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Original Research Article

Effects of resveratrol on oxidative stress in high fat diet /streptozocin induced diabetic wistar albino rats

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ABSTRACT

Background: Diabetes mellitus is a common chronic disease. One of the pathophysiology is found to be oxidative stress. This study aims to evaluate the effect of resveratrol on oxidative stress in high fat diet (HFD)/streptozotocin induced diabetic rats.
Methods: Wistar albino rats, fed with HFD rendered diabetic with streptozotocin, were divided into 6 groups, namely the diabetic control treated with vehicle (DC), standard control which received metformin (SC), test groups treated with 5,10, and 20 mg/kg b.w. of resveratrol and combination of half dose of metformin and resveratrol (10 mg/kg)(TC). A group of six normal animals served as normal control (NC), another six as HFD control. Fasting blood glucose, lipid profile and serum MDA and SOD were measured one week after induction of diabetes. The animals were then treated orally for 2 weeks after which the same parameters were repeated. The in-vivo results were analysed by one way ANOVA followed by Tukey's multiple comparison test.
Results: The DC group demonstrated a increase in the fasting blood glucose compared to NC, HFD control while a significant decrease in the fasting blood glucose was observed with SC, Test groups ($p<0.05$) as compared to the DC group. TC showed a significant improvement in dyslipidemia compared to their baseline values ($p<0.05$). There was significant change in the serum MDA level and SOD activity.
Conclusions: Resveratrol improves oxidative stress in diabetic rats.

Keywords: Diabetes, Oxidative stress, Resveratrol

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the world's most common chronic diseases as changing lifestyles lead to reduced physical activity and increased obesity.[1] Insulin resistance or insufficient insulin secretion not only has negative metabolic consequence but also contributes to subsequent pancreas β-cell exhaustion.[2] Previous experimental study demonstrated that high fat feeding has led to insulin resistance and impaired glucose tolerance.[3] Several authors suggested that increased carbohydrate in form of simple sugar like fructose ingestion may be responsible for the epidemic of obesity and the increased incidence of metabolic syndrome and

diabetes.[4] Hyperinsulinemia is a central pathophysiological feature of NIDDM and has been shown to play a key role in the disease evolution and complications.[5]

Oxidative stress may be a common pathway linking diverse mechanisms of complication of DM, as several experimental animal models of diabetes induced by streptozotocin (STZ), HFD (high fat diet) with STZ and high fructose diet etc exhibit high degree of oxidative stress due to depletion of antioxidative defense system of body such as superoxide dismutase (SOD), catalase and glulathione peroxidase.[6,7]

Resveratrol, a phytoalexin produced naturally by several plants when under attack by pathogens such as bacteria or fungi, has got some beneficial effects like anti-cancer, anti-inflammatory, cardioprotective, blood-sugar-lowering in mouse and rat experimental models.[8] Though there are various approaches are available to treat diabetes and prevent its secondary complications, herbal medications may be used as an alternative therapy as these are well tolerated.

Aims and objectives of the study was in this context, the present study was undertaken to evaluate antioxidant and hypolipidemic properties of the resveratrol which in turn may be helpful in the prevention and ultimately management of high fat diet with low dose streptozotocin induced type-2 DM model in wistar albino rats.

METHODS

Wistar albino rats of either sex weighing between 100-150 g were procured from National Institute of Nutrition, Hyderabad, India. They were housed in clean polypropylene cages (four rats / cage), maintained under controlled room temperature (25±1^{o}) and with relative humidity of 45-55% under 12:12hr light and dark cycle in the central animal house. They were provided with standard lab diet and water ad libitum and kept for 1 week to acclimatize with the laboratory condition before starting the experiment. Prior to the study, the study protocol was approved by I.A.E.C, M.K.C.G. Medical College, Berhampur. The study was carried out as per CPCSEA guidelines.

