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Full Length Research Paper

Impact of Canagliflozin and metformin on metabolic abnormalities in obese diabetic rat models

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This study aimed to investigate the effect of canagliflozin (CAN) or metformin (MET) on investigated biochemical parameters in obese diabetic rat model. Obesity induced by melted butter administration and hyperlipidemic rats were subjected to streptozotocin (STZ; 35 mg/kg i.p) to develop the diabetic model (D). Animals were grouped as control (C), D none treated and diabetic treated with CAN (10 mg/kg) or MET (100 mg/kg) for two and four weeks. Both treated groups showed significant reduction in body weight and CAN group exhibited significant decrease in oral glucose tolerance test (OGTT) as compared to D or MET group in the experimental periods. Both drugs showed hypolipidemic activity by reducing total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) and elevating high-density lipoprotein (HDL) as compared to D group values in the two intervals with advantage in MET effect. Both treatments achieved significant reduction in the diabetic elevated serum values for liver and kidney functions. These results indicate that butter high-fat diet and low dose of STZ makes normal male adult rat associated with hyperlipidemia, glucose intolerance and disturbed liver and kidney functions. Treatment by either CAN or MET resisted the metabolism disturbance of the butter high fat diet/STZ -induced obese type 2 diabetes mellitus rats.

Key words: Canagliflozin, metformin, obese diabetic rats, glucose intolerance, biochemical parameters.

INTRODUCTION

Data from the World Health Organization (WHO) shows that overweight and obesity prevalence has increased dramatically throughout recent decades accompanied by increased rates of type II diabetes (T2DM) in the Middle Eastern/North African region. This is greatest in the Arabian Gulf area characterized by significant changes in socioeconomic status and lifestyle. The International Diabetes Federation (IDF, 2011) statistics for T2DM showed six Arabic-speaking countries, Kuwait, Lebanon, Qatar, Saudi Arabia, Bahrain, and United Arab Emirates among the world's leaders in terms of T2DM prevalence. The prevalence rates among adults showed the highest in Kuwait (Badran and Laher, 2012). Insulin resistance accompanied with obesity for incidence of DM previously discussed (Luis-Rodríguez et al., 2012). According to the National and International Guidelines, metformin (MET) is

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the recommended first-line oral therapy for the treatment of T2DM (Nathan et al., 2009). This is down to several factors, including the safety record of the drug used for over 50 years and the fact that MET treatment is weight neutral or result in weight loss. MET, a partial insulin-sensitizing agent, is the gold standard first-line treatment for T2DM. This recommendation based on data from the UK Prospective Diabetes Study Group (UKPDS, 1998). Invokana (canagliflozin, CAN) is a sodium-glucose co-transporter 2 (SGLT2) inhibitor. SGLT2, expressed in the proximal renal tubules, is responsible for majority of the reabsorption of filtered glucose from the tubular lumen. By inhibiting SGLT2, CAN reduces reabsorption of filtered glucose and lowers the renal threshold for glucose (RTG), and thereby increases urinary glucose excretion. The Food and Drug Administration (FDA) approval of Invokana was 29 March, 2013).

The present study was carried out to investigate the effect of either CAN or MET on some biochemical parameters in blood of insulin resistant rat model induced by high fat fed rats subjected to low dose of streptozotocin (STZ).

MATERIALS AND METHODS

Animals

This study was carried out on adult male albino rats (Wistar strain) (150 ± 10 g) obtained from the animal house of National Organization for Drug Control and Research (NODCAR). The experimental animals were allowed to acclimatize under the laboratory conditions two weeks before the beginning of the experiments and kept under controlled temperature of 21°C and 12 h light/12 h dark cycle throughout the experiment. Animals were feed on AIN-93G diet in pelleted form for complete diet composition as a standard diet (SD) (Reeves et al., 1993). The food debris, feces and urine were removed daily to prevent food and water contamination. The animal care conforms to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Drugs

CAN ($C24H25FO5S \cdot 1/2\ H_2O$) (Invokana), 300 mg tablets manufactured by Janssen Ortho, LLC. Gurabo, PR 00778, for Janssen pharmaceuticals, INC. Titusville, NJ 08560. MET hydrochloride ($C4H11N5 \bullet HCl$) (Glucophage) 1000 mg tablets purchased from Merck Santé S.A.S. STZ, Sigma-Aldrich Co., St. Louis, Mo, USA. Product number: S0130.

Animal model of diabetes

Rats following adaptation period had *ad libitum* access to SD pelleted form. A group of rats left as control normal SD fed. The rest animals were used for the diabetic model using high fat diet manipulation and low dose of STZ. The modification in Srinivasan et al. (2005) model by using butter rather than lard for the high fat feeding as the high availability of saturated fatty acid (SFA)-rich foods in today's obesogenic environment could contribute to develop and maintain obesity. According to the USDA National Nutrient Database Release 27, butter composition reported for SFA (51.368%), monounsaturated fatty acids (MUFA; 21.021%) and polyunsaturated fatty acids (PUFA; 3.043%) (Basic Report 01145, Butter, without salt), meanwhile that in lard reported SFA (39%), MUFA (45%) and PUFA (11%) (Basic Report 04002, Lard).

