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Assessment of the Protective Effect of Resveratrol and Short Term Caloric Restriction on Diabetic Nephropathy in Rats Via SIRT1/AMPK Pathway

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Abstract

Background: Diabetic nephropathy (DN) has been recognized as the leading cause of end-stage renal disease. Resveratrol, a polyphenolic compound, has potential role in diabetes mellitus patients. Caloric restriction (CR) has been shown to reduce the incidence diabetes mellitus (DM) complications.

Aim of Study: This study aims to assess the protective effect of resveratrol (RSV) and short term caloric restriction (CR) on diabetic nephropathy.

Material and Methods: Forty eight rats were divided into control group, diabetic group, Resveratrol group administered RSV 5mg/kg/day orally for 2 months after induction of diabetes, Diet restriction group subjected to 40% Caloric restriction (CR) for 2 months after induction of diabetes. Serum levels of (glucose, insulin, lipid profile, creatinine and urea with 24h micro albuminuria) in addition to assessment of HOMA-IR, sirtuin1 activity, adenosine monophosphate - activated protein kinase (AMPK) and superoxide dismutase (SOD) levels in the kidney with histopathological examination for kidney tissues.

Results: Significant improvement in all parameters in resveratrol group and caloric restriction group compared to diabetic group. Also a Significant increase in SIRT1 activity, AMPK, SOD levels in kidney tissues in resveratrol group and caloric restriction groups compared to diabetic. Pathological examination of kidney tissues in diabetic group showed deterioration in renal tissue. In resveratrol and caloric restriction groups results showed normal structure of glomerular tufts with mild vascular degeneration. Regarding renal tubule there was no protonus cast in the lumen and mild thickening of the glomerular basement membrane.

Conclusion: The SIRT1 activators like resveratrol and CR are of considerable value in protecting kidney from complication of diabetes.

Key Words: *Diabetic nephropathy – Resveratrol – Caloric restriction – SIRT1.*

Introduction

PREVALENCE of type 2 diabetes mellitus (T2DM) has increased dramatically over the past four decades. T2DM is characterized by insulin resistance, hyperinsulinemia and hyperglycemia **[1]**. Diabetes is the major cause of chronic kidney disease which in turn may lead to end-stage renal disease (ESRD) ending up in dialysis. Hemodynamic and structural changes following diabetes are working together in the process of development of diabetic nephropathy **[2]**. While still considered a microvascular complication of diabetes, nephropathy involves more than just kidney capillaries, extending its damage across the various kidney cells and associated extracellular structures **[3]**. The exact cause of diabetic nephr opathy is unknown but various mechanisms are considered such as altered renal hemodynamics, hyperglycemia, advanced glycation end products, activation of cytokines, oxidative stress and inflammation **[4]**. It was by **[5]** shown that SIRT1 is closely related to the occurrence and development of DN. It was revealed by **[6]** that four single nucleotide polymorphisms (SNPs) in SIRT1 are associated with DN. SIRT1 is one of the seven mammalian sirtuins, NAD-dependent deacetylases **[7]** and it regulates various biological functions in several tissues, including cell survival, mitochondria biogenesis **[8]**, insulin secretion **[9]**, and glucose/lipid metabolism **[10]**. The activation of SIRT1 exerts cytoprotective effects through multiple mechanisms, such as antiapoptosis, antioxidative, and antiinflammatory effects and the regulation of mitochondrial biogenesis, autophagy, and metabolism in response to the cellular energy and redox status **[11]**. Considering the previously reported role of SIRT1 in kidney disease, it may become a new therapeutic

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target of kidney disease including DN [12]. Caloric restriction (CR) delays the onset of numerous age-associated diseases including cancer, atherosclerosis, and diabetes, and can greatly increase lifespan [13]. The beneficial effects of CR involve the function of the NAD+-dependent deacetylase, Sirt1, the expression of which is induced by CR. AMPK is another cellular energy sensor that is activated by calorie restriction [14]. Resveratrol is SIRT1 activators and can prevent many diseases, such as diabetes, neurodegenerative disorders, cognitive disorders, cancer and cardiovascular disease through SIRT1 activation [11]. As diabetic nephropathy is one of the common diabetic complications and since amelioration of diabetic nephropathy at the beginning it prevents further renal damage, we aimed in our study to find out the possible mechanisms to stop renal complications of diabetes. Resveratrol is well known to have cytoprotective effects through at least two mechanisms, also it is available and low cost natural medication [11]. The aim of the present study is to investigate for the protective effect of resveratrol and short term caloric restriction in attenuation of diabetic nephropathy in rats.

Material and Methods

Experimental animals & groups:

Forty eight male albino rats, approximately 8 weeks of age and weights ranging from 160-200 gram were included in the study. The animals were purchased and placed under ordinary living conditions in the animal house of Faculty of Medicine Cairo University September 2017. They were housed in wire mesh cages at room temperature and had free access to food and water for 2 weeks. Rats were kept under the same environmental conditions. All experimental procedures were accepted by Ethics Committee of Cairo University.

Animals were randomly divided into the following groups:

Control group (n=12):

The other 36 rats received intra-peritoneal injection of streptozotocin (35mg/kg) for induction diabetes after 2weeks of high fat diet [15].

Diabetic rats will be further subdivided into the following groups:

Diabetic group (DM) (n=12):

Diabetic + resveratrol (DM+RSV) group (n=12): Rats administrated orally by gavage 5mg/kg body weight of resveratrol (Sigma-Aldrich, USA) dissolved in distilled water once daily for two months after induction of diabetes [16].

Diabetic +caloric restriction (DM+CR) group (n=12): Rats were subjected to 40% caloric restriction program for 2 months after induction of diabetes [17].

Induction of diabetes: Diabetes was induced by feeding HFD (diet with 40% kcal fat) for two weeks followed by a single intra-peritoneal (I.P) injection of streptozotocin STZ 35mg/kg [15]. Rats were fasted for 12-h before diabetes was induced using STZ which was freshly dissolved in 0.05M citrate buffer, pH 4.5. For the intra peritoneal injection of STZ, the rat was held in one hand in dorsal position, the injection site was swabbed using povidone-iodine solution and the designated amount of STZ was injected in the caudal abdominal cavity [18]. Glucose was given to rats after STZ injection to avoid sever hypoglycemia.