Study design

There were 54 wistar albino rats were grouped randomly into 9 groups and distributed 6 in each as follows:

Control groups

Gr-I: Normal pellet diet and distilled water *per oral*.

Gr-II: Normal pellet diet and DMSO *per oral*.

Gr-III: High fat diet (HFD) and DMSO *per oral*.

Disease control

Gr–IV: HFD and oral DMSO after induction of diabetes.

Test groups

Gr-V-VII: Resveratrol in 5, 10, 20mg/kg orally for 2 weeks, after induction of diabetes.

Standard group

Gr-VIII: Metformin (0.5gm/kg) orally for 2 weeks after induction of diabetes

Combination of test and standard

Gr–IX: Minimum effective dose of resveratrol (10mg/kg) + half dose of metformin (250mg/kg) orally for 2 weeks after induction of diabetes.

Procedure

The test drug resveratrol was obtained from InvivoGen, streptozotocin from Himedia Lab. and high fat diet was prepared in the Laboratory.[3] The doses of standard and test drugs were selected from the different published literature.[9,10] The test drug resveratrol was dissolved with DMSO before administration whereas distilled water served as vehicle for standard drug metformin.

Induction of diabetes

Diabetes mellitus was induced in overnight fasted wistar albino rats by I.P injection of single dose of freshly prepared solution of streptozotocin (40 mg/kg) which was made by dissolving with 0.1M Citrate buffer solution (pH 4.5) containing 0.9% NaCl after 2 weeks of high fat diet.[11] To avoid an early fatal hypoglycemia 5% glucose solution was fed on 1st day of Streptozotocin administration to all rats. The rats having fasting glucose level ≥200mg/dl after 48 hrs of administration of streptozotocin and persistent after 7th day after administration of STZ were considered diabetic and included in the study groups.[12]

Preparation of stock solution resveratrol

To obtain a 20mg/ml stock solution, 250µl DMSO was added to 5mg resveratrol powder. Solution was then vortexed until complete solubilization, aliquoted and stored at 4^{0}C. Required dose was obtained by dilution with distilled water as per the instruction given by the product manufacturer.

Preparation of high fat diet (HFD)

High fat diet was prepared by adding excess of coconut oil to normal diet so as to provide 42% of total calories from the fat source.[3]

Estimation of biochemical parameters

After 12 hr fasting, about 3 ml of blood was collected in a sterilized test tube containing EDTA through retro-orbital puncture under light ether anesthesia. Assay of different biochemical parameters like Fasting plasma glucose (GOD/POD method), plasma cholesterol (CHOD/PAP method), triglyceride**(**GPO / PAP method) and HDL (Peg precipitation method) were estimated by using commercially available kits (Crest Biosystem). The LDL cholesterol was calculated from the formula of Friedwald et al as given below: [13]

LDL-C = Total cholesterol - [HDL+ VLDL]

Where VLDL = Triglyceride /5

Oxidative stress parameters like SOD activity and MDA were estimated by standard biochemical procedures.[14,15]

Statistical analysis

The data obtained from different parametric tests of this study like fasting plasma glucose, plasma total cholesterol, triglyceride, HDL, LDL, MDA, SOD activity were analyzed by one way ANOVA followed by Tukey's multiple comparison test. The 'P' values less than 0.05 was considered statistically significant. Graph pad prism version- 5.0 software was used for data analysis in a personal computer.

RESULTS

Table 1: Effect of resveratrol on fasting plasma glucose concentrations in HFD+STZ induced diabetic rats (n=6).