Fatty acids analysis of butter sample by gas chromatography

Methyl esters of fatty acids (FAME) prepared by transmethylation of butter sample using sulfuric acid/methanol (5/95 v/v). Fatty acids in butter sample were determined by gas chromatography (GC) (Figure 1) using Agilent GC 7890 system with FID detector and a split/splitless injector port (Palo Alto, CA USA). The column used was HP-5 fused-silica capillary column (30 m × 0.32 mm), coated with 5% phenylmethylsiloxane (0.25 µm). The GC temperature programming using detector temperature 300°C, inlet temperature 250°C and initial oven temperature 100 to 216°C ramping rate (12°C min^{-1}), to 218°C (2°C min^{-1}) and to 240°C (12°Cmin^{-1}). The recorded fatty acids depend on available reference standards.

High fat diet manipulation

Animals in line with the SD feeding administered orally by 1 g of just melted butter for ten days in line with the SD and the dose of butter increased in the next ten days to 2 g according to the animal body weight. At the 21 day, retro-orbital blood samples collected by experienced animal technician under light diethyl ether anesthesia using glass micro-capillary tubes and collected in centrifuge tubes to separate serum to confirm hyperlipidemia.

Lipid profile screening

Serum used to confirm hyperlipidemia through estimation of total cholesterol (TC) and high-density lipoprotein (HDL) using EnzyChrom™ AF Cholesterol and HDL Assay Kit (ECCH-100 and E2HL-100). Triglyceride (TG) using EnzyChrom™ Triglyceride Assay Kit (Cat# ETGA-200), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) calculated based on the Friedewald equation (Friedewald et al., 1972).

LDL (mg/dl) = TC-HDL-TG/5

VLDL (mg/dl) = TC-HDL-LDL.

Development of STZ-treated type 2 diabetic rats

Hyperlipidemic rats injected at day 22 with STZ (35 mg/kg i.p) were dissolved in sodium citrate buffer at pH 4.5. Animals continued butter supplementation in line with SD for another one week. Hence, animals were fed high-fat diet (HFD) for four weeks. After one week from STZ injection, blood samples from lateral tail vein was used immediately for determination of blood glucose level to assess diabetics using glucometer (OneTouch, Johnson& Johnson Medical). The devise was cleaned after each monitoring by 70% ethyl alcohol. Thirty-six animals with blood glucose level >180 mg/dl were selected as diabetics and included in the experiment and 12 animals were used as control.

Experimental groups

Groups were divided into four groups administered daily oral (PO) as control group (C) (2 ml water) and diabetic group (D) a model according to Srinivasan et al. (2005). CAN group (D+CAN) diabetic

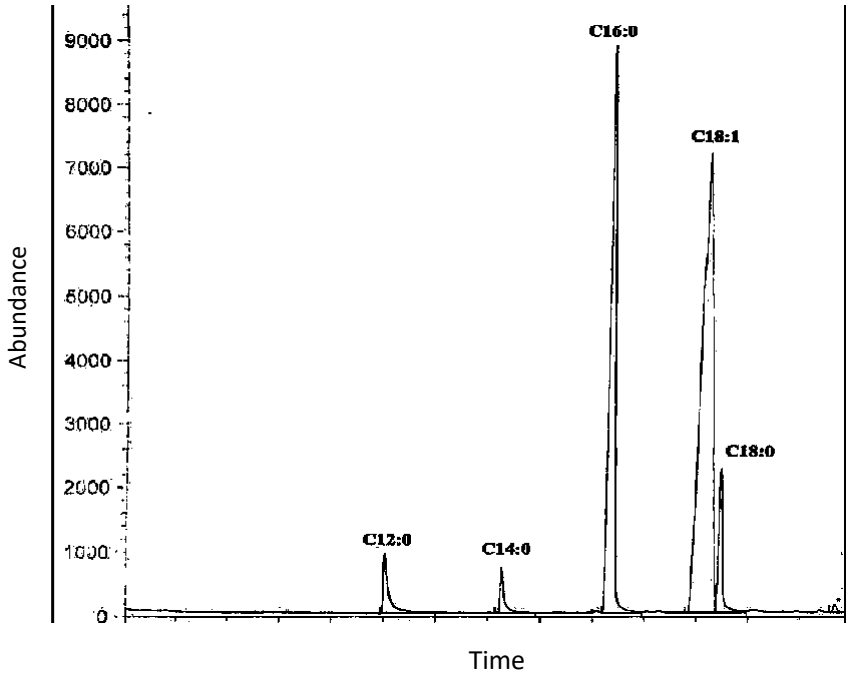

Figure 1. Chromatogram of separated fatty acids of butter sample by GC showing saturated fatty acids, lauric (C12:0), myrestic (C14:0), palmitic (C16:0) and stearic (C18:0) and the unsaturated oleic fatty acid (C18:1).

rats were administered CAN (10 mg/kg) according to Liang et al. (2012) and MET group (D+MET) diabetic rats were administered MET (100 mg/kg) according to the Guidance for Industry and Reviewers by FDA (2002). The administration of butter continued throughout the extended experimental periods (2 and 4 weeks of either CAN or MET treatments). Body weight was recorded weekly from the initial body weight after acclimatization until the end of the experiment.

Glucose tolerance test

Animals were sacrificed at the end of 2 and 4 weeks of treatments and a day before sacrifice they were deprived of food and oral glucose tolerance performed on blood samples from lateral tail vein after 30, 60, 90 and 120 min of oral glucose load (2 g/kg body weight).