Caloric restriction regimen:

The rats were assigned to either a freely eating (ad libitum) or short term CR groups. Food intake of the freely eating rats was measured every. Food intake of the freely eating rats was measured every other day for 2 weeks. Short term CR rats were given food equal to 60% of the average amount of food eaten by the freely eating controls for 2 months [17].

Experimental measurements:

During the experiment blood glucose level was measured after 10 days of STZ injection to check for diabetes and rats below 250mg/dl were excluded. At the end of the experiment 24h urine was collected then animals were sacrificed and fasting blood samples were withdrawn from abdominal aorta and kidneys were removed from all groups of animals then cut vertically as two halves. One half was put in tubes containing formalin (10%) in a ratio of 40% tissue to 60% formalin for pathological examination. The other half was stored at –80° C.

Biochemical analysis:

- Fasting serum glucose level: Was measured using biochemical kits supplied by “Diamond Diagnostics”.
- Fasting serum insulin level: Was measured by enzyme immunoassay using the rat insulin ELISA kits.
- Calculation of Insulin Resistance index (HOMA-IR) [19]: As the product of fasting serum insulin (μIU/L) and fasting serum glucose (mmol/L) divided by 22.5: in cases when HOMA-IR is more than 4.0 this is diagnostic of insulin resistance.

- Measurement of plasma total cholesterol (TC): The total plasma cholesterol was measured by quantitative - Enzymatic - Colorimetric determination of Total cholesterol in plasma.
- Determination of serum level of triglycerides **[20]**: The plasma triglyceride was measured by quantitative - enzymatic - colorimetric determination of triglycerides in serum.
- Calculation of plasma low density lipoproteins cholesterol (LDLC): LDL cholesterol was calculated according to the following equation **[21]**:

LDL Cholesterol (mg/dl) = Total Cholesterol - Triglycerides - HDL Cholesterol

- Determination of serum level of high density lipoproteins cholesterol (HDLC) **[22]**: HDL-Cholesterol is obtained through selective precipitation of LDL and VLDL lipoproteins, thus HDL lipoproteins remain in solution.

Measurement of serum creatinine: Serum creatinine was estimated by QuantiChromTMcreatinine Assay Kit **[23]**.

Measurement of blood urea: Serum urea was estimated by QuantiChromTM Urea Assay kit (DIUR-500) **[24]**.

Estimation of urinary microalbumin: This was done by Enzyme-Linked Immunosorbent assay (ELISA) kit provided by ALPCO diagnostics **[25]**.

Determination of SIRT1 and AMPK (adenosine monophosphate-activated protein kinase) activity in the kidney (ng/dl): SIRT1 activity was assessed by Fluorometric Assay Kit **[26]**.

Determination of kidney superoxide dismutase activity: Superoxide dismutase was assayed in kidney homogenate according to the method **[27]**.

Histopathological examination of kidney: All specimens were collected and preserved in ice box and send to laboratory where they preserved in 10% formalin solution for at least 48hrs before preparation for histopathological processing ,then after fixation, the samples were washed in running water, dehydrated in graduated ethanol 50%, 70%, 95% and 100% 2hrs for each. Then the samples were cleared in 2 changes of xylene 2hrs. for each and embedded in paraffin wax at 70 C (3 changes 2hrs for each). The samples were blocked in paraffin wax and underwent microtomy. Five microns tissue sections were mounted on clean glass slides and stained with Hematoxylin and Eosin stain according to **[28]**.

Statistical methods:

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22. Data was summarized using mean ± standard deviation. Comparisons between quantitative variables were done using one way analysis of variance (ANOVA) with post hoc Tukey test. *p*-values less than 0.05 were considered as statistically significant.

Results

Fig. (1) showed that fasting blood glucose (mmol/L), blood insulin (µIU/L) and (HOMA) levels were significantly increased ($p<0.05$) by 148.64% ,72.77% and 347.39% in diabetic group compared to control group. Mean values in diabetic group were 15.49±3.16, 15.67±3.81 and 11.14± 4.31 versus 6.23±0.83, 9.07±1.06 and 2.49±.10 in control group respectively.

As depicted from Fig. (2) that TG, TC and LDLC levels (mg/dl) were significantly increased ($p<0.05$) in diabetic group by 57.16%, 49.17% and 137.35% respectively compared to control group. Mean values oh TC were 145.01±14.17, 229.28± 21.27and 168.07±21.34 in diabetic group versus 92.27±13.51, 153.70±16. 08 and 70.81±17.02 in control group respectively, while HDLC (mg/dl) was significantly decreased ($p<0.05$) by 50.02% in diabetic group compared to control group. On the other hand mean value of LDLC was 32.20± 3.93 in diabetic group versus 64.43±4.70 in control group respectively.

As shown in Fig. (3) that urea, creatinine (mg/dl) levels and 24h microalbuminuria were significantly increased ($p<0.05$) in diabetic group by 137.35%, 212.78% and 1094.1% respectively compared to control group. Mean values were 102.94±15.50, 4.16 ±1.29 and 200.25±34.35 in diabetic group versus 43.37±12.38, 1.33±.49 and 16.77±3.30 in control group respectively.

On assessment the mechanistic of diabetic nephropathy, it was shown in Fig. (4) that SIRT 1activity, AMPK and SOD were significantly decreased ($p<0.05$) in diabetic group by 78.64%, 75% and 89.23% respectively compared to control group. Mean values were 0.22±0.10, 0.26±0.14 and 0.32±0.15 in diabetic group versus 1.03±0.03, 1.04±0.03 and 12.97±0.74 in control group respectively.

Effect of resveratrol on blood glucose (mmol/L), blood insulin levels (µIU/L) and HOMA-IR in diabetic male rats.

In Fig. (1) diabetic rats that was given taken resveratrol had significant ($p<0.05$) improvement regarding fasting serum glucose (mmol/L), serum insulin (μIU/mL) levels and HOMA-IR. Fasting serum glucose (mmol/L), serum insulin (μIU/mL) levels and HOMA were significantly decreased by 37.25%, 31.21 %, and 58.62% in respectively compared to Diabetic. Mean values of Insulin levels in Diabetic + resveratrol were 9.72 ±1.35#, 10.78± 1.91# and 4.61 ±0.80# versus 15.49±3.16, 15.67±3.81 and 11.14±4.31 in diabetic respectively. Serum glucose, serum insulin levels and HOMA levels were insignificantly increased in Diabetic + resveratrol compared to control with mean values 9.72±1.35#, 10.78±1.91# and 4.61 ±0.80# in Diabetic + resveratrol versus 6.23 ±0.83, 9.07±1.06 and 2.49±.10 in control respectively.

