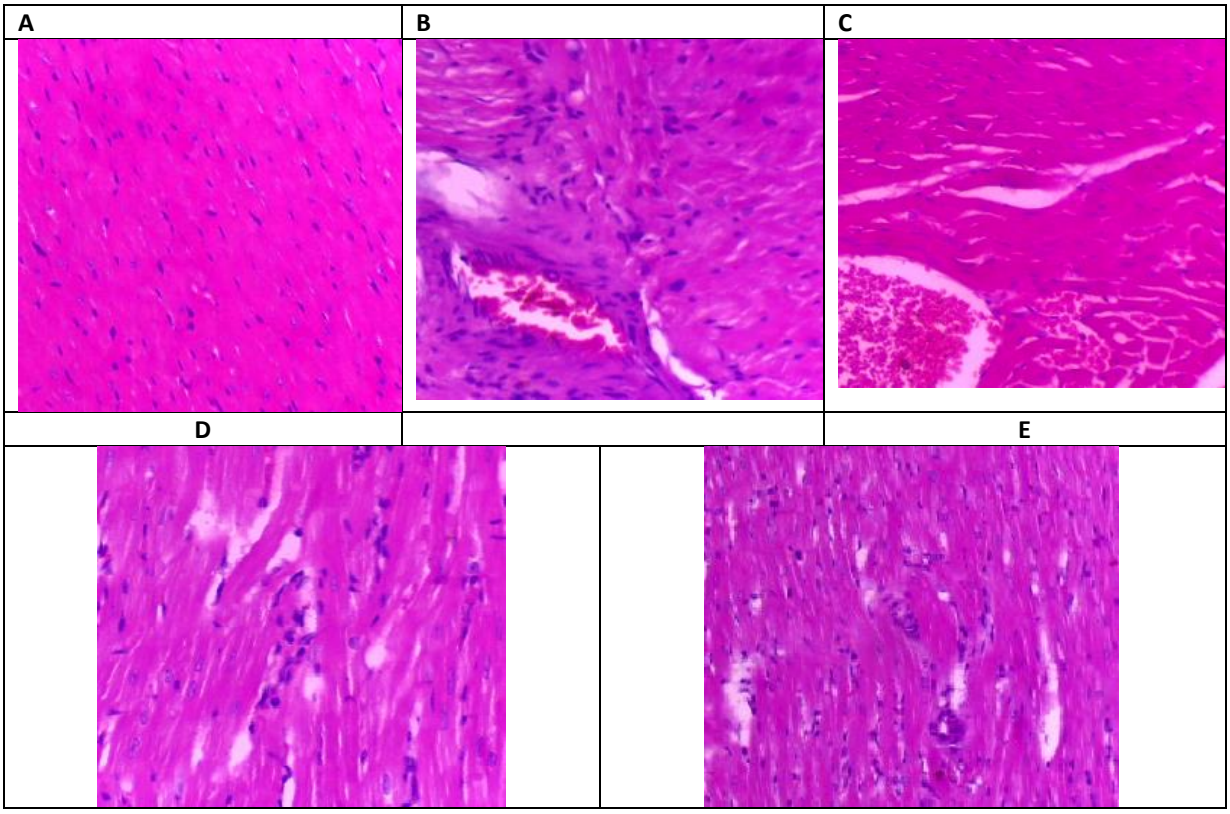
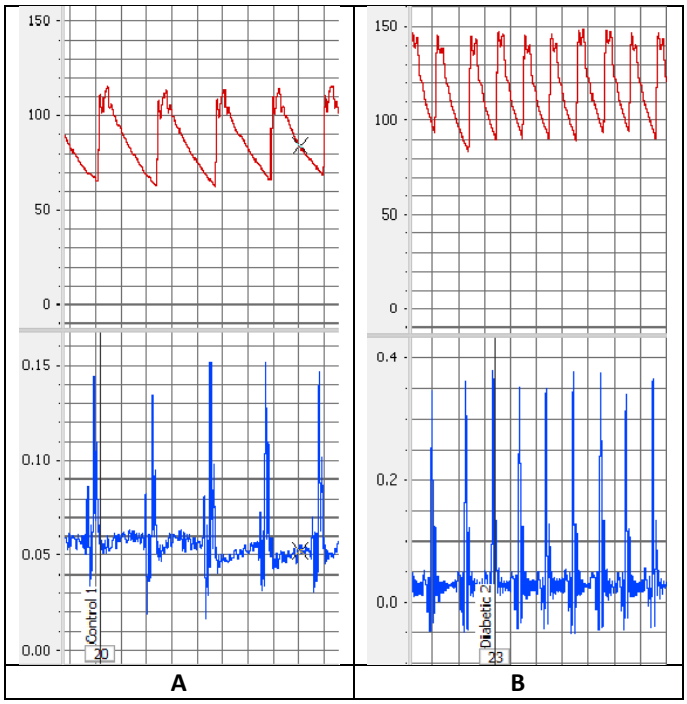


