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Original Article

ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF RESVERATROL AND VITAMIN-C COMBINATION ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Objective: Resveratrol and vitamin-C combination was evaluated for its antidiabetic and antioxidant activity in streptozotocin (STZ) induced diabetic rats.

Methods: The wistar rats were challenged with a single intra peritoneal injection of STZ (50 mg/kg). The rats were treated with graded oral dose of 10 mg/kg of resveratrol and 0.9 g/kg of Vitamin C for 21 days. The fasting blood glucose levels were monitored for all animals in 1,7,14 and 21 days of drug treatments by using glucometer. The liver homogenate was used for estimation of proteins, malondialdehyde (MDA), lipid hydroperoxide (LH), Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione reductase (GSSH) and Reduced glutathione (GSH).

Results: Resveratrol with vitamin C combination showed significant ($P<0.01$) increase in body weight, total protein, and significant ($P<0.01$) decrease in fasting blood sugar level, MDA and LH, when compared to diabetic control. Resveratrol and vitamin C significantly ($P<0.01$) restored the levels of both enzymatic (SOD, CAT, GSSH) and non enzymatic antioxidant enzymes (GSH) which are almost similar to the control.

Conclusion: Resveratrol and vitamin C both is having antioxidant activity, but the combination showed synergistic antioxidant & antidiabetic activity. Thus, the present study provides a significant rationale for the management of diabetes.

Keywords: Streptozotocin, Diabetes, Synergistic activity, Resveratrol.

INTRODUCTION

Diabetes Mellitus, a metabolic disorder of multiple etiologies, is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism that result from imperfections in insulin secretion, insulin action or both [1]. The increase in the incidence of diabetes is due to longevity of life, changing lifestyle, obesity, sedentary work, changing dietary patterns and low birth weight [2]. The poly phenolic compound resveratrol which is also known as 3,4',5 trihydroxy stilbene [3] and 3,4',5-stilbenetriol, exists in cis-and trans stereo isomeric forms commonly found in grapes and wines particularly red wine. It is synthesized from pcoumaroyl CoA and malonyl CoA. It is a naturally occurring phytochemical found in many plants.

The antioxidant role of resveratrol supports recent research in a protective mechanism through increasing endogenous cellular antioxidant defences. Resveratrol has been discovered as an antiproliferative agent for cancer [4], the cardiac protective ability [5] effective against diabetes [6] and also protective effect on most of the vital organs including kidney, heart, and brain from ischemic reperfusion injury. The protective mechanism of resveratrol includes its role as intracellular antioxidant, anti-inflammatory agent and its ability to induce nitric oxide synthase (NOS) expression with a dose of (10 mg/kg) [7]. Most of biological protective actions of resveratrol have been associated with its antioxidative, anti-inflammatory, and antiapoptotic properties and other indirect pathways [8]. Continued public interest and increasing resveratrol supplements on the market warrant a review of the available *in vitro* and *in vivo* science reported in the Diabetic-related literature. Rigorous clinical trials evaluating the effects of resveratrol in diabetes mellitus are absent, though the general population consumption appears to be relatively safe. Resveratrol has shown potential for treating diabetes in laboratory animals.

Vitamin C or L-ascorbic acid is an essential nutrient for humans. It has several medicinal properties like antioxidant, anticancer, effect on coronary artery diseases [9], diabetes [10] with the dose of (0.9g/kg) etc. So the present study was undertaken to evaluate the synergistic effect of resveratrol and vitamin-c the combination in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

The chemicals required for this study were obtained from (Resveratrol and Vitamin-C) Sigma chemicals, Streptozotocin from Himedia.

Preparation of resveratrol and vitamin C suspensions

The Resveratrol suspension was prepared by dissolving Resveratrol in 0.5% carboxymethylcellulose solution. Vitamin C suspension also prepared in 0.5% CMC solution. All these drugs were administered in a constant volume of 0.5 ml/100g body weight of the rat.

Experimental animals

Wistar rats (120-150g) were obtained from the animal house and they were maintained in clean, sterile, polypropylene cages with paddy husk as bedding. Animals were housed at temperature of 25±2 °C and relative humidity of 30-60%. A 12:12 h light and dark cycle was followed. The animals had free access to food and de mineralised drinking water ad libitum. All the experimental procedures and protocols used in this study were reviewed by the Institutional animal ethics committee (688/2/C-CPCSEA) of NCP and were in accordance with the guidelines of the IAEC.