Effect of resveratrol on TG,TC, LDLC and HDLC (mg/dl) in diabetic male rats.

As shown in Fig. (2) diabetic rats that were given resveratrol showed significant decrease ($p<0.05$) in TG, TC and LDLC levels (mg/dl) by 27.58%, 16.35% and 25.91% respectively compared to diabetic group. Mean values in Diabetic + Resveratrol group were 105.01 ±10.68, 191, 80±7.84 and 124, 52±9.52 and 46.28±5.70 versus .01± 14.17, 229.28±21.27 and 168.07±21.34 in diabetic. Meanwhile HDLC level (mg/dl) was significantly increased ($p<0.05$) by 43.73% in Diabetic + Resveratrol compared to diabetic with mean value 46.28±5.70 versus 32.20±3.93 respectively.

However, TC and LDLC levels were significantly increased but TG was insignificantly increased in Diabetic + resveratrol compared to control with mean values 191,80±7.84, 124, 52±9.52 and 105.01±10.68 in diabetic + resveratrol versus 153.70±16. 08, 70.81±17.02 and 92.27± 13.51 in control group while HDLC level (mg/dl) was significantly decreased ($p<0.05$) in Diabetic + resveratrol compared to control group with mean value 46.28±5.70 in Diabetic + resveratrol versus 64.43±4.70 in control group.

Effect of resveratrol on serum creatinine, blood urea and 24h microalbumenaria (mg/dl) in diabetic male rats.

As shown in Fig. (3) diabetic + resveratrol group should significant decreases ($p<0.05$) regarding serum urea, creatinine (mg/dl) levels and 24h microalbuminuria by 37.64%, 51.2% and 52.99% in diabetic + resveratrol compared to diabetic group with mean values 64.19±9.57, 2.03±0.84 and 94.13±16.65 in diabetic + resveratrol versus 102.94±15.50, 4.16±1.29 and 200.25±34.35 in Diabetic group, while serum urea, creatinine (mg/dl) levels were insignificantly increased but 24h microalbuminuria significantly increased ($p<0.05$) in Diabetic + resveratrol compared to control with mean values 64.19 ±9.57#, 2.03±0.84# and 94.13±16.65*# in diabetic+resveratrol group versus 43.37±12.38,1.33±.49 and 16.77±3.30 in control group.

Effect of resveratrol on SIRT1 activity, AMPK level and SOD activity in the kidney tissues of diabetic male rats.

Fig. (4) showed that resveratrol had significant improvement ($p<0.05$) regarding SIRT1activity, AMPK and SOD level by 209.09%, 169.23% and 393.75% in Diabetic + resveratrol compared to Diabetic with mean values 0.68±0.22, 0.70±0.16 and 1.58±0.45 in diabetic +resveratrol versus 0.22± 0.10, 0.26±0.14* and 0.32±0.15 in diabetic. Also SIRT1activity, AMPK and SOD level significantly decreased ($p<0.05$) in diabetic + resveratrol compared to control with mean values 0.68 ±0.22, 0.70±0.16 and 1.58±0,45 in diabetic + resveratrol versus 1.03±0.03, 1.04±0.03 and 12.97±0.74 in control respectively.

Effect of caloric restriction on blood glucose (mmol/L), blood insulin levels (μIU/mL) and HOMA-IR in diabetic male rats.

It was shown in Fig. (1) that diabetic rats which undergo caloric restriction had significant improvement ($p<0.05$) regarding fasting serum glucose (mmol/L), serum insulin (μIU/mL) levels and HOMA. fasting serum glucose (mmol/L), serum insulin (μIU/mL) levels and HOMA were significantly decreased by 36.93%, 33.31% and 59.07% respectively in Diabetic + caloric restriction compared to diabetic group. Mean values in Diabetic + Caloric restriction were 9.77±1.58, 10.45±1.59 and 4.56±1.20 versus 15.49±3.16, 15.67±3.81 and 11.14±4.31 * in diabetic group respectively while serum glucose, serum insulin levels and HOMA-IR levels were insignificantly increased in Diabetic +caloric restriction compared to control with mean values 9.77±1.58,10.45±1.59 and 4.56±1.20 in Diabetic + caloric restriction versus 6.23 ±0.83, 9.07±1.06 and 2.49±.10 in control group respectively.

Effect of caloric restriction on TG (mg/dl), TC (mg/dl), LDLC(mg/dl) and HDLC (mg/dl) in diabetic male rats.

Fig. (2) showed that diabetic rats which undergo caloric restriction had significant decrease ($p<0.05$)

regarding TG, Cholesterol and LDL levels (mg/dl) by 32.19%, 14.11% and 20.85% in diabetic + caloric restriction compared to diabetic group with mean values 98.33±7.79, 196.94±8.49 and 133.02± 9.95 in diabetic + caloric restriction versus .01 ± 14.17, 229.28±21.27 and 168.07±21.34 in diabetic group, while HDL level significantly increased (p<0.05) by 37.42% in diabetic + caloric restriction group compared to diabetic with mean values 44.25±7.57 in Diabetic + caloric restriction versus 32.20±3.93 in diabetic group.

However, TC and LDLC levels were significantly decreased (p<0.05) but TG was insignificantly increased in diabetic+caloric restriction compared to control group with mean values 196.94±8.49, 133.02±9.95 and 98.33±7.79 in diabetic + caloric restriction versus 153.70 ± 16.08, 70.81 ± 17.02 and 92.27 ± 13.51 in control respectively while HDLC level (mg/dl) was significantly decreased (p<0.05) in diabetic + caloric restriction compared to control group with mean value 44.25 ± 7.57 in diabetic + caloric restriction versus 64.43 ± 4.70 in control group.

Effect of caloric restriction on serum creatinine, blood urea and 24h microalbumenaria (mg/dl) in diabetic male rats.

