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COMPARATIVE EFFECTS OF CANAGLIFLOZIN AND METFORMIN ON CARDIAC DYSFUNCTION AND TESTICULAR DAMAGE IN DIABETIC RATS

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ABSTRACT

Purpose: The current study was carried out to investigate the antidiabetic effect of canagliflozin and metformin on induced cardiac dysfunction and testicular damage in diabetic rats. **Methods:** Male albino rats were classified into 4 groups. Control group received distilled water(C). Diabetes was induced in second, third and fourth groups rats by feeding them with a high-fat diet(HFD) for 3 weeks followed by intraperitoneal injection of a low dose of streptozotocin (35 mg/kg) and continued with butter supplementation in line with SD for another one week. Second group remained as the untreated diabetic group(D). The third group received orally (10 mg/kg) daily dose of canagliflozin (D + CAN) for 4 weeks. The forth group received orally (100 mg/kg) daily of Metformin (D + MET) for 4 weeks. **Results**: CAN and MET treatment significantly ($P<0.05$) increased superoxide-dismutase (SOD), glutathione oxidized (GSSG) activities andglutathione Reduced (GSH) total nitric oxide(TNO)contents with a concomitant decrease in Malondialdehyde (MDA), Interlukin-6 (IL-6) and Tumor necrosis factor alpha(TNFα), lipid profile in both heart and testis tissues. On the other hand, cardiac enzymes C-reactive protein (CRP), Creatine Kinase (CK) and Aspartate aminotransferase (AST) and testicular enzymes Acid phosphatase (ACP), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH) and total protein showed marked improvement in their levels after treatment with CAN than MET. **Conclusion:** On conclusions, CAN has the antidiabetic effect and corrects complications associated with diabetes better than metformin such as, cardiac dysfunction and testicular damage. So,

Canagliflozin represent a novel therapeutic approach for antidiabetic medications and needed more studies.

KEYWORDS: Diabetes, Canagliflozin, Metformin, Heart, Testis, Rats.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a serious metabolic disorder with numerous complications. It is defined as a metabolic dysfunction manifested by chronic hyperglycemia resulting from defects in insulin metabolism and impaired function in carbohydrate, lipid and protein metabolism leads to long-term complications.[1] Patients with diabetes have a significantly increased risk of morbidity and mortality associated with cardiovascular disease and stroke, causing 75 % of all deaths.[2] Furthermore, T2DMis usually associated with other factors like hypertension, dyslipidemia, obesity and accelerated atherosclerosis risk that contributes to an even higher morbidity and cardiovascular mortality.[3] Hyperglycemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. So, oxidative stress is thought to be a major contributor to cardiovascular disease in diabetes mellitus.[4] Oxidative stress known to play a major role in the fertility related health complications in diabetic patients[5], via action at multiple levels including altered spermatogenesis,degenerative and apoptotic changes in testes, altered glucose metabolism in Sertoli/ blood testes barrier, reduced testosterone synthesis and secretion.[6]

Metformin is commonly used in the treatment of T2DM. The drug is known to decrease the production of glucose in liver[7], reduce the absorption of glucose from the intestine, and improve insulin sensitivity by increasing peripheral glucose uptake and utilization, chiefly muscle.[8] Several epidemiological studies have found that in diabetic patients, metformin improves vascular function and reduces cardiovascular events and mortality through its antihyperglycemic effects.[9] Despite all its benefits, metformin is contraindicated in patients with heart failure due to the potential risk of developing lactic acidosis, a rare but potentially fatal metabolic condition resulting from severe tissue hypoperfusion.[10]

Canagliflozin trade name (Invokana) is an orally anti-diabetic drug of a newer medication class called gliflozin drugs.Canagliflozin is an inhibitor of subtype 2 sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of renal glucose reabsorption. Blocking this transporter causes up to 119 grams of blood glucose per day to be

eliminated through the urine.Additional water is eliminated by osmotic diuresis, resulting in a lowering of blood pressure.[11]

The diuretic action of SGLT2 inhibitors may also have advantages for heart failure. Recently, it has been reported that administration of empagliflozin or canagliflozin significantly reduced cardiovascular adverse outcomes in T2DM patients.[12]

MATERIALS AND METHODS

Drugs: Canagliflozin ($C_{24}H_{25}FO_5S \cdot 1/2\ H_2O$) (Invokana), 300 mg tablets manufactured by Janssen Ortho, LLC. Gurabo, PR 00778, for Janssen pharmaceuticals, INC. Titusville, NJ 08560. Metformin MET hydrochloride ($C_4H_{11}N_5 \bullet HCl$) (Glucophage) 1000 mg tablets purchased from Merck Santé S.A.S. Streptozotocin STZ, Sigma-Aldrich Co., St. Louis, Mo, USA. Product number: S0130.