Experimental protocol

30 Male Wister albino rats were divided into five groups of 6 animals in each group.

Induction of diabetes mellitus

Male wistar rats (120-150g) were fasted for overnight before challenging with a single injection of freshly prepared streptozotocin (50 mg/kg) intraperitonially, and injected within 5 min of preparation to prevent degradation. After administration of streptozotocin, the animals had free access to food and dematerialized drinking water ad libitum. The development of hyperglycemia in rats was confirmed by fasting serum glucose estimation of 48 h on post streptozotocin injection. The rats with fasting serum glucose level of above 200 mg/dl were considered diabetic and included in the study. The test dose was administered orally for 21 days as follows;

Group-I	Vehicle control received Normal saline (10 ml/kg)
Group-II	Rats received streptozotocin (50 mg/kg)
Group-III	STZ induced diabetic rats received Glibenclamide (0.5 mg/kg)
Group-IV	STZ induced diabetic rats received Resveratrol (10 mg/kg)
Group-V	STZ induced diabetic rats received Vitamin C (0.9g/kg)
Group-VI	STZ induced diabetic rats received Resveratrol (10 mg/kg) with Vitamin C 0.9g/kg) combination

The fasting blood glucose levels were monitored for all animals in 1,7,14 and 21 days of drug treatments by using glucometer. Blood was collected from tail tip of the animals after 3 hours of drug administration and the fasting blood glucose levels were compared with that of diabetic control group animals.

The rats were sacrificed on the 22th day and their liver was excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCL buffer. The homogenate was used for estimation of proteins [11], malondialdehyde and lipid hydroperoxide [12]. Part of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for the estimation of GSSH [13] and GSH [14]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4 ° C. The supernatant thus obtained was used for the estimation of SOD [15] and CAT [16].

RESULTS AND DISCUSSION

The normal control rats were gained body weight during the experimental period. STZ induced diabetic rats showed a severe loss of body weight throughout the experimental period. Administration of standard drug glibenclamide, resveratrol, vitamin C and resveratrol with vitamin C combination showed significant (P<0.01) increase in body weight compared to diabetic rats. Results suggested that resveratrol alone, vitamin C alone and resveratrol with vitamin C treatment has a positive effect on maintaining body weights in diabetic rats. The resveratrol with vitamin C combination treated rats gained more weight than the standard drug (table 1).

The Resveratrol (10 mg/kg) and Vitamin-C (0.9g/kg) treated groups shown a significant (P<0.01) decrease in fasting blood sugar level, when compared to diabetic rats. The resveratrol & Vit C combination exhibited maximum glucose lowering effect in diabetic rats compared to resveratrol and Vit C treated rats. Glibenclamide exhibited a % reduction in blood glucose levels at the end of the study when compared to diabetic control (table 2).

Treatment with STZ showed a significant (P<0.01) decrease in the level of total protein and an increase in the level of malondialdehyde (MDA) and lipid hydroperoxides (LH) when compared to normal control. Treatment with the resveratrol and vitamin C caused a significant (P<0.01) decrease in the MDA and LH and an increase in total protein content (table 3).

Treatment with STZ produced a significant (P<0.01) decrease in the enzymatic antioxidants like catalase, superoxide dismutase, and glutathione reductase and the non-enzymatic antioxidant reduced glutathione in the lens homogenate when compared to normal control. Treatment with the resveratrol and vitamin C significantly (P<0.01) restored the levels of both enzymatic and non enzymatic antioxidant enzymes which is almost similar to the control group (table 4).

Table 1: Effect of resveratrol and vitamin-C combination on body weight changes in streptozotocin induced diabetic rats

Groups	Change in body weight (g)
Normal	36.8±3.6
Diabetic	20.6±2.6^{a}
Diabetic+Glibenclamide	35.4±4.6^{b}
Diabetic+Resveratrol	32.8±1.8^{b}
Diabetic+Vit C	34.6±3.4^{b}
Diabetic+Resveratrol+Vit C	35.6±3.4^{b}

Values are mean±SD; n=6 in each group; ^{a}P<0.01 when compared to normal control; ^{b}P<0.01, when compared to diabetic control (one way ANOVA followed by Dunnett's test).