It was shown in Fig. (3) that caloric restriction had significant decrease (p<0.05) regarding serum urea, creatinine (mg/dl) levels and 24h microalbuminuria by 29.81 %, 45.19% and 42.41 % in diabetic + caloric restriction compared to diabetic with mean values 72.25 ± 17.23, 2.28±0.50 and 115.33± 12.90 in diabetic + caloric restriction versus 102.94± 15.50, 4.16± 1.29 and 200.25±34.35 in diabetic group respectively. Serum urea and 24h microalbuminuria (mg/dl) levels were significantly increased (p<0.05) but serum creatinine insignificantly increased in diabetic + caloric restriction compared to control with mean values 72.25 ± 17.23, 115.33± 12.90 and 2.28±0.50 in Diabetic + caloric restriction versus 43.37± 12.3, 8.33±.49 and 16.77±3.30 in control group.

Effect of caloric restriction on SIRT1 activity, AMPK level and SOD activity in kidney tissues of diabetic male rats.

Fig. (4) showed that caloric restriction significantly increased (p<0.05) SIRT1 activity, AMPK and SOD by 163.64%, 153.85% and 546.88% in diabetic + Caloric restriction compared to diabetic with mean values 0.58±0.17, 0.66±0.15 and 2.07± 0.48 in diabetic + Caloric restriction versus 0.22 ± 0.10, 0.26±0.14 and 0.32±0.15 in diabetic group respectively. Also SIRT1activity, AMPK and SOD level significantly decreased (p<0.05) in diabetic + caloric restriction compared to control group with mean values 0.58±0.17, 0.66±0.15 and 2.07±0.48 in diabetic + caloric restriction versus 1.03±0.03, 1.04±0.03 and 12.97±0.74 in control group.

Histopathological examination:

The pathological examination to the kidney tissues from rats of untreated diabetic group with haematoxylin and eosin (H &E) stain revealed that renal cortex contain some renal corpsules have variable degree of glomerular atrophy, renal tubules show degenerative changes in the form of (cloudy swelling and hydropic degeneration), protonus cast in their lumen, sever vascular degeneration in tunica media of the blood vessel, congested blood vessel, heamorrage, destincted lobulation of glomerular tuft (platinum lobe) and thickening of the glomerular basement membrane. Also periodic acid schiff (PAS) stain for untreated diabetic group showed + reaction sever thickening of the basement membrane in both glomerular tuft and renal tubule (membranous glomerulonephritis).

On the other hand the pathological examination to the kidney tissues of diabetic rats orally treated with RSV haematoxylin and eosin (H&E) stain showed normal structure of glomerular tufts with mild vacular degeneration normal renal tubule with no protonus cast. In addition periodic acid schiff (PAS) stain showed mild thickening of the glomerular basement membrane. And their was significant decrease in thickening of the glomerular basement membrane between Diabetic + Resveratrol compared to diabetic with mean value 39.92 ±6.14 for diabetic and 46.02±2.22 for Diabetic + resveratrol.

Also pathological examination to the kidney tissues of diabetic rats treated with 40% caloric restriction haematoxylin and eosin (H&E) stain showed normal structure of glomerular tufts with mild vacular degeneration normal renal tubule with no protonus cast. In addition periodic acid schiff (PAS) stain showed mild thickening of the glomerular basement membrane. And their was significant decrease in thickening of the glomerular basement membrane between Diabetic + caloric restriction compared to diabetic with mean value 39.9 ±6.15 for diabetic and 46.04 ±2.25 for diabetic + caloric restriction (Figs. 5-8).

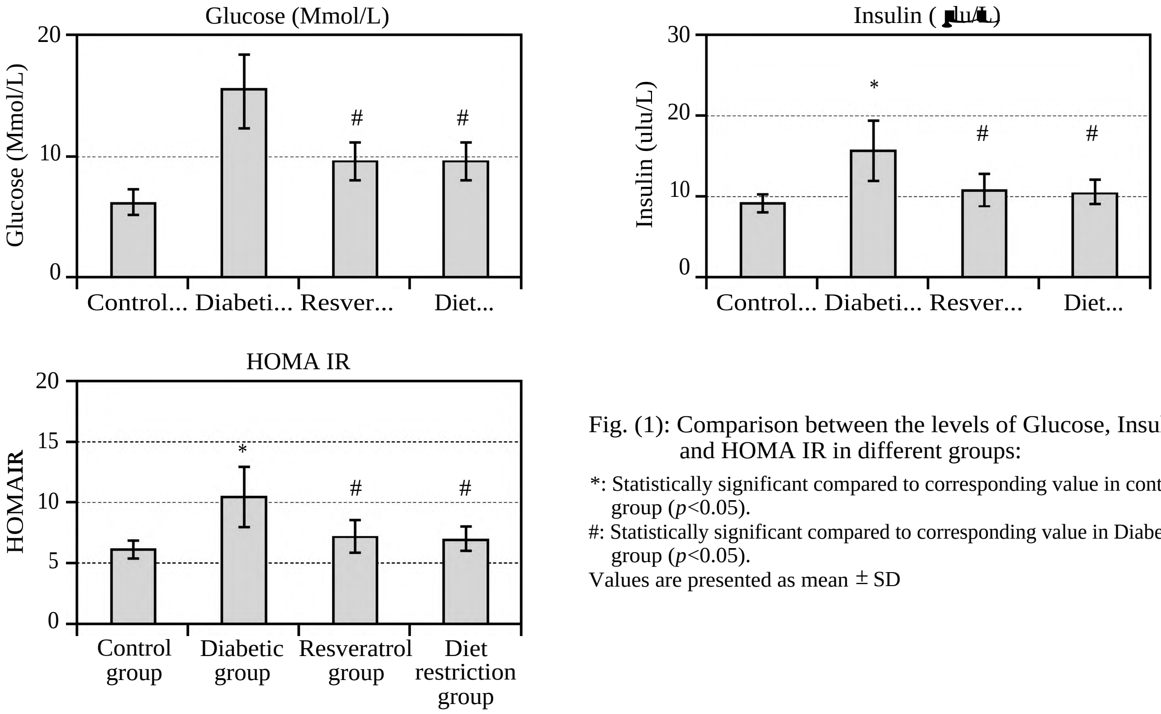

Fig. (1): Comparison between the levels of Glucose, Insulin and HOMA IR in different groups:

*: Statistically significant compared to corresponding value in control group ($p<0.05$).

#: Statistically significant compared to corresponding value in Diabetic group ($p<0.05$).