Biochemical parameters

Animals were sacrificed after 12 h from the last administered dose by rapid decapitation and blood glucose level determined using test strips of a glucometer (One Touch Ultra, LifeScan, Milpitas, California, USA). Serum cholesterol, HDL, LDL and VLDL were determined. Aspartate aminotransferase (ASAT), alanine amino-transferase (ALAT) and alkaline phosphatase (ALP) activities were determined using DiaSys Diagnostic kit. Creatinine was determined using Spinreact kit and serum uric acid assayed enzymatically using DiaSys Diagnostic kit.

Statistical analysis

Reported values were represented as means ± standard error (SE). Statistical analysis was evaluated by one-way analysis of variance (ANOVA) with least significant difference (LSD) post hoc test and comparisons were performed to assess the significance of differences among various groups at $p<0.05$ using Statistical Package for Social Science "SPSS" for Windows software, Release 20.0 (SPSS, Chicago, IL).

RESULTS

Effect of high fat diet on serum lipid parameters

Data presented in Figure 2A showed significant increase in serum TC, TG, LDL and VLDL as compared to the corresponding control values recording 25.70, 24.05, 185.19 and 24.04% as percentage difference from control value, respectively. Meanwhile, HDL is not statistically different with a decrease (-11.82%) as compared to control value.

Effect of treatments on body weight

Results in Figure 2B showed significant increase in the body weight of all groups as compared to their corresponding initials. Diabetic and treated groups from the 4th week exhibited significant decrease in the body weight as compared to control values until the 8th week of the experiment. Treatment with MET was recorded significant decrease in the body weight as compared to D group or D+CAN group in the corresponding period,

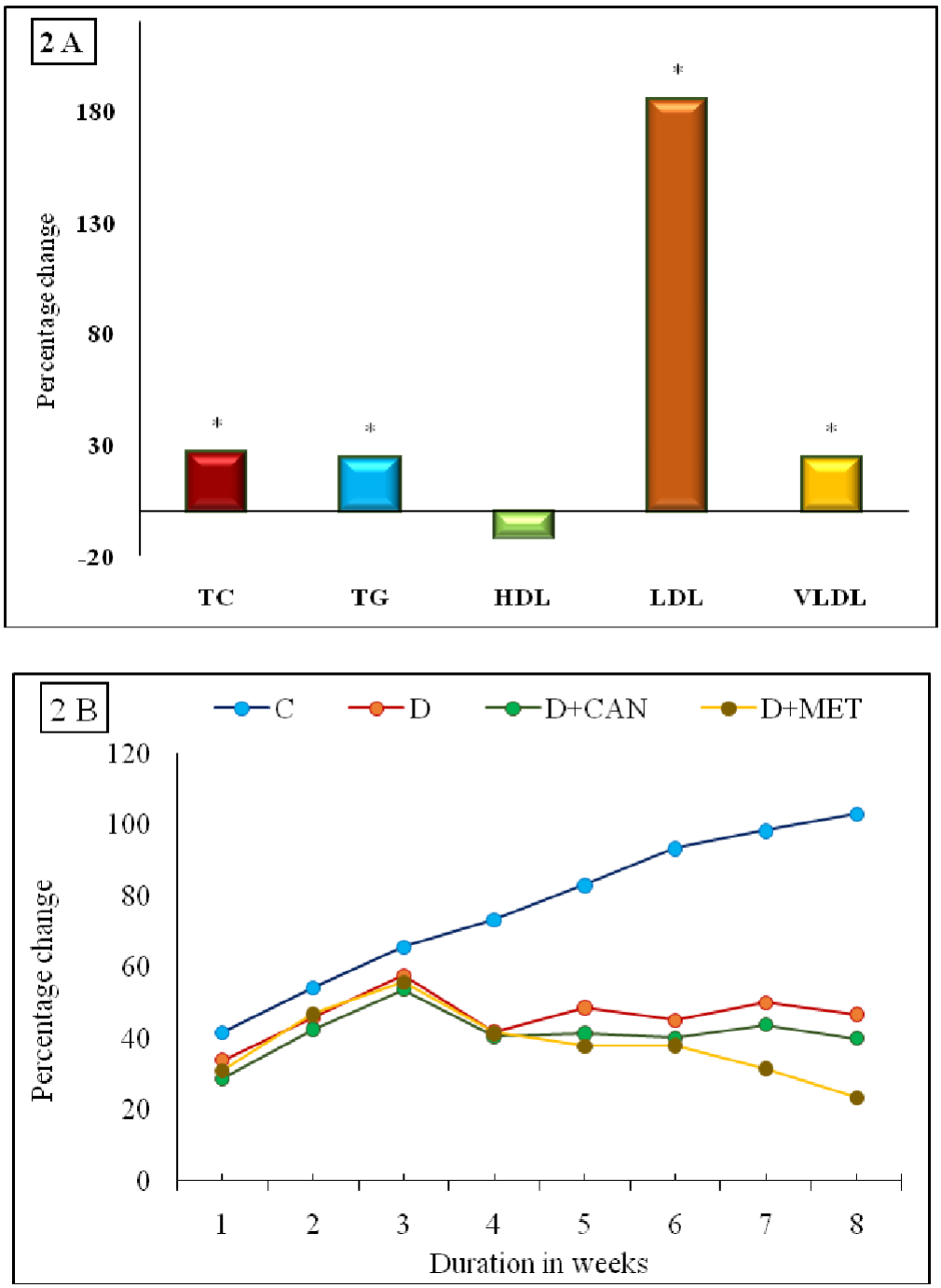

Figure 2. A: Effect of high fat diet on percentage change from control of serum lipid parameters. *Significance level of 0.05 from control. B: Percentage change of body weight gain from initial experimental groups, normal control (C), diabetic (D), diabetic treated with canagliflozin (D+CAN) and diabetic treated with metformin (D+MET).

especially at the last two weeks.