Drug and dose	FPG (mg/dl)
Control (DMSO) - 0.5ml	90±4.26
HFD + DMSO	103.83±2.85#
HFD+STZ + DMSO	274.83±16.98$a
HFD+STZ + RES (5mg/kg)	298.2±7.67
HFD+STZ + RES (10mg/kg)	150±4.6*
HFD+STZ + RES (20mg/kg)	120±4.0**@
HFD+STZ + METFORMIN (500mg/kg)	120±2.5**@
HFD+STZ+MET (250mg/kg) +RES (10mg/kg)	120±3.54**

Data expressed as Mean±SE, # (p<0.05) (NC vs HFD), $ (p<0.001) (NC Vs DC), a (HFD vs DC) *(p<0.05), **(p<0.001) (DC vs treatment groups), @ (p>0.05) (STD vs test drug).

It is evident from the above table that the mean fasting plasma glucose (FPG) level in HFD / streptozotocin induced diabetic rats treated with resveratrol at doses 10mg/kg and 20mg /kg were significantly decreased in comparison to that of disease control which is comparable to standard treated groups. But 5mg/kg dose of resveratrol does not produce any significant effect on hyperglycemia. Minimum effective dose of resveratrol (10mg/kg) with half dose of metformin (250mg/kg) produced significant decrease in FPG compared with that of disease control.

Table 2: Effect of resveratrol on plasma lipid profile in HFD+STZ induced diabetic rats (n=6).

Groups	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal control (DMSO)	66.11±1.57	82.32±1.54	24.43±1.36	25.22±0.72
HFD+ DMSO	81.28±3.35@	121.5±2.25@	44.31±3.69@	12.67±0.32@
HFD+STZ (40mg/kg) + DMSO	125±2.69$,*	216.3±2.16$*	72.44±2.71$*	9.27±0.28$*
Diabetic+ Res (5mg/ kg)	119.9±3.27	215.7±2.13	67.39±3.26	9.4±0.43
Diabetic+ Res (10mg/ kg)	71.3±1.95#	117.7±2.21#	25.82±6.30#	25.77±1.44#
Diabetic+ Res (20mg/kg)	67.81±1.34#	109.9±2.69#	15.61±1.60#	31.48±0.38#
Diabetic+ Metformin (500mg/kg)	63.67±1.89#@	92.78±1.23#@	18.19±1.93#@	26.92±0.52#@
Diabetic+ (Res 10mg+ met 250mg/kg)	65.49±1.09#	99.90±1.59#	16.22±1.29#	29.30±0.86#

Data are expressed as Mean±SEM, @ (p<0.05) (NC Vs HFD), *(P<0.001) (NC vs DC), $ p<0.05 (HFD Vs DC), # (p<0.05) (DC vs treatment groups), @ (p>0.05) STD vs test drug

As the Table 2 shows plasma concentration of total cholesterol (TC) was significantly increased in HFD treated group as well as in disease control group respectively compared with that of normal control treated with DMSO. The diabetic rats received resveratrol in 10 and 20mg/kg showed significant decrease in plasma TC level respectively in comparison to disease control. The observed effects of test drug at higher doses are not significantly different from that of normal control and metformin treated group. The combined effect of minimum effective dose of resveratrol (10mg/kg) with half dose of metformin (250mg/kg) also showed significant decrease. The plasma concentration of TG was significantly increased in HFD treated group as well as in disease control group compared with that of normal control treated with DMSO. The diabetic rats received resveratrol in 10 and 20mg/kg showed significant decrease in plasma TG level in comparison to disease

control. The observed effects of test drug at higher doses are not significantly different from that of normal control and metformin treated group. The combined effect of minimum effective dose of resvertrol (10mg/kg) with half dose of metformin (250mg/kg) also showed significant decrease in comparison to disease control. The plasma concentration of LDL is significantly increased in HFD treated group as well as in disease control group respectively compared with that of normal control treated with DMSO. The diabetic rats received resveratrol in 10 and 20 mg/kg showed significant decrease in plasma LDL level in comparison to disease control. There were no significant difference between resveratrol treated group at 10 and 20mg/kg and metformin treated group. The combined effect of minimum effective dose of resvertrol (10mg/kg) with half dose of metformin (250mg/kg) also showed significant decrease in comparison to that of disease control and is similar to normal control. The plasma concentration (HDL) is significantly decreased in HFD treated group as well as in disease control group respectively compared with that of normal control treated with DMSO. The diabetic rats received resveratrol in 10 and 20mg/kg showed significant increase in plasma HDL level in comparison to disease control. The observed effects of test drug at higher doses are not significantly different from that of normal control and metformin treated group. The combined effect of minimum effective dose of resvertrol (10mg/kg) with half dose of metformin (250mg/kg) also showed significant increase in plasma HDL level compared with disease control.