Values are presented as mean ± SD

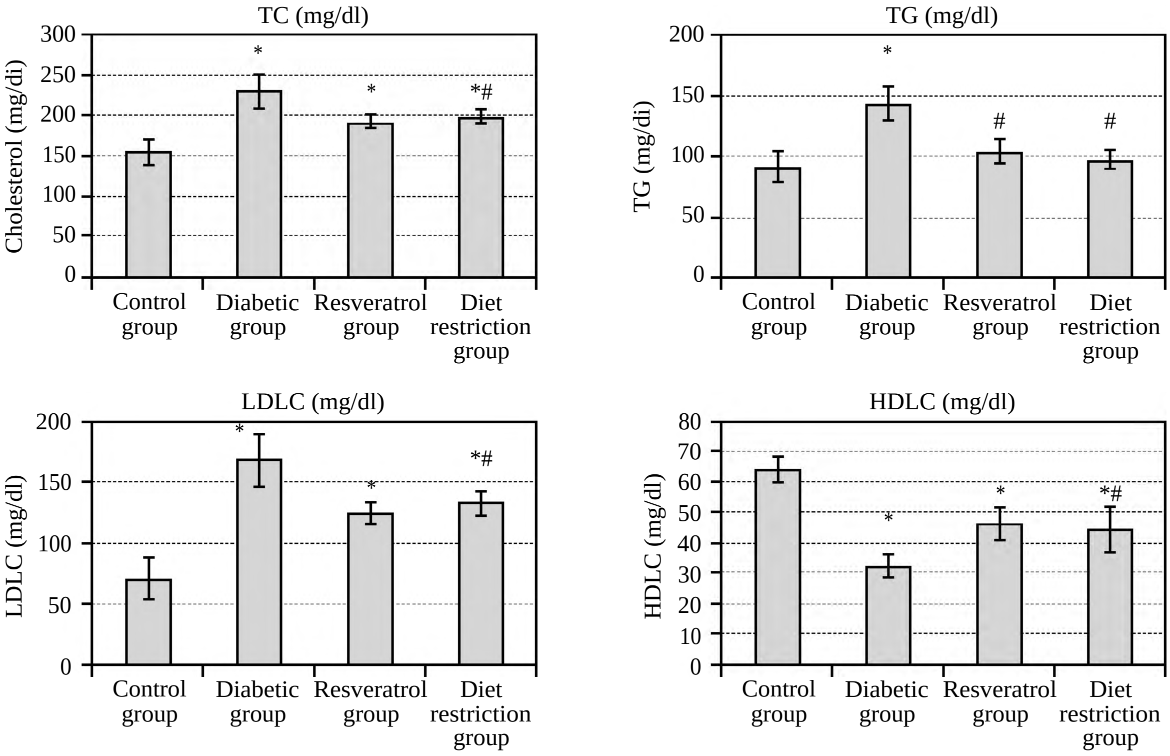

Fig. (2): Comparison between the levels of TC (total cholesterol), TG (triglycerides), low density lipoproteins cholesterol (LDLC) and high density lipoproteins cholesterol (HDLC) in different groups:

*: Statistically significant compared to corresponding value in control group ($p<0.05$).

#: Statistically significant compared to corresponding value in Diabetic group ($p<0.05$).

Values are presented as mean ± SD

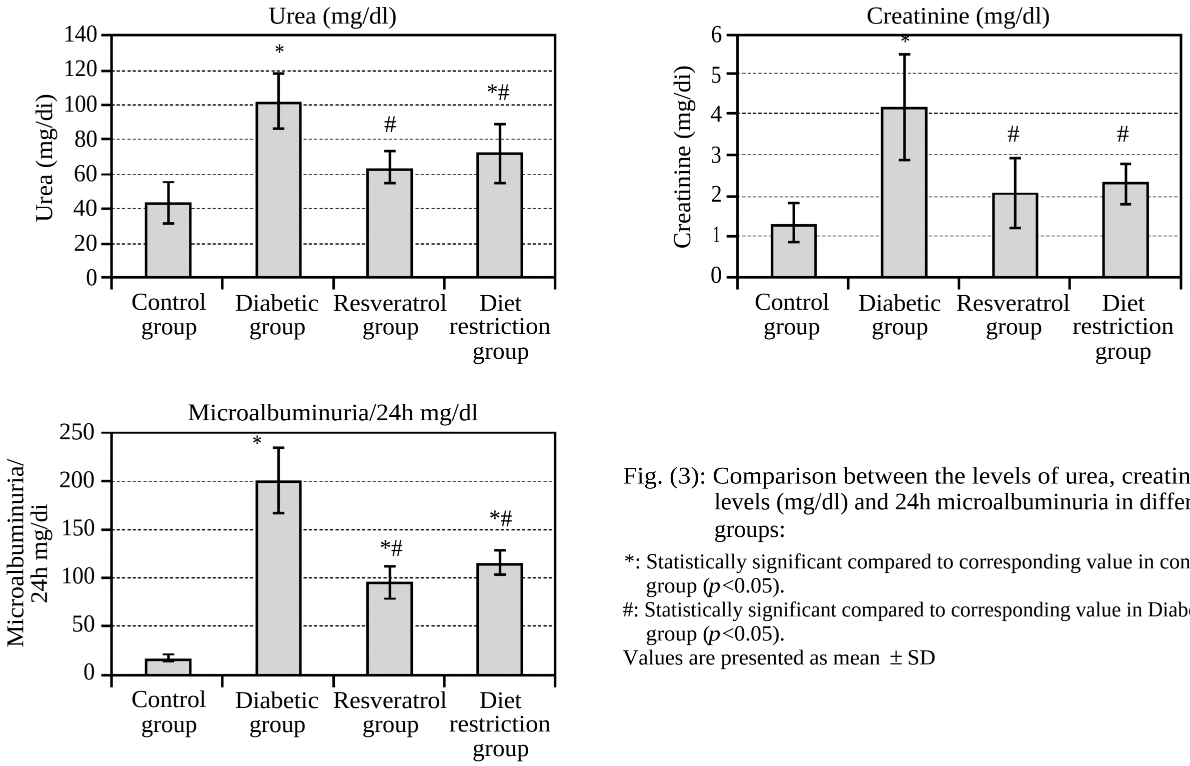

Fig. (3): Comparison between the levels of urea, creatinine levels (mg/dl) and 24h microalbuminuria in different groups:

*: Statistically significant compared to corresponding value in control group (*p*<0.05).