Effect of treatments on oral glucose tolerance test (OGTT)

The oral glucose tolerance results are as shown in Figure 3. Fasting glucose levels of normal rats reached their peak values at 60 min following glucose intake. The glucose levels recorded in diabetic group was significantly elevated as compared to the control ones from fasting until 2 h of glucose estimation, representing peak values at 60 min following glucose. Data about D+CAN groups in the two time intervals were significantly

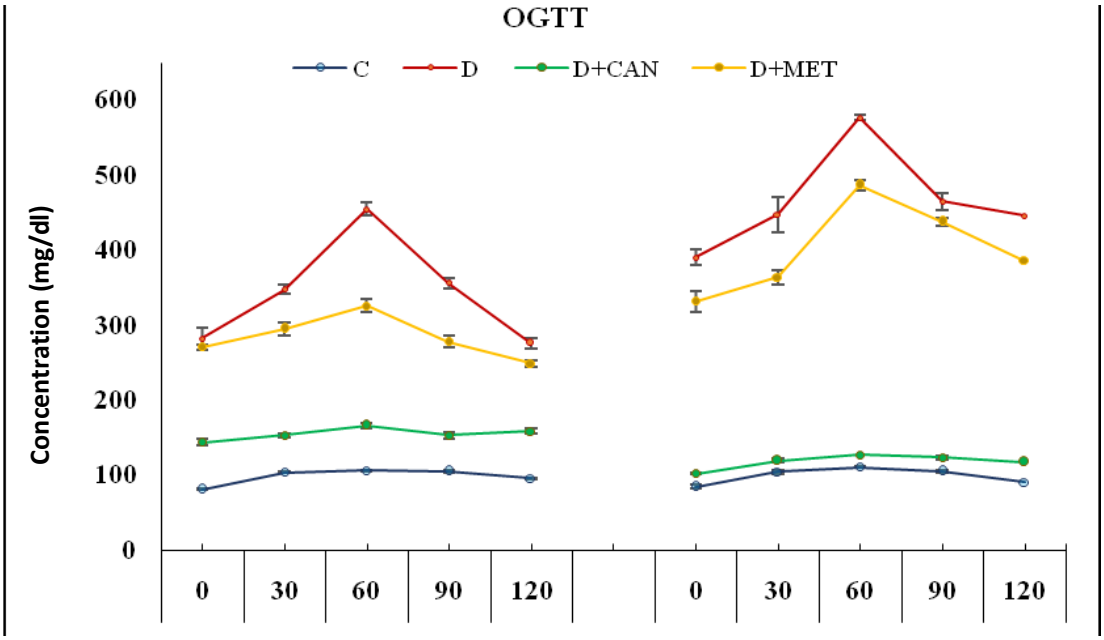

Figure 3. The oral glucose tolerance curve after two and four weeks of treatment in control (C), diabetic (D), diabetic treated with canagliflozin (D+CAN) and diabetic treated with metformin (D+MET) at different time interval. Data expressed as mean ± SE.

Table 1. Effect of treatments on serum lipid profile.

Duration	Group	TC	TG	HDL	LDL	VLDL
2 weeks	C	102.44±2.18	99.53±2.37	68.40±1.65	14.13±1.14	19.91±0.47
	D	147.59±3.01*	141.11±2.09*	50.89±1.81*	68.48±3.63*	28.22±0.42*
	D+CAN	138.73±2.83*^{a}	124.76±3.72*^{a}	48.12±1.73*	65.67±3.96*	24.95±0.74*^{a}
	D+MET	118.18±4.62*^{ab}	122.34±4.95*^{a}	56.86±2.95*^{ab}	36.85±7.58*^{ab}	24.47±0.99*^{a}
4 weeks	C	101.40±1.24	94.13±1.91	68.71±1.16	14.51±0.97	18.83±0.38
	D	157.17±2.32*$^{\#}$	156.50±2.35*$^{\#}$	33.79±1.60*$^{\#}$	92.07±2.58*$^{\#}$	31.53±0.47*$^{\#}$
	D+CAN	132.02±1.95*^{a}	132.67±3.73*^{a}	37.90±1.52*	67.59±1.89*^{a}	26.53±0.75*^{a}
	D+MET	124.78±3.38*^{a}	112.90±2.82*^{ab}	40.70±2.02*^{a}	61.50±5.15*^{a}	22.58±0.56*^{ab}

Data expressed as Mean ± SE. (n=6). One Way analysis performed between groups. Multiple range Duncan test with significance level 0.05. Significant indicated by asterisk (*) as compared to control, (a) as compared to diabetic group (D), (b) as compared to D+CAN group within the same duration of treatment. $^{\#}$Significance between two diabetic non-treated groups.

lower than that of the D group throughout all the detected times for glucose level. Meanwhile, data about D+MET groups in the two time intervals was significantly higher than that of D+CAN and significantly lower than that of the D groups throughout all the detected times of glucose level.