Biological assay

Animals: All experiments including animals were conducted in accordance with the principles and guidelines for the care and use of laboratory animals of the National Organization for Drug Control and Research (NODCAR) (NIH Publication No. 85-23, revised 1996). Male albino rats (Wistar strain) (150 ± 10 g) were got from the animal house of NODCAR. Animals were housed throughout the experiment (6rats/cage) in standard polypropylene cages and maintained at controlled room temperature (21 ± 2 ◦C) and humidity (55%) under a 12 h dark-light cycle and acclimated to the laboratory conditions for 2 weeks prior to the experiments.Rats were feed the AIN-93G diet in pelleted form for complete diet composition as a standard diet (SD) (Reeves et al., 1993). The food debris, feces, urine were uprooted every day to prevent food and water contamination.

Animal model of diabetes: A group of rats left as control normal SD fed. Animals in diabetic groups were fed a standard diet supplemented with 1g butter administered orally for 10 days, and the amount of butter increased to 2 g over the next 10 days.[13] With respect to day 22, hyperlipidemia was confirmed in rats that had received injections of STZ (35 mg/kg) (i.p.)[13,14] dissolved in sodium citrate buffer at pH 4.5.The butter supplemented standard diet was continued for 1 extra week, amounting to a total of 4 weeks of the HFD.One week after STZ injection, the progress of diabetes was confirmed by measuring the glucose level in fasting blood samples taken from a tail vein using Accu-Chek glucometer (Roche, Germany). Rats with blood glucose concentrations >190 mg/dl or high were considered diabetic and

included in the subsequent study. Rats in the diabetic groups were fed the butter-supplemented diet throughout the entire experimental period.

Experimental design: The animals were divided into four groups (n=6)Group 1(Con gr): animals were administered daily oral (2 ml water) as a control. Group 2(Db group): diabetic group a model.Group 3 CAN group: (Db+CAN) diabetic rats were orally administered with CAN (10 mg/kg) according to Liang *et al.*[15] and Group 4MET group :(Db+MET) diabetic rats were orally administered MET (100 mg/kg) according to the Guidance for Industry and Reviewers by FDA (2002).

Sample collection: At the end of the experiment(20 h after the administration of the last dose), blood samples from the lateral tail vein were obtained and immediately used to determine blood glucose levels using a glucometer. Animals were sacrificed and heart and testes were immediately removed and thoroughly washed with ice-cold isotonic saline, blotted dry and then weighed. Then the tissues were immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing phosphate buffer (pH 7.4). Then, the homogenates were centrifuged at 1800 xg for 10 min at 4 ◦C and the resultant supernatant was used for the determination of different biochemical analysis.

Biochemical assays: LDH was assayed by a colorimetric method using kits purchased from BioSystems Co. (Egypt) according to the methods of Young.[16] Triglycerides, Total Cholesterol, Low-density lipoprotein(LDL) and High-density lipoprotein(HDL) contents were determined by colorimetric methods using kits purchased from Bio Med diagnostic Co. (Egypt) according to the methods of Fossati and Principe[17], Tietz[18], Wieland and Seidel[19] and Lopes-Virella *et al*[20], respectively. MDA content, SOD, GSH and GSSG activities were determined by colorimetric methods using kits purchased from Oxford Biomedical following the manufacture instructions.IL-6 and TNFα were determined using ELISA kit purchased from Immuno-Biological Laboratories CO., LTD. AST was determined by a colorimetric method of Reitman and Frankel.[21] TNO contents were determined using kits purchased from Bio vision. CRP was determined by CRP-HS II LT (Latex Turbidimetric Immunoassay) kit purchased from Wako Chemicals GmbH, according to Whicher.[22] CK was determined by kit purchased from Diagnosticum Zrt., according to Mathieu *et al*.[23] Total protein, ACP and ALP activity were determined by kits purchased from Bio diagnostic Co. (Egypt) following the methods of Young[24], Kind and King[25] and Belfield and Goldberg[26], respectively. Total lipid was determined by colorimetric methods using kit purchased from BQ Kits, following

the methods of Knight *et al.*[27] Free fatty acids were determined by kit purchased from Wako Chemicals GmbH.

Statistical analysis: The results of present study were expressed as mean ± S.E. of the mean. Statistical Package for the Social Sciences (SPSS) program, version 16.0 was used to compare significance between each two groups.The difference was considered significant when P <0.05. Percentage difference representing the percent of variation with respect to the corresponding control group was calculated according to the following formula.

$$\% \text{ Difference} = \frac{\text{Treated value} - \text{Control value}}{\text{Control value}} \times 100$$

RESULTS

The results in Table (1) showed the effect of Canagliflozin and Metformin treatment on serum blood glucose levels in diabetic rats. Diabetic group displayed significant elevation (P < 0.05) in the blood glucose level indicating severe hyperglycemia when compared with non-diabetic groups. Furthermore, treatment of Db group with either CAN or MET showed significant depletion (P<0.05) in the levels blood glucose when compared to diabetic group.