#: Statistically significant compared to corresponding value in Diabetic group (*p*<0.05).

Values are presented as mean ± SD

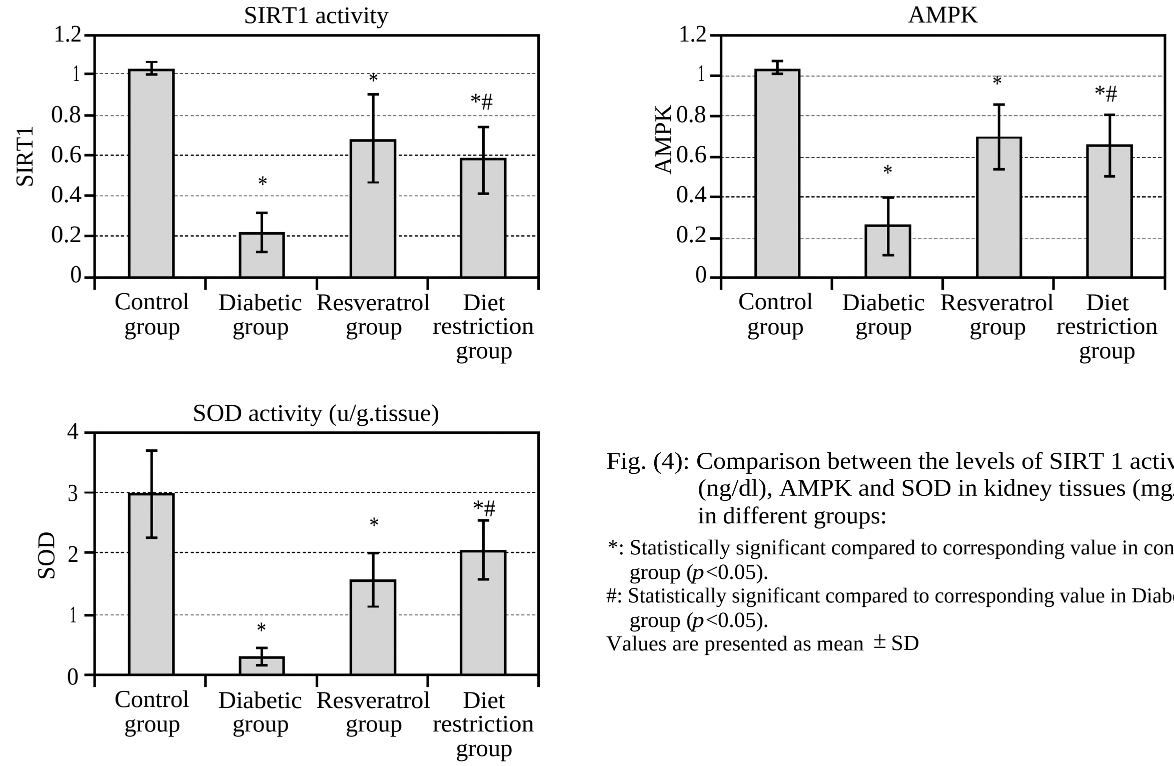

Fig. (4): Comparison between the levels of SIRT 1 activity (ng/dl), AMPK and SOD in kidney tissues (mg/dl) in different groups:

*: Statistically significant compared to corresponding value in control group (*p*<0.05).

#: Statistically significant compared to corresponding value in Diabetic group (*p*<0.05).

Values are presented as mean ± SD

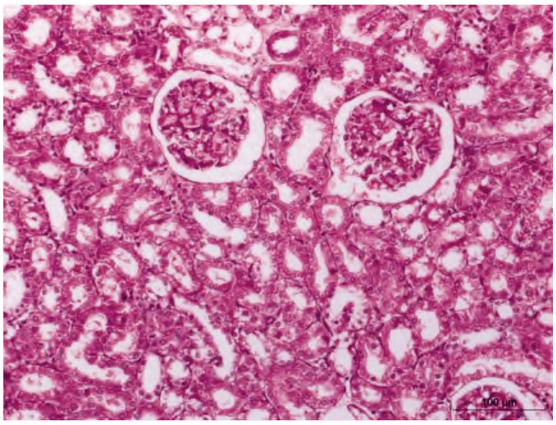

Fig. (5): H&E stain of control group show normal renal corpuscle and renal tubule (x200).

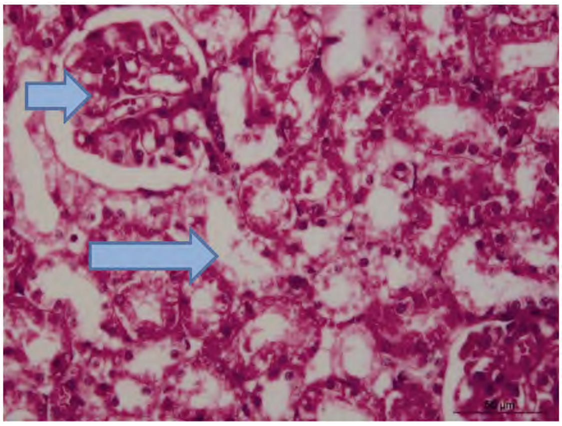

Fig. (6): H&E stain of diabetic group show destincted lobulation of glomerular tuft (platinum lobe) with thickening of basement membrane, renal tubules show degenerative changes (cloudy swelling and hydropic degeneration) (X400).

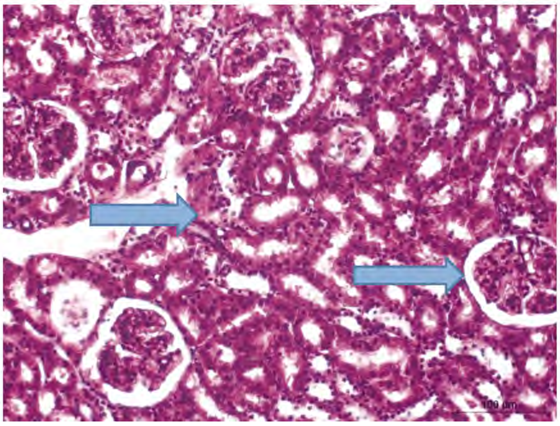

Fig. (7): H&E stain of diabetic group received resveratrol show normal structure of glomerular tufts with mild vacular degeneration and normal renal tubule with no protonus cast (X200).