Fig. (1): A: A Photomicrograph of a cut section in the left ventricle of control group showing normal myocardial cells were neatly and tightly arranged, with clear structure and less extracellular matrix. B: None treated diabetic group showing hypertrophy and distortion in the myocardial cells, which were irregularly arranged, with increased intercellular gap and extracellular matrix. C and D: CAN and MIT treated diabetic groups respectively, show less hypertrophied myocardial cells. The intercellular gap and extracellular matrix were slightly reduced. E: CAN+ MIT treated diabetic group shows regularly arranged myocardial cells. The intercellular gap and extracellular matrix were dramatically reduced (H & E x 200).



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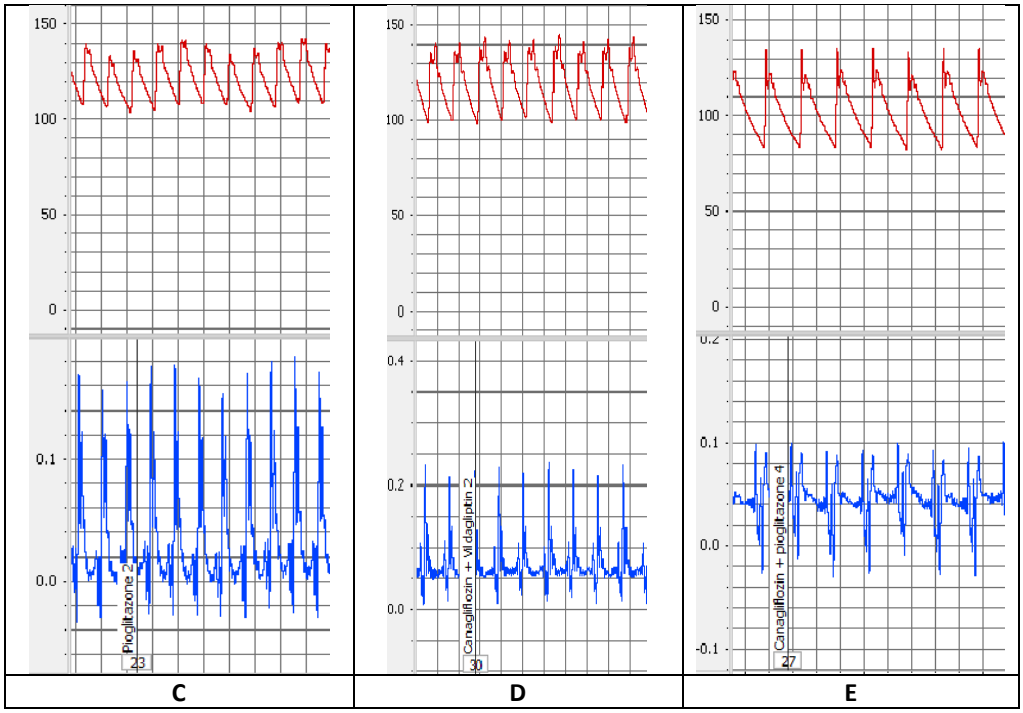

Fig. (2): Labchart ECG and blood pressure records for control group (A), non treated diabetic group (B), CAN treated diabetic group (C), MIT treated diabetic group (D), CAN+MIT treated diabetic group (E).

DISCUSSION

In the present work, oral fructose produced insulin resistance evidenced by marked increase in FBG and serum insulin. This is in agreement with **(27)** who showed that such model produces insulin resistance rather than insulin deficiency.

The main mechanism by which excessive fructose consumption produced insulin resistance may be due to ATP consumption as a result of metabolism of fructose to fructose -1- phosphate by fructose kinase and ketohexokinase enzymes in uncontrollable non negative feedback regulated reaction. This may result in excessive hydrolysis of ATP to ADP. The latter activates adenosine deaminase which initiates cascade of reactions ending by formation of uric acid and free radicals. The latter two substances are responsible for insulin resistance as well as most of the manifestations of metabolic syndrome **(28).** This is in agreement with the clinical study of **Le et al., (2006**) who showed that triglycerides, serum leptin, and fasting blood glucose levels are higher in humans provided with a high fructose diet either as crystalline pure fructose **(29).** Or high fructose corn syrup **(30**) for four weeks compared with those on a starch-based diet.