Table. 1: Effect of treatment with Canagliflozin and Metaformin on serum glucose levels of control and diabetic rats.

Groups / Parameters	Con group	Db group	Db+CAN group	Db+MET group
Glucose(mg/dl)	97.25±7.11	325.50±50.87[ac] C= 234.70%)	137.66±15.21[ab] D= (-57.70%)	273.16±16.57[ac] D =(-16.07%)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

The data illustrated in Table (2) revealed that the levels of LDH, TG, total cholesterol and LDL showed a significant increase (P<0.05) in the diabetic group (51.47%, 181.76%, 30.42% and 34.18%, respectively) and a decrease in HDL(-24.16%) compared with control group.However, treatment of Db group with either CAN or METshowed significant reduction (P<0.05) in the levels of LDH (-20.57%and-15.08%, respectively), TG (-51.61% and -9.67%, respectively), Cholesterol (-30.42% and-16.67%, respectively) and LDL(-21.87% and16.36, respectively) versus Db group.On the other hand, treatment of Db group with CAN and MET

showed a significant increase in the levels of HDL (38.93% and 22.12%, respectively) compared with Db group.

Table. 2: Effect of treatment withCanagliflozinand Metaformine on heart lipid profile of control and diabeticrats.

Parameters / Groups	Lactate dehydrogense LDH (U/g)	Triglycrides TG(mg/g)	Total cholesterol (mg/g)	High density lipoprotein HDL (mg/g)	Low density lipoprotein LDL (mg/g)
Con group	2.236 ± 0.039	2.292 ± 0.348	7.667 ± 0.614	0.149 ± 0.004	5.544 ± 0.616
Db group	3.387 ± 0.071 [a] C= (51.47%)	6.458 ± 0.502 [a] C =(181.76%)	10.00 ± 0.730 [a] C= (30.42%)	0.113 ± 0.003 [a] C= (-24.16%)	7.439 ± 0.745 C=(34.18%)
Db+CANgroup	2.690 ± 0.007 [ab] D= (-20.57%)	3.125 ± 0.279 [b] D= (-51.61)	7.667 ± 0.802 [b] D=(-30.42%)	0.157 ± 0.007 [b] D= (38.93%)	5.812 ± 0.814 D= (-21.87%)
Db+ MET group	2.876 ± 0.015 [abc] D= (-15.08%)	5.833 ± 0.264 [ac] D= (-9.67%)	8.333 ± 0.398 D= (-16.67%)	0.138 ± 0.011 [b] D= (22.12%)	6.222 ± 0.802 D=(-16.36)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

The results in Table (3) showed the effect of treatment with CAN and MET on cardiac MDA content and enzymatic antioxidants activities in diabetic rats. In comparison with the control group, there was a significant increase (P<0.05) in MDA content (156.67%) in Db group. On the other hand, the treatment with either CAN or MET resulted in a significant decrease in the MDA content (-40.24% and -17.16%, respectively) compared with Db group. Moreover, the contents of SOD, GSSG and GSH in heart homogenate significantly decreased (P<0.05) in Db group (-44.68%,-50.96% and -46.37%) when compared with that of the control group. A significant increase (P<0.05) in the activities of SOD, GSSG and GSH heart homogenate were recorded following the treatment of either CAN (48.63%,75.19% and 51.94%, respectively) or MET (15.82%, 36.61% and 30.96%, respectively) compared to diabetic group (Table 3).

Table. 3: Effect of treatment with Canagliflozin and Metformine on heart MDA content and antioxidant enzymes activities in diabeticrats.

Parameters / Groups	MDA (nmol/g)	SOD (U/g)	GSSG (mM/g)	GSH (Mm/g)
Con group	3.190±0.109	13.317±0.245	8.633±0.225	17.467±0.571
Db group	8.188±0.137 ^{a} C=(156.67%)	7.367±0.274 ^{a} C=(-44.68%)	4.233±0.212 ^{a} C= (-50.96%)	9.367±0.423 ^{a} C=(-46.37%)
Db+ CAN group	4.893±0.137 ^{ab} D=(-40.24%)	10.950±0.330 ^{ab} D=(48.63%)	7.416±0.236 ^{ab} D= (75.19%)	14.233±0.404 ^{ab} D=(51.94%)
Db+ MET group	6.783±0.102 ^{abc} D= (-17.16%)	8.533±0.237 ^{abc} D= (15.82%)	5.783±0.183 ^{abc} D=(36.61%)	12.267±0.165 ^{abc} D=(30.96%)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

As appeared in Table (4) cardiac inflammatory cytokine IL-6, TNF-α were significantly increased with the exception of total nitric oxide(TNO) levels significantly decreased(P<0.05) in diabetic rats compared to the control group. However, treatment with CAN or METsignificantly showed depletion(P<0.05) in the inflammatory cytokines compared to the diabetic group.In contrast, the treatment of Db group with either CAN or MET resulted in a significant elevation in the level of TNO (71.26% and 47.78%, respectively) compared with Db group.