Effect of treatments on serum lipid profile

Data in Table 1 showed that D group recorded significant increase in the serum TC, TG, LDL, and VLDL and significant decrease in HDL as compared to the control level after two and four weeks with significance between results of the 2nd and 4th week of diabetes. Treatment with CAN achieved significant decrease in TC, TG, and VLDL as compared to the corresponding D group values after two and four weeks of treatment. However, LDL was significantly decreased in D+CAN group after the 4th week of treatment compared to the corresponding D group value. Treatment with MET achieved significant reduction in TC, TG, LDL and VLDL as compared to the corresponding D group values in the two intervals. Meanwhile, D+MET group exhibited significant increase in HDL as compared to the corresponding D group value after two and four weeks of treatment. MET treated group

Table 2. Effect of treatments on kidney and liver functions parameters.

Duration	Group	Creatinine	Urea	Uric acid	ASAT	ALAT	ALP
2 weeks	C	0.88±0.2	20.86±0.97	4.76±0.11	19.50±0.92	16.80±0.53	105.17±4.21
	D	1.46±0.09*	40.39±1.54*	5.68±0.15*	48.33±1.43*	30.33±0.56*	216.17±6.52*
	D+CAN	1.04±0.02*^{a}	30.43±1.46*^{a}	4.37±0.07^{a}	35.83±1.33*^{a}	26.33±0.88*^{a}	149.50±5.57*^{a}
	D+MET	0.97±0.04^{a}	25.17±1.18*^{ab}	4.75±0.14^{a}	26.67±0.61*^{ab}	28.50±1.51*	142.50±3.10*^{a}
4 weeks	C	0.97±0.01	17.52±0.85	4.40±0.19	23.33±0.92	16.50±0.76	102.77±3.75
	D	1.41±0.03*	42.23±1.25*	8.07±0.31*$^{\#}$	54.67±1.28*$^{\#}$	36.25±0.67*$^{\#}$	229.67±8.95*
	D+CAN	1.11±0.01*^{a}	25.12±0.69*^{a}	5.42±0.23*^{a}	34.50±1.10*^{a}	35.67±0.73*	178.67±6.46*^{a}
	D+MET	1.28±0.03*^{ab}	22.47±0.79*^{a}	5.13±0.19*^{a}	29.75±0.98*^{ab}	31.56±0.73*^{ab}	176.67±5.28*^{a}

Data expressed as Mean ± SE (n=6). One Way analysis performed between groups. Multiple range Duncan test with significance level 0.05.
*Significant as compared to control, (a) as compared to diabetic group (D), (b) as compared to D+CAN group within the same duration of treatment.
$^{\#}$Indicate significance between two diabetic non-treated groups.

exhibited significant reduction in TC, TG, and LDL level as compared to that recorded for the D+CAN group after two weeks of treatments and significant decrease was observed in VLDL level as compared to D+CAN group at the 4th week. D+MET group exhibited significant increase in the level of HDL as compared to that of the D+CAN at 2nd week.

Effect of treatments on kidney and liver functions parameters

Data in Table 2 showed significant increase in the serum creatinine, urea, uric acid levels and ASAT, ALAT and ALP enzymes activity in diabetics from the corresponding control values after two and four weeks, respectively. Results of diabetic group at the 4th week showed significant increase in uric acid contents and ASAT and ALAT enzymes activities as compared to that recorded at the 2nd week of diabetes induction. Treatment with CAN and MET achieved significant reduction in the aforementioned parameters except for ALAT as compared to the corresponding diabetic group values. MET treated group exhibited significant increase in creatinine level as compared to that recorded for the D+CAN group after four weeks of treatments and significant decrease was observed in urea level as compared to D+CAN group after two weeks. In the meantime, MET treated group exhibited significant decrease in the ASAT activities as compared to D+CAN group after two and four weeks of treatments and in ALAT activities as compared to D+CAN group after four weeks.