Table 3: Effect of resvertrol on superoxide dismutase activity and plasma MDA level in HFD+STZ induced diabetic rats (n=6).

Groups	SOD (U/ml)	MDA (nmol/ml)
Control (DMSO)	31.20±2.07	3.08±0.16
HFD + DMSO	22.39±1.38@	4.58±0.16@
DC (HFD+STZ-40mg/kg)+DMSO	14.78±0.86*	6.79±0.23*,#
Diabetic +Resv (5mg/kg)	15.07±0.86	6.41±0.24
Diabetic +Resv (10mg/kg)	32.67±1.11$$$	3.95±0.23$
Diabetic +Resv (20mg/kg)	34.89±1.92$$$	3.62 ±0.15$
Diabetic + Met (500mg/kg)	26.97±1.03$	2.95±0.10$
Diabetic + (Resv 10mg/kg + Met 250mg/kg)	25.38 ±1.19$	3.45 ±0.16$

Data expressed as Mean ± SEM,@p<0.01 (Normal control Vs HFD control),*p <0.001(NC Vs DC),# p<0.05(HFD Vs DC), $ p<0.05, $$$ p <0.001 (Diabetic group Vs treatment group)

As the Table shows plasma SOD activity was significantly decreased in HFD control as well as in disease control respectively compared with normal control received DMSO. Groups treated with resveratrol 10mg/kg and 20mg/kg produced significant increase in plasma SOD activity respectively which were similar with that of metformin treated group and not significantly different from normal control. The combined effect of minimum effective dose of resveratrol with half effective dose of metformin showed significant increase in plasma SOD activity compared with disease control and is similar to that of group treated with metformin alone. The plasma MDA level was significantly increased in HFD control as well as in disease control respectively compared with normal control received DMSO. Groups treated with resveratrol 10mg/kg and 20mg/kg produced significant decrease in plasma MDA level respectively which are similar with that of metformin treated group and not significantly different from normal control. The combined effect of minimum effective dose of resveratrol with half effective dose of metformin showed significant decrease in plasma MDA level compared with disease control and is similar to that of group treated with metformin alone.

DISCUSSION

The present study was undertaken to evaluate the effect of resveratrol on lipid profile and oxidative stress in term of blood glucose profile of HFD and low dose STZ (40mg/kg) model of T2DM in wistar albino rats. The effect of resveratrol in different doses like 5, 10 and 20mg/kg, were studied by estimating biochemical parameters (FPG, lipid profile, MDA and SOD activity) in plasma. The results were compared with that of standard drug (metformin).

Wistar albino rats were selected for this study as the rodents (rats/mice) are standardized experimental animals both for behavioral study and used for the effect of a candidate compound on intermediary metabolism in liver muscle and adipose tissue with subsequent effects on metabolic blood parameters like glucose, lipids, etc. There are reports suggesting streptozotocin induced diabetic wistar rats showing significantly higher fasting plasma glucose, higher area under curve of an oral glucose tolerance test, and higher lipid abnormalities in comparison to other strains.[16]

The dose and route of streptozotocin for induction of diabetes were taken from the previous published articles[2]. HFD was prepared by adding excess of coconut oil (42%v/w) to standard laboratory diet and fed to the experimental animals for 2 weeks prior to STZ.[3]Dose and route of resveratrol were chosen from the similar type of published work.[10]