Increased hepatic and visceral fats promotes the formation of inflammatory cytokines namely TNF-α which may induce insulin resistance probably through inhibition of insulin receptor auto phosphorylation and activation of insulin receptor substrate -1 **(31)** as well as decreasing insulin receptor number and sensitivity **(32).** Moreover, persistent hyperglycemia may generate free radicals through non enzymatic glycosylation of cellular lipid and protein contents which may promote formation of inflammatory cytokines **(33).**

Moreover, this work showed association between fructose induced insulin resistance and free radical formation as evidenced by significant reduction of the endogenous antioxidant (GSH) in non-treated fructose fed animal group. This clearly indicated excessive production of free radicals. The source of them may be the above explained release of inflammatory mediators or insulin receptor dysfunction which shifted signaling pathway at PI3-kinase step from normal activation of phosphoinsitol diphosphate to Rac, which amplifies the activity of NADH2 oxidase enzyme. The latter is one of the most potent mitochondrial oxidizing enzymes **(34).**

This work revealed that high fructose dose caused marked disturbance in fat metabolism manifested as increase in body weight and dyslipidemia in the form of increase serum triglycerides, total and LDL cholesterol and lowering in HDL cholesterol. This deleterious effect may be related to above mentioned adipocyte dysfunction and free radical formation that participates in hypertriglyceridemia. In addition, increase hepatic triglycerides may promote production of triglyceride rich VLDL which exchanges its triglyceride contents for cholesteryl esters from small LDL and HDL via CETP. Triglyceride-rich HDL or LDL can undergo hydrolysis by hepatic lipase or lipoprotein lipase which is upregulated in cases of insulin resistance **(35)** which allows HDL related apoprotein A-1 to be filtered by renal glomeruli and degraded by renal tubular cells.

High fructose diet produced harmful effect on both blood vessels and heart. Its harmful vascular effect was represented by increase in SBP, DBP and MABP. This is in agreement with clinical study of **Johnson et al., (2007)** who showed that fructose increases the incidence of hypertension and diabetes. Endothelial dysfunction as well as chronic sympathetic over activity are probably the main mechanisms contributing to high fructose induced hypertension **(36).** Moreover, animal studies of **Huang et al. (2004) and Reungjui et al. (2007)** supported the view of sustained fructose enriched diet as cause hypertensive insulin resistant rats **(37,38).**

In addition, the present work showed that high fructose diet produced structural and functional features similar to diabetic cardiomyopathy namely cardiac hypertrophy as evidenced by increase left ventricular wall thickness, contractile disorders in the form of significant increase in height of R wave in lead II of ECG as well as structural changes in the form of distortion and irregular arrangement of myocardial cells, increase intercellular gaps and increase interstitial matrix.

Regarding increased the height of R wave in lead II of ECG and left ventricular increase in wall thickness. This is in agreement with **Van Hoose et al. (2010)** who recorded similar ECG changes in zucker diabetic fatty rats and concluded that the increase in R wave amplitude indicates a change in left ventricular mass **(39). Okin et al., (2001)** attributed it to the cardiac strain which delayed repolarization of hypertrophied left ventricle. Moreover, it may be a manifestation of diabetic cardiomyopathy which caused sarcoplasmic reticulum dysfunction which impaired calcium handling **(40).**

The most prominent microscopic structural changes in cardiac tissue in non-treated high fructose fed animals were the accumulation of extracellular and interstitial matrix. This may be explained by multiple mechanisms among which is increased synthesis as a result of either non enzymatic glycation of membrane amino acid which resulted in formation of AGEs which accumulated in extracellular and interstitial spaces as well as increase synthesis of collagen IV as a result of increased expression of the enzyme responsible for its synthesis mainly lysosyl oxidase enzyme **(41).**

This work revealed that canagliflozin treatment partially reduced but did not completely relieved insulin resistance parameters. This may be explained by reduction of FBG through promoting its renal excretion as a result of inhibition of its tubular reabsorption through inhibition of SGLT-2 in the renal tubules. It may have minimal effect on intestinal glucose absorption because such transporter is only expressed in high density in renal tubules and in low density in intestinal mucosa **(42).** Amelioration of insulin resistance may be attributed to its beneficial effects on both glycemic control and lipid metabolism. Blood glucose reducing effect may attenuate positive feedback of glucose on insulin secretion as well as non-enzymatic glycosylation of HbA1c and hence HOMA-IR ratio. Moreover, it may reduce formation of intracellular AGEs, an important source of free radical and inflammatory mediators that block autophsophorylation of insulin receptor **(34).**