DISCUSSION

The study investigated the fatty acid composition of butter sample showing high content of SFA, lauric (C12:0), myristic C14:0 and palmitic C16:0 and a higher content of MUFA oleic acid C18:1 which in line with previous investigations of butter samples (Ledoux et al., 2005; Rutkowska and Adamska, 2011). The body weight significant increment was expected due to the imbalance in energy homeostasis developed in HFD fed rats, which is consistent with previous studies demonstrating that fat accumulation is greater when more energy comes from dietary fat than from carbohydrate or protein (Woods et al., 2003). The increment in lipid parameters over normal values induced due to the butter consumption in rats support the influence of SFAs on serum TC, TG and lipoprotein metabolism previously discussed (Ledoux et al., 2005; Prasanna and Narsimha, 2014). Lin et al. (2005) showed that diets rich in SFAs might mediate their hyperlipidemic effects in mice via peroxisome proliferator-activated receptor γ coactivator-1β (PGC-1β). PGC-1β expression greatly increased in cultured rat primary hepatocytes treated with individual SFAs, particularly after incubation with palmitic acid. Results of our rat model is in line with previous studies that reported that rats fed with HFD are already mildly hyperglycemic and more susceptible to develop significant hyperglycemia and hyperlipidemia with low doses of STZ, which mimics the human T2DM (Reed et al., 2000; Srinivasan et al., 2005; Bansal et al., 2012). As predicted in our study, previously, rat diabetic model showed significant increase in measures for oxidative stress metabolite, liver and kidney functions (Sahin et al., 2007). Rat treatment with HFD and small dose of STZ showed significant increase in the insulin resistance index, and significant decrease in insulin-sensitivity index as compared to normal control rats indicating the development of insulin resistance in the diabetic rat model like in our model, which showed impairment in glucose tolerance (Sasidharan et al., 2013). Randle et al. (1963) suggested the role of elevated free fatty acid (FFA) availability and insulin resistance and speculated that high plasma concentration of FFA is one of the common characteristics in patients with either diabetes or other carbohydrate disorders. This hypothesis was supported by studies which established that insulin resistance could induced within hours through

lipid infusion or weeks through a high fat feeding regimen (Randle et al., 1963, 1964). Rats fed with high fat diet developed insulin resistance by different mechanisms mainly through Randle or glucose-fatty acid cycle (Zhang et al., 2003). STZ destroys beta cells that increased blood glucose level and decreased insulin secretion. In addition, STZ induces DNA methylation in beta cells that lead to beta cell death and protein methylation that lead to beta cells dysfunction as well as other deleterious effects by means of the production of ROS (Eleazu et al., 2013).

CAN significantly lowered body weight and the most likely mechanism of weight loss is the caloric loss related to renal glucose wasting (Yang et al., 2014). Polidori et al. (2013) concluded that CAN 300 mg reduces postprandial plasma glucose and insulin concentration in healthy subjects by two distinct mechanisms first by increasing urinary glucose excretion (UGE) due to renal SGLT2 inhibition and secondly by delaying the rate of end, which is likely due to transient intestinal SGLT1 inhibition. The inhibition of SGLT2 by CAN provides a novel mechanism for control of hyperglycemia in patients with diabetes, enhancing urinary glucose excretion and promoting a natriuretic and diuretic action (Komala et al., 2013).

This unique mechanism of action results in decreased blood glucose levels (via glucose excretion) and weight loss via caloric deficit (Nisly et al., 2013). CAN monotherapy reported on the improvement of fasting lipid values (HDL-C and TG), while LDL-C values increased modestly and the decreasing effect on uric acid as compared to placebo value (Stenlöf et al., 2013). CAN monotherapy provides improvement in weight, blood pressure, and lipid parameters (Vivian, 2014; Scheen, 2015). Liang et al. (2012) suggested about reduced body weight gain in CAN -treated animals obesity rodent models by increased fatty acid metabolism and/or reduced *de novo* lipogenesis. The mechanism by which CAN reduces serum uric acid was postulated to involve the renal SLC2A9 transporter, which, is known to exchange glucose for uric acid and this potential mechanism is supported by evidence of trans-stimulation of uric acid efflux with high glucose concentrations in Xenopus oocytes expressing SLC2A9b (Chino et al., 2014). SGLT2 inhibitors proposed may improve glomerular filtration rate and might possibly protect long-term renal function (Stanton, 2014).

The results about liver and kidney functions showed improvement and consistent with previous studies (Inagaki et al., 2015; Ji et al., 2015). Meanwhile increasing severity of renal impairment, requiring dosage adjust-ments or restrictions with moderate-to-severe renal dysfunction in addition to cautions in liver function have been discussed (Halimi and Vergès, 2014).

MET is widely used for the treatment of diabetes and studies on rat diabetic models created as MET treatment (200 mg/kg) for 4 weeks in rat model of HFD+STZ diabetes induced significant reduction of body weight and glucose level in OGTT test and serving significant improvement in insulin resistance (Saad et al., 2015). MET treatment (180 mg/kg) for three weeks significantly reduced body weight, fasting blood glucose and excursion (OGTT) as well as significant reduction in serum lipid profile in high fat diet and low dose STZ - induced T2DM in rats (Bhandari et al., 2013).

The weight loss effect of MET may be due to the improvement of the metabolic changes in diabetic patients with decreased synthesis and increased clearance of VLDL as described by Wiernsperger and Bailey (1999). Lobato et al. (2012) recorded that MET treatment decreased cholesterol, TG and LDL and increased HDL levels, which disturbed in monosodium glutamate-induced obese non-diabetic rats to restore the normal values. Zhou et al. (2001) found out that the major cellular regulator of lipid and glucose metabolism 5' adenosine monophosphate-activated protein kinase (AMPK) activation is required for MET's inhibitory effect on glucose production by hepatocytes.

Geerling et al. (2014) discussed the importance of brown adipose tissue (BAT) in the lipid-lowering action and weight loss effect of MET by lowering plasma TG, through a selective BAT-mediated increase in VLDL-TG uptake/lipolysis. The mechanism postulated by increased AMP-activated protein kinase α1 (AMPKα1) expression and activity of the lipolytic enzyme hormone sensitive lipase (HSL) and mitochondrial content in BAT.