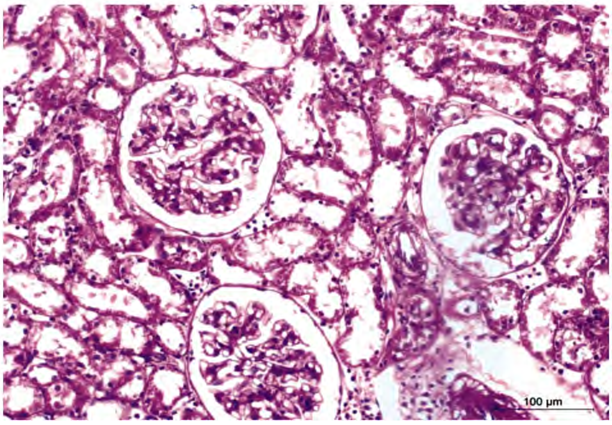

Fig. (8): H&E stain of diabetic group underwent 40% caloric restriction show normal structure of glomerular tufts with mild vacular degeneration and normal renal tubule with no protonus cast (X200).

Discussion

Diabetes mellitus (DM) is an epidemic medical challenge that threatens the health and life quality of people world wide. DM impairs metabolic, neural, and vascular function, and thus has profound impacts on different systems and organs in the body **[1]**. The present work has been designed to investigate the possible link between the action of resveratrol and CR on SIRT1 in kideny of diabetic rats. Results showed that the fasting serum glucose level was elevated by (148.64%) indicating significant ($p<0.05$) hyperglycemia compared to control group. Moreover, the present results also showed a significant increase in fasting serum insulin level by (72.77%) and HOMA-IR by (347.39%) confirming the characteristic features of T2DM. The present results were in agreement with **[29]** who stated that feeding HFD induces tissue insulin resistance through accumulation of lipids such as free fatty acids, their CoA esters and triglycerides in the adipose, skeletal muscle and liver in experimental animals. Furthermore, low dose STZ treatment mediates partial destruction of β-cells which may be responsible for the long-term glycemic imbalance in rats. The present results showed significant elevation ($p<0.05$) of serum TG, TC and LDLC level by 57.16%, 49.17% and 137.35% with significant reduction ($p<0.05$) of serum HDLC level by 50.02% in diabetic rats versus the control group. The present study results showed significant elevation ($p<0.05$) of serum urea and creatinine levels by 137.35% and 68.03% in diabetic rats versus the control group. The present results were in agreement with **[30]** who said that serum urea and creatinine are known to be raised with hyperglycemia in uncontrolled diabetics and usually correlate with severity of kidney damage. This also matched with the present study results which show significant elevation in 24h microalbuminuria by 1094.1 % in diabetic group compared with control group. It was mentioned by [31] said that microalbuminuria is an important therapeutic target for improving the prognosis of renal and cardiovascular risk in diabetic patients. The present study results were in agreement with [32] who demonstrated that SIRT1 expression decreases in proximal tubules before albuminuria in a mouse model of diabetic nephropathy, and that albuminuria is suppressed in proximal tubule-specific mice overexpressing SIRT1. These findings suggest that decreased SIRT1expression in proximal tubular cells causes abnormal nicotine metabolism and reduces the supply of nicotinamide mononucleotide from renal tubules to glomeruli. The present results showed a significant decrease (p<0.05) in AMPK level in kidney tissues by 75% in diabetic group compared with control group. The results were in agreement with [33] who demonstrated that dysregulation of (AMPK) in relevant tissues was crucial to the development of metabolic syndrome and diabetes. Also [34] found that AMPK protein phosphorylation and expression levels were remarkably reduced in diabetic renal tissues. The present results showed a significant decrease in SOD level by 89.23% in diabetic group compared with control group. The present results were in agreement with [35] who found that SOD, CAT and GSH activities were significantly decreased in the kidney of diabetic rats as compared to the normal control rats. On the contrary [36] revealed that no significant change was observed in superoxide dismutase (SOD) activity in diabetic rat kidney tissue. In view of this, the present study was done to assess the protective effect of resveratrol in diabetic nephropathy. Administration of resveratrol showed significant decrease (p<0.05) in serum glucose level, serum insulin level and HOMA-IR in which these parameters became lower than those in diabetic group by 37.25%, 31.21 % and 58.62% respectively. The present study results were in agreement with [37] who said that the regular consumption of resveratrol has been known to improve glucose homeostasis and reverse insulin resistance in type 2 diabetes mellitus. In contrast to the present study results [38] found that there were no significant differences in blood glucose levels between diabetic rats treated with resveratrol and without treatment. The present study results showed that diabetic rats which received resveratrol had a significant decrease (p<0.05) in triglycerides, total cholesterol, and LDLC levels by 27.58%, 16.35% and 25.91% respectively. While HDLC significantly increased (p<0.05) by 43.73% in (DM+RSV) compared to diabetic group. The present results were in agreement with [39] who said that Resveratrol treatment decreased triglycerides, cholesterol, and LDLC levels while HDLC was significantly increased. In the present study serum ceratinine, blood urea nitrogen and urinary albumin results showed a significant decrease (p<0.05) in their levels by 51.2%, 37.64% and 52.99% in treated diabetic group DM+RSV compared to diabetic group respectively. In agreement with the present results [40] showed that when diabetic rats treated with resveratrol for 4 weeks serum creatinine and urinary albumin/24h levels significantly decreased. In the present study oral administration of resveratrol by the diabetic rats in dose 5mg/kg/day for 2 months showed significant increase (p<0.05) in SIRT1 activity by 209.09% in (DM+RSV) compared with diabetic group. In agreement with the present study results [41] demonstrated that resveratrol (RSV) may prevent T2DM by targeting Sirtuin type 1 (SIRT1), indicating that SIRT1 may be a novel therapeutic target for T2DM prevention. The present study revealed that the level of phospholyrated AMPK in kidney tissues increased significantly (p<0.05) in diabetic rats received resveratrol DM+RSV by 169.23% compared to diabetic group. The present study results were in agreement with [42] who observed that Resveratrol treatment restored the phosphorylated AMPK in kidney of diabetic mice. In the present study SOD activity increased significantly (p<0.05) by 393.75% in diabetic rats received resveratrol (DM+RSV) compared to diabetic group (GPII).In agreement with the present study results [43] found that Oral treatment of RSV significantly increased the altered SOD activity to near control values. In the present work, 40% CR in (DM + CR) caused a significant decrease (p<0.05) in serum glucose level, serum insulin level and consequently HOMA IR by 36.93%, 33.31 % and 59.07% respectively compared to diabetic group. In the present study results were in agreement with [44] who examined 25% CR for 6 months in obese type 2 diabeteic patients and found significant decrease in blood glucose level. The present study results revealed a significantly (p<0.05) lower serum triglycerides, total cholesterol, and LDLC levels by 32.19%, 14.11% and 20.85% respectively While HDLC significantly (p<0.05) increased by 37.42% in (DM+CR) compared to diabetic group respectively. Similarly [44] in his study on type 2 diabetic patients found that 25% CR significantly lower LDLC and significantly increase HDLC. In addition to the beneficial effect of CR on serum glucose, insulin, TC, TG, LDLC and HDLC, the present work showed highly improvement in kidney function in the form of significant reduction ($p<0.05$) in serum creatinine, blood urea nitrogen and 24h microalbuminuria by 45.19%, 29.81% and 42.41% in (DM+CR) compared to diabetic group respectively. The present study results were in agreement with **[45]** who said that 50-72% of the caloric intake of ad libitum-fed to rodent with chronic kidney disease significantly improved serum creatinine, blood urea nitrogen and urinary protein. The present study results showed that Short term CR in GPIV (DM + CR) significantly ($p<0.05$) increased phospholyrated AMPK by 153.85% compared to diabetic group. In agreement with the present study results **[46]** found that rats which underwent 8-weeks CR with 60% of the food intake of the ad libitum increased level of SIRT1 and AMPK in aged kidney. The present study results show that short term CR significantly ($p<0.05$) increased SOD level by 546.88% compared to diabetic group which indicates that caloric restriction has antioxidant activity. In agreement with the present study results **[47]** said that daily ingestion of 60-70% caloric needs increased SOD activity in rats.