For induction T2DM, a single low dose of STZ at 40mg/kg body weight was injected after 2 weeks of HFD feeding. High doses of STZ (>40 mg/kg body weight i.e. 60-80mg/kg) is well known to be taken by pancreatic β-cells via GLUT2 and to induce severe damages of pancreatic β-cells, mimicking Type-I DM.[17] But the combination of HFD and low doses of STZ resulted in characteristic of type-2DM where insulin resistance plays

a major role in pathophysiology leading to various metabolic alterations like increased blood glucose level, hyperinsulinemia, and dyslipidemia.[12] Oxidative stress is one of the major predisposing factors in this experimental model. So, in this present study HFD/ low dose STZ model was selected.

Early treatment with antidiabetic dugs and lifestyle modification such as losing weight, exercising, and watching the diet are often recommended for prevention and control of diabetes and related complications. Though there are a good number of pharmaceutical products (medicines) developed day by day to control the disease but most of them are not free from dangerous unwanted effects. Thus, there has been a growing interest in herbal remedies that can be introduced into the general population with the least side effects and the maximal preventive outcome.[18]Resveratrol which is used as nutritional supplements for some of its beneficial effect, are not cleared**.** In this present study, rats of different control groups administered with distilled water and DMSO did not produce any significant change in the mean fasting plasma glucose level and also there is no significant change between control group and HFD group.[2]

Disease control group received HFD/ STZ produced significant increase in plasma glucose level.[2] Resveratrol treated groups in doses 10 and 20mg/kg for a period of 14 days produced significant decrease in FPG in comparison to disease control and remained within the normal range.[19] This effect is similar to standard drug metformin. The combined effect of resveratrol with metformin showed significant decrease in plasma glucose level which is comparable with normal control group.[20]

Resveratrol in doses 10 and 20mg/kg produced significant decrease in TC, TG and LDL level with increase in HDL level in a dose dependant manner in comparison to disease control which showed beneficial effect on dyslipidemia due to diabetes. This result of the present study corroborates with observations made by Shahi et al.[19] It also noted that metfomin alone as well as in combination with resveratrol showed significant improvement in plasma lipid profile in diabetic rats which is comparable with normal control.

The result of this study showed significant increase in plasma SOD activity and decrease in plasma MDA level in resveratrol treated groups in of 10 and 20mg/kg dose level. The standard drug metformin alone as well as combination with resveratrol showed significant improvement in plasma SOD activity and MDA level in diabetic rats and is comparable with normal control group.[21]

CONCLUSION

Resveratrol, a phytochemical present in grape skin, red wine, purple grape juice, peanuts, and some berries, is commonly used as nutritional supplement and proved in some studies for its beneficial effects like anti-inflammatory, blood sugar lowering in some mouse and rat experimental models. Though some of its effects are proved in few clinical studies, its effects are not yet fully established for its use in therapy. Therefore, the present study has been undertaken to evaluate the potential role of resveratrol against hyperglycemia, dyslipidemia, oxidative stress HFD/STZ induced T2DM in wistar albino rats.

The result of this study revealed that resveratrol at a dose of 10 and 20 mg/kg showed significant antihyperglycemic effect, favourable effect on lipid profile with improvement in oxidative stress parameter which are comparable with that of metformin. These effects are may be due to its enhancement of peripheral glucose utilization by increasing insulin sensitivity. Though it is being used as nutritional supplement, this study result may be due to action on sirtuin 1 which was proved by other similar studies.

On the basis of these observed results of this present study, it can be concluded that resveratrol can be a better candidate to control T2DM and may prevent its complications like diabetic neuropathy. Also along with other oral antidiabetic agents like metformin, resveratrol can have potential adjuvant value. By this the dose of antidiabetic agent can be reduced and may limit the burden of ADR related to that particular drug. Therefore it can be suggested that more and more animal studies and further clinical trials are needed to establish its clinical use in future.

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