MET at a dose of 125 mg/kg restored the levels of ALAT, ASAT and ALP in rats chemically induced hepatocarcinogenesis (Afzal et al., 2012). A novel mechanism reported about MET may protect against apoptosis, involving the induction of hemeoxygenase-1 (HO-1) and bcl-xl expression and the reduction of mitogen-activated protein kinase (JNK) activation and the modulation of JNK activity represents a target for the treatment of these disorders (de la Rosa et al., 2015). MET ameliorates functional defects, activation of caspases and apoptosis in pancreatic islets from type 2 diabetic patients (Marchetti et al., 2004).

The study suggests that increase consumption of butter prompted obesity and the risk of insulin resistance and treatment with either CAN or MET can reduce the body weight, blood glucose level, resist the lipid metabolism disturbance by different mechanisms and improve liver and kidney functions of the obese T2DM rat model. This with the advantage of MET on body weight loss and HDL and TG improvement effect over that of CAN treatment and the advantage of CAN in blood glucose tolerance due to the higher amount of glucose to be excreted within the urine. Long-term studies should be conduct to evaluate the efficacy and safety of CAN.

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Conflict of interests

The authors have not declared any conflict of interests

REFERENCES

Afzal M, Kazmi I, Gupta G, Rahman M, Kimothi V, Anwar F (2012). Preventive effect of Metformin against N-nitrosodiethylamine-initiated hepatocellular carcinoma in rats. Saudi Pharm. J. 20(4):365-370.

Badran M, Laher I (2012). Type II Diabetes Mellitus in Arabic-Speaking Countries. Int. J. Endocrinol. 11 p.

Bansal P, Paul P, Mudgal J, Nayak PG, Pannakal ST, Priyadarsini KI, Unnikrishnan MK (2012). Antidiabetic, antihyperlipidemic and antioxidant effects of the flavonoid rich fraction of Pileamicrophylla (L.) in high fat diet/streptozotocin-induced diabetes in mice. Exp. Toxicol. Pathol. 64(6):651-658.

Bhandari U, Chaudhari HS, Khanna G, Najmi AK (2013). Antidiabetic effects of Embeliaribes extract in high fat diet and low dose streptozotocin-induced type 2 diabetic rats. Front Life Sci. 7(3-4):186-196.

Chino Y, Samukawa Y, Sakai S, Nakai Y, Yamaguchi J, Nakanishi T, Tamai I (2014). SGLT2 inhibitor lowers serum uric acid through alteration of uric acid transport activity in renal tubule byincreased glycosuria. Biopharm. Drug Dispos. 35(7):391-404.

de la Rosa LC, Vrenken TE, Buist-Homan M, Faber KN, Moshage H (2015). Metformin protects primary rat hepatocytes against oxidative stress-induced apoptosis. Pharm. Res. Perspect. 3(2):e00125.

Eleazu CO, Eleazu KC, Chukwumam S, Essien UN (2013). Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J. Diabetes Metab. Disord. 12(1):60.

Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the ultracentrifuge. Clin. Chem. 18:499-502.

Geerling JJ, Boon MR, van der Zon GC, van den Berg SA, van den Hoek AM, Lombès M, Princen HM, Havekes LM, Rensen PC, Guigas B (2014). Metformin lowers plasma triglycerides by promoting VLDL-triglyceride clearance by brown adipose tissue in mice. Diabetes 63(3):880-91.

Guidance for Industry and Reviewers (2002). Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers. Food Drug Adm. pp. 1-26.

Halimi S, Vergès B (2014). Adverse effects and safety of SGLT-2 inhibitors. Diabetes Metab. 40(6-1):28-34.

Inagaki N, Kondo K, Yoshinari T, Kuki H (2015). Efficacy and safety of canagliflozin alone or as add-on to other oral antihyperglycemic drugs in Japanese patients with type 2 diabetes: A 52-week open-label study. J. Diabetes Invest. 6(2):210-218.

Ji L, Han P, Liu Y, Yang G, Dieu Van NK, Vijapurkar U, Qiu R, Meininger G (2015). Canagliflozin in Asian patients with type 2 diabetes on metformin alone or metformin in combination with sulphonylurea. Diabetes Obes. Metab. 17(1):23-31.

Komala M, Panchapakesan U, Pollock C, Mather A (2013). Sodium glucose cotransporter 2 and the diabetic kidney. Curr. Opin. Nephrol. Hypertens 22(1):113-119.

Ledoux M, Chardigny JM, Darbois M, Soustre Y, Sébédio JL, Laloux L (2005). Fatty acid composition of French butters, with special emphasis on conjugated linoleic acid (CLA) isomers. J. Food Compost. Anal. 18:409-425.

Liang Y, Arakawa K, Ueta K, Matsushita Y, Kuriyama C, Martin T, Du F, Liu Y, Xu J, Conway B, Conway J, Polidori D, Ways K, Demarest K (2012). Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models. PLoS One 7(2):e30555.

Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, Yang W, Pei L, Uldry M, Tontonoz P, Newgard CB, Spiegelman BM (2005). Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. Cell 120(2):261-273.

Lobato NS, Filgueiram FP, Hagihara GN, Akamine EH, Pariz JR, Tostes RC, Carvalho MH, Fortes ZB (2012). Improvement of metabolic parameters and vascular function by metformin in obese non-diabetic rats. Life Sci. 90(5-6):228-235.