In the present study pathological examination of kidney of diabetic group showed thickening of the basement membrane in both glomerular tuft and renal tubule, sever vascular degeneration in tunica media of the blood vessel. Also it showed congested blood vessel, there is heamorrage, pressure atrophy in some renal capsules and vaccular degeneration in other. Some renal tubules showed protonus cast in their lumen and other showed degenerative changes (cloudy swelling and hydropic degeneration). On the other hand the pathological examination to the kidney tissues of diabetic rats treated with RSV showed normal structure of glomerular tufts with mild vaccular degeneration normal renal tubule with no protonus cast and mild thickening of the glomerular basement membrane. Similarly pathological examination to the kidney tissues of diabetic rats treated with 40% caloric restriction showed normal structure of glomerular tufts with mild vacular degeneration normal renal tubule with no protonus cast and mild thickening of the glomerular basement membrane. It was found by **[43]** in their study of effect of RSV on diabetic nephropathy that renal section of diabetic rats portrays marked thickening of the glomerular basement membrane, cystic dilation of renal tubules (asterisks), marked vacuolation of the epithelial lining of renal tubules, hyaline cast in the renal tubules and presence of few pyknotic nuclei in renal epithelium. The section of renal tissues of diabetic rats orally treated with RSV showed normal structure of glomerular tufts with moderate vacuolation of the tubular lining epithelium of the distal convoluted tubules type 2 diabetic rats while Calorie restriction significantly decreased the number of glomerulosclerotic lesions on PAS-stained tissue sections in diabetic rats.

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تقييم التأثير الوقائى للريسفيراترول وتقييد السعرات الحرارية على لمدى القصير على اعتلال الكلية السكرى فى الفئران عبر مسار SIRT1/AMPK

تم التعرف على اعتلال الكلية السكرى باعتباره السبب الرئيسى لمرض الكلى فى مرضى السكرى. الريسفيراترول، مركب لوليفينول، له دور فعال فى مرضى السكرى. ثبت أن تقييد السعرات الحرارية يقلل من حدوث مضاعفات داء السكرى.

الهدف من هذه الدراسة تقييم التأثير الوقائى لريسفيراترول وتقييد السعرات الحرارية على المدى القصير على اعتلال الكلية السكرى.

قمنا باستخدام ثمانية وأربعون فأراً وتم تقسيمهم إلى مجموعة تحكم، مجموعة مصابة بداء السكرى، مجموعة ريسفيراترول تم إعطاؤها ٥ مجم/كجم/يوم عن طريق الفم لمدة شهرين، مجموعة تقييد النظام الغذائى تخضع لـ ٤٠٪ من السعرات الحرارية (CR) لمدة شهرين. تم قياس مستويات مصل الدم من الجلوكوز، الأنسولين، الدهون، الكرياتينين واليوريا مع بيلة زلالية دقيقة لمدة ٢٤ ساعة بالإضافة إلى تقييم HOMA-IR، نشاط sirtuin1، بروتين كيناز أدينوزين أحادى الفوسفات (AMPK) ومستويات ديسموتاز الفائق (SOD) فى الكلى مع فحص الأنسجة لأنسجة الكلى.

توصلت النتائج إلى تحسن معنوى فى جميع المتغيرات فى مجموعة ريسفيراترول ومجموعة تقييد السعرات مقارنة بمجموعة مرضى السكرى. أيضاً زيادة فى نشاط SIRT1، AMPK، ومستويات SOD فى أنسجة الكلى فى مجموعة ريسفيراترول ومجموعات تقييد السعرات الحرارية مقارنة بمرضى السكرى. أظهر الفحص الباثولوجى لأنسجة الكلى فى مجموعة مرضى السكرى تدهور فى الأنسجة الكلوية. أظهرت النتائج فى مجموعات الريسفيراترول ومجمو عات تقييد السعرات الحرارية التركيب الطبيعى للخصلات الكبيبية مع تنكس الأوعية الدموية الخفيف. فيما يتعلق بالنبيب الكلوى، لم يكن هناك بروتونات مصبوبة فى التجويف وسماكة خفيفة فى الغشاء القا عدى الكبيبى.

\الخلاصة : زيادة نشاط SIRT1 فى مجموعات ريسفيراترول و CR لها قيمة كبيرة فى حماية الكلى من مضاعفات مرض السكرى.