Table. 4. Effect of treatment with Canagliflozin and Metformine on heart IL-6,TNF-α and TNO levels in diabitic rats.

Parameters / Groups	IL-6 (AU/g)	TNF-α (pg/g)	Total Nitric oxide (TNO) (Mm/g)
Con group	39.533±0.766	36.450±1.107	279.933 ± 5.236
Db group	139.667±4.207 ^{a} C=(253.29%)	136.633±5.273 ^{a} C=(274.850%)	131.067 ± 7.196 ^{a} C=(-53.17%)
Db+ CAN group	79.500±2.423 ^{ab} D=(-43.07%)	78.266±4.082 ^{ab} D=(-42.71%)	224.467 ± 3.438 ^{ab} D=(71.26%)
Db+ MET group	105.133±2.521 ^{abc} D=(-24.72%)	109.400±2.737 ^{abc} D=(-19.93%)	193.700 ±2.662 ^{abc} D=(47.78%)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

The data in Table (5) illustrated the effect of treatment with CAN and MET on cardiac CRP, CK and AST levels in diabetic rats. diabetic group showed significant elevation (P<0.05) in the heart levels of CRP (122.78%), CK (88.10%) and AST (31.85%) as compared to the control group. In the contrary, treatment of diabetic group with either CAN or MET resulted in significant reduction in the levels of CRP (-31.81% and-15.15%, respectively), CK (-12.65% and-10.54%, respectively) and AST (-22.22% and-16.91%, respectively) compared with Db group.

Table. (5). Effect of treatment with Canagliflozin and Metformine on heart CRP, CK and AST levels in diabitic rats.

Parameters / Groups	C-reactive protein CRP (mg/g)	creatine kinase CK (mg/g)	Aspartate amino- transferase AST (mg/g)
Con group	0.237 ± 0.006	0.933 ± 0.020	0.157 ± 0.000
Db group	0.528 ±0.019 [a] C=(122.78%)	1.755 ± 0.024 [a] C=(88.10%)	0.207 ± 0.001 [a] C=(31.85%)
Db+ CAN group	0.360 ± 0.012 [ab] D=(-31.81%)	1.533 ± 0.022 [ab] D=(-12.65%)	0.161 ± 0.000 [ab] D=(-22.22%)
Db+ MET group	0.448 ± 0.009 [abc] D=(-15.15%)	1.570 ± 0.0219 [ab] D=(-10.54%)	0.172 ± 0.001 [abc] D=(-16.91%)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

As shown in Table (6) the activities of testicular acid phosphatase(ACP) and lactate dehydrogenase(LDH) were significantly increased (P<0.05) whereas, alkaline phosphatase(ALP) and total protein(TP) level were significantly decreased (P<0.05) in diabetic group compared to the control group. Treatment of Db group with either CAN or MET significantly restored the levels of ACP, ALP, TP and LDH approximately to normal levels compared to diabetic group and this effect was more pronounced in CAN group than MET suggesting an antioxidant effect of CAN.

Table. 6: Effect of treatment with Canagliflozin and Metaformine on testicular levels of ACP, ALP, total protein and LDH of control and diabetic rats.

Parameters / Groups	Acid phosphatase ACP (U/gm)	Alkaline phosphatase ALP(U/gm)	Total protein TP(mg/g)	Lactate dehydrogenase LDH(U/g)
Con group	0.306±0.004	0.012±0.002	160.000±33.466	150.032±14.106
Db group	0.394±0.005[a] C= (28.75%)	0.001±0.001[a] C= (-91.66%)	70.000±20.298 [a] C= (-56%)	184.033±53.596 C= (22.66%)
Db+ CAN group	0.288±0.011[b] D= (-26.90%)	0.011±0.000[b] D= (1.000%)	156.000±25.884[ab] D = (122.85%)	110.095±13.635 D= (-40.17%)
Db+ MET group	0.324±0.007[bc] D= (17.76%)	0.008±0.000[abc] D= (700%)	140.000±26.985[ac] D= (100%)	139.238±23.647 D= (-24.34%)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