Luis-Rodríguez D, Martínez-Castelao A, Górriz JL, De-Álvaro F, Navarro-González JF (2012). Pathophysiological role and therapeutic implications of inflammation in diabetic nephropathy. World J. Diabetes 3(1):7-18.

Marchetti P, Del Guerra S, Marselli L, Lupi R, Masini M, Pollera M, Bugliani M, Boggi U, Vistoli F, Mosca F, Del Prato S (2004). Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. J. Clin. Endocrinol. Metab. 89(11):5535-5541.

Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B (2009). Medical management of hyperglycaemia in type 2 diabetes mellitus: a consensus algorithm for the initiation and adjustment of therapy. A consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 32(1):193-203.

Nisly SA, Kolanczyk DM, Walton AM (2013). Canagliflozin, a new sodium-glucose cotransporter 2 inhibitor, in the treatment of diabetes. Am. J. Health Syst Pharm. 70(4):311-319.

Polidori D, Sha S, Mudaliar S, Ciaraldi TP, Ghosh A, Vaccaro N, Farrell K, Rothenberg P, Henry RR (2013). Canagliflozin Lowers Postprandial Glucose and Insulin by Delaying Intestinal Glucose Absorption in Addition to Increasing Urinary Glucose Excretion: Results of a randomized, placebo-controlled study. Diabetes Care 36(8):2154-2161.

Prasanna K, Narsimha R (2014). Evaluation of clarithromycin pharmacokinetics after single and repeated oral administration of atorvastatin in hyperlipidemicWistar rats. Afr. J. Pharm. Pharmacol. 8(15):399-407.

Randle P, Garland P, Hales C, Newsholme E (1963).The glucose fatty-acid cycle its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 281(7285):785-789.

Randle P, Newsholme E, Garland P (1964). Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. Biochem. J. 93(3):652-665.

Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, Reaven GM (2000). A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism 49(11):1390-1394.

Reeves PG, Nielsen FH, Fahey GCJr (1993). AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J. Nutr. 123(11):1939-1951.

Rutkowska J, Adamska A (2011). Fatty Acid Composition of Butter Originated from North-Eastern Region of Poland. Pol. J. Food Nutr. Sci. 61(3):187-193.

Saad M, Kamel M, Hanafi M (2015). Modulation of Adipocytokines Production and Serum NEFA Level by Metformin, Glimepiride, and Sitagliptin in HFD/STZ Diabetic Rats. Biochem Res Int. 7p.

Sahin K, Onderci M, Tuzcu M, Ustundag B, Cikim G, Ozercan IH, Sriramoju V, Juturu V, Komorowski JR (2007). Effect of chromium on carbohydrate and lipid metabolism in a rat model of type 2 diabetes mellitus: the fat-fed, streptozotocin-treated rat. Metabolism 56(9):1233-1240.

Sasidharan S, Joseph J, Anandakumar S, Venkatesan V, AriyattuMadhavan C,Agarwal A (2013). An experimental approach for selecting appropriate rodent diets for research studies on metabolic disorders. Biomed Res Int. 2013:752870.

Scheen AJ. (2015). Pharmacodynamics, efficacy and safety of sodium-glucose co-transporter type 2 (SGLT2) inhibitors for the treatment of type 2 diabetes mellitus. Drugs 75(1):33-59.

Srinivasan K, Viswanad B, Asrat L, Kaul C, Ramarao P (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol. Res. 52(4):313-320.

Stanton R (2014). Sodium Glucose Transport 2 (SGLT2) Inhibition Decreases Glomerular Hyperfiltration: Is There a Role for SGLT2 Inhibitors in Diabetic Kidney Disease? Circulation 129(5):542-544.

Stenlöf K, Cefalu WT, Kim KA, Alba M, Usiskin K, Tong C, Canovatchel

W, Meininger G (2013). Efficacy and safety of canagliflozinmonotherapy in subjects with type 2 diabetes mellitus inadequately controlled with diet and exercise. Diabetes Obes. Metab. 15(4):372-382.
UK Prospective Diabetes Study (UKPDS) Group (1998). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 352(9131):854-865.
Vivian E (2014). Sodium-glucose co-transporter 2 (SGLT2) inhibitors: a growing class of antidiabetic agents. Drugs Context 3:212264.
Wiernsperger N, Bailey C (1999). The antihyperglycaemic effect of metformin: therapeutic and cellular mechanisms. Drugs 58(1):31-39.
Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P (2003). A controlled high-fat diet induces an obese syndrome in rats. J. Nutr. 133(4):1081-1087.
Yang XP, Lai D, Zhong XY, Shen HP, Huang YL (2014). Efficacy and safety of canagliflozin in subjects with type 2 diabetes: systematic review and meta-analysis. Eur. J. Clin. Pharmacol. 70(10):1149-1158.
Zhang F, Ye C, Li G, Ding W, Zhou W, Zhu H, Chen G, Luo T, Guang M, Liu Y, Zhang D, Zheng S, Yang J, Gu Y, Xie X, Luo M (2003). The rat model of type 2 diabetes mellitus and its glycometabolism characters. Exp. Anim. 52(5):401-407.
Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE (2001). Role of AMP-activated protein kinase in mechanism of metformin action. J. Clin. Invest. 108(8):1167-1174.