The beneficial effect of canagliflozin on lipid metabolism was demonstrated by **Nishitani et al (2018)** who showed that other SGLT2 inhibitors like dapagliflozin and empagliflozin promote adiponectin gene expression through increasing the formation of 3 hydroxy butyric acid which modify histone acetylation at H3K9bHB Peptide locus **(43).** The latter reduces adipocyte differentiation, increase energy expenditure through promotion of mitochondrial uncoupling of oxidation phosphorylation **(44)** as well as anti-inflammatory and antiathrogenic properties **(44).** It acts synergistically with leptin to decrease appetite **(45).** Such effects collectively reduced intracellar triglyceride accumulation and formation of abnormal large adipocytes which contributed in insulin resistance as previously explained **(46).**

In accordance, canagliflozin treatment has beneficial effect on cardiovascular system reflected as, reduction in R wave height, left ventricular wall thickness, SBP, DBP and MABP compared with non-treated groups. Such beneficial effect was attributed to diuretic effect of the drug or possibly reduction of vascular resistance mediated by hypolipidemic action. Moreover, this work showed that the tested drug produced anti-free radical effect manifested as elevation of GSH which counteracted the harmful free radicals produced by AGEs. **Verma and McMurray (2018)** proposed that canagliflozin had a uricosuric effect. This clearly explain both antioxidant and cardio protective effect of the drug in fructose model of insulin resistance in which uric acid play an important role in vascular damage, hypertensive, oxidative stress as previously described **(47). Uthman et al. (2018)** proposed a (sodium/ hydrogen exchanger) SHE inhibition as a plausible explanation of canaglfilozin induced cardio protection **(48).** Inhibition of cardiomyocyte variety of such exchanger may reduce cytosolic sodium and calcium. On the other hand, down regulation of renal variety of such exchanger (NHE3) in the proximal convoluted tubules may partially explain the diuretic effect of tested drug. Over expression of cardiomyocyte variety was implicated in myocardial hypertrophy **(49).**

The data of the present work revealed that, canagliflozin produce slight structural improvement manifested by less hypertrophied myocardial cells with slight reduction of intercellular gap and extracellular matrix. These results were in agreement with **Lee et al. (2017)** who showed that dapagliflozin, another member of SGLT-2 inhibitor, produces marked cardiopretective effects by suppressing collagen synthesis through increasing the activation of macrophages and inhibiting differentiation of myofibroblast **(50).**

Metformin exerted marked reduction in insulin resistance. In addition to suppress hepatic glucose production through AMPK activation **(51 &52)** metformin increase insulin sensitivity, enhance peripheral glucose uptake, decrease insulin- induced suppression of fatty acid oxidation and decrease absorption of glucose from gastrointestinal tract **(53).** Also, metformin reduces cholesterol synthesis capabilities by phosphorylation of its rate limiting enzyme namely HMG CoA reductase **(54)** as well as reduction in the expression and activity of sterol regulatory protein element binding protein-1 (SREBP-1) by SREBP cleavage activating proteins **(55).**

Moreover, Metformin produced significant cardiopretective action manifested as decrease SBP, DBP, MABP, R wave height and left ventricular wall thickness. This may be attributed to improvement of hyperlipidemic induced vascular dysfunction and as a result of decrease insulin resistance which may exert a vasodilator effect through increase expression of nitric oxide (NO) and endothelium–derived hyperpolarization factor (EDHF) **(56).** In addition metformin inhibited Angiotensin II- induced endoplasmic reticulum stress via an AMPK∞2-PLB-SERCA pathway which may provide a novel therapeutic target for hypertension treatment **(57, 58).**

Metformin treatment resulted in significant antioxidant and anti-inflammatory activities manifested as increase of GSH and significant reduction of NF-kB levels. This may be attributed to increase glutathione activity, modulation of ferritin heavy chain which responsible for sequestration of any excess intracellular iron **(59).**

Canagliflozin / Metformin combination was proved to be having more powerful inhibitor than either of its single components as regards amelioration of insulin resistance, lipid profile, more powerful reduction of R wave height as well as suppression of left ventricular wall thickness. This may imply that this combination may be less likely to produce heart failure. Other beneficial effects include more elevation in reduced glutathione and more reduction of NF-kB levels which increase antioxidant and anti-inflammatory effects. This may be attributed to different mechanism of action and synergistic effects of both drugs.

CONCLUSION

Based on above mentioned data about tested drug combination, one may conclude that canagliflozin / Metformin combination is very promising tool in treatment of insulin resistance because it exerted the most powerful effects regarding reversion of the tested parameters regarding insulin resistance, lipid profile and diabetic cardiomyopathy. Moreover, it was the only therapeutic tool that reversed the structural changes associated with diabetic cardiomyopathy.

This study highlighted the crucial role played by NF-kB in pathogenesis of insulin resistance and diabetic cardiomyopathy. Canagliflozin / Metformin combination attenuated its level compared with each drug alone which may be a positive point in favor of former drug combination. Yet, its level was far more normal value. It remains to be a plausible target for future drugs.

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