The data illustrated in Table (7) revealed that the testicular levels of total lipid, free fatty, triglycerides, total cholesterol, and LDL showed significant increase (P<0.05) in the diabetic group (39.56%, 104.30%, 208.32%, 106.66% and 153.45%, respectively) compared with control group. However, treatment of Db group with either CAN or MET showed significant reduction (P<0.05) in the levels of total lipid (-19.97% and -12.02%, respectively), free fatty(-30.11% and -20.40%, respectively), triglycerides (-45.94% and -16.21%, respectively), total cholesterol (-35.48% and -16.12%,respectively) and LDL(-41.98 % and -19.27 %, respectively) versus Db group. On the other hand, the treatment of Db group with either CAN or MET resulted in a significant elevation in the testicular level of HDL (46.26 % and4.47%, respectively) compared with Db group.

Table. 7: Effect of treatment withCanagliflozin and Metaformine ontestecular lipid profile in control and diabetic rats.

Parameters / Groups	Total lipid (mg/g)	Free fatty acid (mg/g)	Triglycrides (mg/g)	Total cholesterol (mg/g)	High density lipoprotein HDL(mg/g)	Low density lipoprotein LDL(mg/g)
Con group	12.8±0.096	1.185±0.011	2.500±0.323	5.000±0.856	0.076±0.0103	3.573±0.872
Db group	17.864±0.229 $_{a}$ C= (39.56%)	2.421±0.042 $_{a}$ C= (104.30%)	7.708±0.817 $_{a}$ C= (208.32%)	10.333±0.955 $_{a}$ C= (106.66%)	0.067±0.003 C= (-11.84%)	9.056±0.958 $_{a}$ C=(153.45%)
Db+ CAN group	14.297±0.099$_{ab}$ D =(-19.97%)	1.692±0.038 $_{ab}$ D = (-30.11%)	4.167±0.618 $_{b}$ D = (-45.94%)	6.667±0.989 $_{b}$ D = (-35.48%)	0.098±0.002 $_{ab}$ D = (46.26 %)	5.254±0.978 $_{b}$ D =(-41.98 %)
Db+ MET group	15.715±0.085 $_{abc}$ D = (-12.02%)	1.927±0.040 $_{abc}$ D = (-20.40%)	6.458±0.751 $_{ac}$ D = (-16.21%)	8.667±1.229 $_{a}$ D = (-16.12%)	0.070±0.0029 D = (4.47%)	7.311±1.173 $_{a}$ D =(-19.27 %)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

In comparison with the negative control group, there was a significant increase (P<0.05) in MDA content (156.67%) in Db group. On the other hand, the treatment of Db group with either CAN or MET resulted in significant decrease in the MDA content(-40.24% and -17.16%, respectively) when compared with Db group. Furthermore, the contents of, GSSG and GSH in testis homogenate significantly decreased (P<0.05) in Db group (-44.68%,-50.96% and -46.37%, respectively) compared with that of normal control group. A significant increase (P<0.05) in the testicular levels of SOD, GSSG and GSH were recorded in groups with either CAN (48.63%, 75.19%, and 51.95%, respectively) and MET (15.83%, 36.61% and 30.96%, respectively) compared to Db group.

Table. 8: Effect of treatment with Canagliflozin and Metformin on testicular MDA content and antioxidant enzymes activities in diabetic rats.

Parameters / Groups	MDA (nmol/g)	SOD (U/g)	GSSG (mM/g)	GSH (Mm/g)
Con group	3.190±0.109	13.317±0.245	8.633±0.225	17.467±0.571
Db group	8.188±0.137[a] C= (156.67%)	7.367±0.274[a] C= (-44.68%)	4.233±0.212[a] C= (-50.96%)	9.367±0.423[a] C= (-46.37%)
Db+ CAN group	4.893±0.137[ab] D = (-40.24%)	10.950±0.330[ab] D = (48.63%)	7.416±0.236[ab] D =(75.19%)	14.233±0.404[ab] D = (51.95 %)
Db+ MET group	6.783±0.102[abc] D = (-17.16%)	8.533±0.237[abc] D = (15.83%)	5.783±0.183[abc] D = (36.61 %)	12.267±0.165[abc] D =(30.96%)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

Levels of testicular inflammatory cytokine IL-6 and TNF-α were significantly increased (P<0.05) in diabetic group compared with non-diabetic control group (253.29% and 274.85%, respectively). These IL-6 and TNF-α levels were significantly decreased by MET treatment which recorded (-24.72% and-19.93%, respectively), and more marked reductions were seen in the CAN-treated group (-43.08% and -42.72%, respectively).Conversely, testicular NO levels were significantly decreased (P<0.05) in Dp group (-53.89%) when compared with that of the control group. A significant increment (P<0.05) in the testicular levels of NO were recorded following the treatment of inducted groups with either CAN (73.91%), or MET (48.49%) compared to Db group (Table 9).

Table. 9: Effect of treatment with Canagliflozin and Metformine on testicular IL-6, TNF-α and TNO levels in control and diabetic rats.

Parameters / Groups	IL-6 (AU/g)	TNFα (pg/g)	Total Nitric oxide (TNO) (Mm/g)
Con group	39.533±0.766	36.450±1.107	279.933±5.236
Db group	139.667±4.207[a] C= (253.29%)	136.633±5.273[a] C= (274.85%)	129.066±5.286[a] C= (-53.89%)
Db+ CAN group	79.500±2.423[ab] D = (-43.08%)	78.266±4.082[ab] D =(-42.72%)	224.466±3.438[ab] D = (73.91 %)
Db+ MET group	105.133±2.521[abc] D = (-24.72%)	109.400±2.737[abc] D =(-19.93%)	191.650±2.208[abc] D = (48.49%)

Data were represented as Mean ± S.E of 6 rats /group. C%=Percentage of change from the normal control group. D%=Percentage of change from the diabetic group. a = significant

change at P> 0.05 in comparison with control group. b = significant change at P> 0.05 in comparison with Db group. c= significant change at P> 0.05 in comparison with CAN group.

DISCUSSION

The present work was performed to explore the potential mechanisms of Canagliflozin and metformin against diabetes-associated cardiovascular risk and testes dysfunction. Streptozotocin-induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia which contributes to the development of diabetic complications.[28] Consistent with previous studies, diabetic rats in the present study showed a significant elevation in glucose levels after STZ injection.

The present findings have further shown that CAN treatment decreased significantly serum glucose level as compared to diabetic control.Consistent with these results, Messana et al.[29] reported that canagliflozin plays an essential role in reducing postprandial sugars more effectively than others in normal subjects.Canagliflozin reduce hyperglycemia in patients with T2DM by reducing renal glucose reabsorption and increasing urinary glucose excretion, mild osmotic diuresis, promote weight loss and exert a modest diuretic effect with blood pressure reduction.[30,31]

The present studies indicate that MET treatment caused down-regulation in serum glucose level of diabetic rats and this hypoglycemic action may be due to thatMET stimulates the insulin-induced component of glucose uptake into skeletal muscle and adipocytes.[32] Moreover, Malin and Kashyap[33] reported that metformin has glucose-lowering effects on rats with type 2 diabetes, through transporter-stimulated tissue uptake of glucose which improves peripheral insulin sensitivity. The present findings suggesting that CAN has strongly anti-hyperglycemic effect on diabetic rats than MET. This effect might have been clear from the depletion observed in glucose level of diabetic rat model The current results indicated that STZ administration to normal rats caused a significant increase in lipid profile levels in cardiac and testicular tissue accompanied by decreases in the levels of HDL indicating the progress of dyslipidemia, as a result, of excessive deposition of lipids which enhances the risk of T2DM. These findings are in accordance with the results of Ceriello and Motz[34] who reported that diabetes mellitus caused impaired glucose metabolism which in effect adversely alters the intermediary metabolism of lipids and proteins.Formation of protein glycation products releases free radicals; consequently causing oxidative stress which in turn increased lipid profile. Furthermore, patients with diabetes have a characteristic ‘lipid

triad' of HDL -cholesterol levels, high triglyceride levels and normal or slightly raised LDL -cholesterol levels, with a preponderance of small, dense LDL-C particles.[35,36] The current results are concordant with these results, where a recorded increase in the cardiac and testicular lipid profile levels in diabetic rats and these results may be due to the oxidative damage which resulted from progression of diabetes by streptozotocin.[37,38]

On another hand, administration of CAN significantly ($P<0.05$) decreased the cardiac and testicular levels of total lipid profile compared with the diabetic group. The present results are consistent with those of Inzucchi et al.[39] and Messana et al.[29] who observed that in patients with T2DM the administration of CAN reduced the overall weight, adiposity and blood pressure without compensatory increases in heart rate and have some effects on plasma lipids (slightly decrease in triglycerides, increase in HDL-C and small increase in LDL-C, with no change in HDL-C/LDL-C). Besides its advantage on reducing cardiovascular (CV) risk beyond glucose lowering effects.

Moreover, MET-treated diabetic group exhibited a significant reduction in total lipid profile levels reflected by an increase in levels of HDL in both heart and testes. This could be attributed to that MET has been found to cause a mild and transient inhibition of mitochondrial complex I which decreases ATP levels and activates AMPK-dependent catabolic pathways, increasing lipolysis and ß-oxidation in white adipose tissue.[40,41] Interestingly, several studies confirmed that MET caused improvements in lipoprotein metabolism, including decreases in LDL-C, fasting and postprandial TG, and free fatty acids.[42,43]

In this study, the diabetic rat model exhibited a significant increase in the lipid peroxidation levels and a decrease in the enzymatic activities of SOD, GSSG and GSH in both heart and testes throughout the experimental period when compared with the normal control rats that indicating the development of diabetic complications and increasing oxidative stress. In accordance with the present results, Rahimi et al.[44] noticed that in diabetes the hypo insulinemia increases the activity of the enzymes such as fatty acyl-coenzyme-A oxidase, which initiates beta-oxidation of fatty acids, resulting in LPO.An increase in lipid peroxides in plasma may be one of the important factors in the development of vascular complications and atherosclerosis in diabetes mellitus.[45] Also, the increased testicular MDA content of diabetic rats could be attributed to the peroxidative injury which led to the progress of disease.[46] In the current study, The depletion in endogenous antioxidant activities of the tests

including SOD, GSSG, and GSH may be due to oxidative stress associated with diabetes which led to peroxidative damage and impairment of defense system.In accordance with the present results, Aitken and Roman[47] reported that the testicular damage which precipitated by temporary ischemia is associated with oxidative stress and supported by the sudden induction of lipid peroxidation which concomitant suppression of endogenous antioxidant activities including SOD, catalase, and glutathione peroxidase.

The administration of CAN to diabetic rats markedly reduced the elevated levels in activities of SOD, GSSG, and GSH content accompanied with significant depletion in MDA content compared with Db group. The present results are consistent with those of Osorio et al.[48] and Abdel-Wahab et al.[49] who found that CANtreatmentcan attenuates diabetes-induced oxidative stress in the kidney of diabetic rats through decreasing MDA levels and increasing the activities of antioxidant enzymes GH-Px and SOD. The present data are in agreement with this view since the improvement observed in SOD, GSSG and GSH and the decrease in MDA levels indicating the synergistic action of CAN on improving endogenous antioxidant activities of diabetic rats suggesting its role in protecting cells against oxidative stress thereby reducing diabetic complications.

Moreover, treatment of diabetic rats with MET results in a significant increase in the levels of SOD, GSSG, and GSH accompanied by significant decrease in MDA content in heart and testes. The present data are in agreement with Rojas and Gomes[41] who indicated that metformin possesses antioxidant properties through it reduces ROS by inhibiting mitochondrial respiration and decreases advanced glycosylation end product (AGE) indirectly through reduction of hyperglycemia and directly through an insulin-dependent mechanism.It has also been described that long-term treatment with metformin increases antioxidant enzymatic activities and serum glutathione levels, thereby improving the antioxidant status.[50]

Diabetic rat model in this study showed a significant increase in the levels of cardiac and testicular pro-inflammatory cytokines IL-6 and TNF-αaccompanied by decreases in the level of TNOas compared to control group.This could be attributed to that hyperglycemia results in oxidative stress and inflammation, and it is considered a risk factor for cardiovascular disease.[51] The present findings were confirmed with Mruk and Cheng[52] who reported that nitric oxide synthase/ nitric oxide (NOS/NO) contribute in controlling the levels of cytokines and hormones in the testes. On the other hand, NO is playing a unique role in modulating

germ cell viability and development, and indirectly acting on some aspects of male infertility and testicular pathological conditions. It plays an important role in protection against the progression of CVD by regulating vascular tone, inhibition of platelet aggregation and prevention of smooth muscle proliferation.[53] It has been demonstrated that increased levels of cardiac pro-inflammatory cytokines (TNF-α, IL-1β) leading to activation of immunocompetent cells (macrophages, T lymphocytes (consequently, abnormal structural alterations in diabetic rats which led to impaired cardiac performance.[54] Moreover, prospective studies have confirmed that increased concentrations of markers of the acute phase response including CRP, TNF-α, IL-1β and IL-6, and chemokines in patients with obesity and T2DM.[55] Inflammatory factors, which play a critical role in the development of atherothrombosis, was found to be elevated in patients suffering from diabetes.[56]

The administration of CAN significantly reduced cardiac and testicular levels of IL-6 and TNF-α accompanied by an improvement in the level of TNO level in a diabetic rat model.In view of the present data, several lines of evidence have shown that canagliflozin and Ipragliflozin appear to can reduce plasma and liver inflammatory markers (IL-6, TNF-α and CRP) in high-fat diet and streptozotocin–nicotinamide-induced type 2 diabetic mice and rats.[57,56] Moreover, SGLT-2 inhibitors reduce leukocytosis induced by hyperglycemia and reduce inflammation and oxidative stress which are processes involved in the pathophysiology of atherosclerosis.[48,58] Furthermore, Db+MET group showed a reduction in the proinflammatory cytokines IL-6, TNF-α level and raise in TNO level in both heart and testes. MET can show anti-inflammatory action through inhibition of advanced glycation end products which advance inflammation and ROS.[59]

On the other hand, METindicates plasminogen activator inhibitor 1 and macrophage migratory inhibition factor from the plasma of obese patients; this drug may in this way have anti-inflammatory activity and reduce cardiovascular injury/mortality.[60] Several authors have demonstrated that, reduction in the proinflammatory cytokines IL-6 and TNF-αconcentrations and elevation in TNO level after treatment with METresulting from inhibiting of pro-inflammatory responses through direct inhibition of NF-KB by blocking the PI3K–Akt pathway resulting in the reduction of cardiovascular events.[61,62] According to previous researches hyperglycemia-induced oxidative stress which is an achieve pathogenic factor in the development of cardiac fibrosis. There is an increasing interest in suppressing oxidative stress as an effective strategy for reducing cardiac fibrosis.[63,64] Furthermore, CRP

has been reported to promote atherosclerosis, metabolic disorders, CV disease and dyslipidemia by directly increasing the transcytosis of LDL across vascular endothelial cells and increasing LDL retention in vitro and in vivo and impairs nitric oxide (NO) production, resulting in endothelial dysfunction[65,66] CRP is also a marker of low-grade inflammation, especially when associated with visceral adipose tissue.[67] Creatine kinase (CK) is the enzyme responsible for supplying the working muscle of the heart with a continuous supply of ATP which is used in the contraction process.[68] In the view of aforementioned studies, the present results suggesting that increased cardiac enzymes, as a result of hyperglycemia, that increased free radicals resulting in myocardial cell damage.

The observed amelioration of the cardiac enzymes of diabetic rats administered CAN be due to the ability of CAN to protect against tissue damage resulting in values near to normal levels of cardiac CRP, CK, and AST.It has been illustrated by Zimlichman[69] that, SGLT-2 inhibitors may improve endothelial capacity or the vascular architecture contributing to CV risk reduction.In this line, administration of ipragliflozin inhibited the progression of atherosclerosis in a mouse model of repetitive glucose spikes.[70,71] This observation was similar to the present suggesting where that anti-atherosclerotic role of CAN was attributed to its antioxidant properties that reducing CV disease risk in diabetic rats. Moreover, the present data indicated that treatment of diabetic group with MET resulted in significant decrease in the levels of cardiac enzymes as compared to control group.In agreement with previously published studies which demonstrated that MET reduces CV morbidity and mortality in overweight T2DM subjects and protective effect on the vascular wall and the inflammatory state.[72,73]

The elevation observed in ACP and LDHactivities and the depletion in ALP activities and total protein level may be due to the damage in testicular enzyme levels in the diabetic rat model which resulting from the progress of diabetes. The present results are more compatible with Karimi et al.[74] and Mallidis et al.[75] who reported that oxidative stress may play an important role in the pathophysiology of diabetes-related male reproductive dysfunction and abnormalities.

CAN treatment restore the levels of ACP, ALP, LDH and total protein(TP) approximately to normal levels. As, Akhtar,[76] and Sabry and Sakr[77] showed that administration of canagliflozin to diabetic animals reduced the incidence of testicular lesions caused by streptozotocin or metformin treatment, and showed round seminiferous tubules with thick

basal laminae. The present study has been suggested that CAN has a therapeutic efficacy in preventing damage could be induced in the testicular tissue of diabetic rats. Also, treatment with MET significantly ameliorated testicular damage in the levels of ACP, ALP, LDH, and TP when compared to diabetic rats.Such results are in agreement with the findings of Alves et al.[78] who demonstrated that MET is a drug involved in metabolic homeostasis and Sertoli cell metabolism and it has been found to be essential for spermatogenesis. Furthermore, Rabbani et al.[5] announced that treatment with MET prevented the nicotinamide (NA)-STZ induced defects in sperm shape, sperm count, the weight of testis and oxidative stress besides its antidiabetic activity and also, suggest that MET might have stimulated the proliferation of cells of testicles in the diabetic animals.

CONCLUSION

In conclusion, the current study provided experimental evidence for the potential role of Canagliflozin and Metformin in the regression of diabetes in male rats.This effect was achieved through the antioxidant and anti-inflammatory effects of the selected treatment play a key role in their therapeutic potential. Noteworthy, Canagliflozin provided significant hypoglycemic efficacy and corrects complications associated with diabetes than Metformin such as, cardiac dysfunction and testicular damage. So, Canagliflozin represents a novel therapeutic approach for antidiabetic medications and needed more studies.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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