Table 2: Effect of resveratrol and vitamin-C combination on fasting blood glucose level of streptozotocin induced diabetic rats

Groups	3rd day	7th day	14th day	21th day
Normal	82.5±7.3	84.9±6.2	81.3±8.8	83.9±7.8
Diabetic	238.5±13.6^{a}	256.8±18.9^{a}	271.5±23.2^{a}	261.7±3.4^{a}
Diabetic+Glibenclamide	216.3±14.9^{c}	185.3±12.5^{c}	152.3±10.3^{c}	118.7±1.6^{c}
Diabetic+Resveratrol	230.6±9.8^{b}	201.4±12.4^{c}	185.1±12.6^{c}	174.1±5.3^{c}
Diabetic+Vit C	226.8±10.4^{c}	198.1±11.6^{c}	192.2±15.7^{c}	138.7±1.8^{c}
Diabetic+Resveratrol+Vit C	216.8±12.4^{c}	181.1±13.8^{c}	162.5±13.4^{c}	126.7±1.8^{c}

Values are mean±SD; n=6 in each group; ^{a}P<0.01 when compared to normal control; ^{b}P<0.05,^{c}P<0.01, when compared to diabetic control (one way ANOVA followed by Dunnett's test).

Table 3: Effect of resveratrol and vitamin-C combination on liver protein, MDA and LH in control and experimental animals

Groups	Protein (moles/min/mg wet tissue)	MDA (nmoles/min/mg protein)	LH (nmoles/min/mg protein)
Normal	1.78±0.07	0.72±0.005	0.54±0.03
Diabetic	1.02±0.06^{a}	1.26±0.08^{a}	1.06±0.02^{a}
Diabetic+Glibenclamide	1.45±0.08^{c}	0.91±0.02^{c}	0.68±0.06^{c}
Diabetic+Resveratrol	1.28±0.05^{c}	1.08±0.06^{b}	0.92±0.05^{c}
Diabetic+Vit C	1.39±0.1^{c}	0.94±0.04^{c}	0.76±0.02^{c}
Diabetic+Resveratrol+Vit C	1.51±0.09^{c}	0.88±0.05^{c}	0.72±0.04^{c}

Values are mean±SD; n=6 in each group; ^{a}P<0.01 when compared to normal control; ^{b}P<0.05,^{c}P<0.05, when compared to diabetic control (one way ANOVA followed by Dunnett's test).

Table 4: Effect of resveratrol and vitamin-C combination on liver enzymatic and non enzymatic antioxidants in control and experimental animals

Groups	CAT (µmoles/min/mg protein)	SOD (nmoles/min/mg protein)	GSSH (nmoles/min/mg protein)	GSH (nmoles/min/mg protein)
Normal	48.6±3.8	4.6±0.3	32.8±3.2	12.6±0.9
Diabetic	36.2±4.2[a]	3.2±0.2[a]	21.2±1.6[a]	9.2±0.92[a]
Diabetic+Glibenclamide	46.3±3.8[c]	4.4±0.4[c]	32.6±3.1[c]	12.2±1.1[c]
Diabetic+Resveratrol	39.6±2.9	3.9±0.3[c]	29.5±2.8[c]	10.9±0.8[c]
Diabetic+Vit C	42.6±4.1[b]	4.1±0.4[c]	30.9±3.2[c]	11.2±1.2[c]
Diabetic+Resveratrol+Vit C	44.6±4.3[c]	4.3±0.2[c]	30.6±2.7[c]	11.8±0.7[c]

Values are mean±SD; n=6 in each group; [a]P<0.01 when compared to normal control; [b]P<0.05,[c]P<0.01, when compared to diabetic control (one way ANOVA followed by Dunnett's test).

Diabetes mellitus (DM) is a metabolic disorder principally characterized by elevated blood glucose levels and by micro vascular and macro vascular complications that considerably increase the morbidity and mortality related to the disease[17]. Around 150 million peoples are suffering from diabetic complications in the world. This disorder was associated with an increased production of reactive oxygen species (ROS) in both humans and animals. Induction of oxidative stress is a key process in the onset on diabetic complications. The mechanisms and pathways involved are complex. ROS are produced by mitochondria and various enzymes, including NADPH oxidase, xanthine oxidase, and NOS. The STZ induced diabetes provides a relevant example of endogenous chronic oxidative stress and hyperglycemia. STZ induced diabetes provides a relevant example of endogenous chronic oxidative stress and hyperglycemia.

Oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. In-vivo studies of the effect of various herbal drugs on diabetes mellitus with the aim of establishing the relationship between the free radicals, diabetes and its complication. In diabetes hypo insulinemia increases the activity of the enzymes which initiate oxidation of fatty acids, results in lipid peroxidation. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity that leads to cell injury and damage [18] STZ-induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycemic agents [19]. STZ selectively destroyed pancreatic insulin secreting β–cells, leaving less active cell resulting in a diabetic state [20].

Many secondary metabolites participate in a variety of anti-diabetic functions *in vivo* [21]. Resveratrol is a poly phenolic compound and Vitamin-C; both are antioxidants and showed antidiabetic activity. Therefore the present study is designed to evaluate the synergistic effect of resveratrol and vitamin-C combination in STZ-induced diabetic rats.

In the present study, the groups of normal rats have shown a gain body weight and fasting blood glucose levels were maintained in the normal range. STZ-injected rats showed the symptoms of diabetes mellitus such as hyperglycemia and growth retardation. It has also been observed that significantly increased fasting blood glucose in this group. The improvement in body weight gain in diabetic rats supplemented with resveratrol, vitamin C and resveratrol with vitamin C combination highlight the blood glucose homeostasis which in turn promotes the body weight gain.

Elevated blood glucose levels observed in the diabetic rats were significantly decreased in resveratrol, vitamin C and resveratrol with vitamin C combination treated groups suggesting insulin stimulated effect of resveratrol, vitamin C and resveratrol with vitamin C combination from the remnant β-cells. This was further evidenced that these drugs have anti diabetic property.

CONCLUSION

The experimental evidence obtained in the present laboratory animal study indicates that resveratrol with vitamin C combination was much more effective in diabetic complications compared to resveratrol and vitamin C alone. This may be due to enhancing effect on cellular antioxidant defences to protect against oxidative damage of resveratrol and vitamin C.

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CONFLICT OF INTERESTS

Declared None

REFERENCES

1. American Diabetes Association, Diagnosis and classification of diabetes mellitus. Diabetes Care 2007;30:42–7.
2. Pavkov ME, Hanson RL, Knowler WC, Bennett PH, Krakoff J, Nelson RG. Changing patterns of type 2 diabetes incidence among pima Indians. Diabetes Care 2007;30:1758–63.
3. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res 2004;24:2783-840.
4. Busquets S, Ametller E, Fuster G, Olivan M, Raab V, Argiles JM, *et al*. Resveratrol, a natural diphenol, reduces metastatic growth in an experimental cancer model. Cancer Lett 2007;245:144-8.
5. Chanvitayapongs S, Draczynska-Lusiak B, Sun AY. Amelioration of oxidative stress by antioxidants and resveratrol in PC12 cells. NeuroReport 1997;8:1499-502.
6. Chi TC, Chen WP, Chi TL, Kuo TF, Lee SS, Cheng JT, *et al*. Phosphatidylinositol-3-kinase is involved in the anti hypoglycemic effect induced by resveratrol in streptozotocin-induced diabetic rats. Life Sci 2007;80:1713-20.
7. Dipak K Das, Nilanjana Maulik. Resveratrol in cardioprotection: a therapeutic promise of alternative medicine. Mol Interv 2006;6:36-47.
8. Shankar S, Singh G, Srivastava RK. Chemoprevention by resveratrol: molecular mechanisms and therapeutic potential. Front Biosci 2007;12:4839-54.
9. Howerde E. Sauberlich: pharmacology of vitamin C. Annu Rev Nutr 1994;14:371-91.
10. Patriciaz craven, Valerian E, Kagan T, Rebecca K. Studer. Effects of supplementation with vitamin C or E on Albuminuria, Glomerular TGF-β, and Glomerular size in diabetes. J Am Soc Nephrol 1997;8:1405-14.
11. Lowry OH, Rosenbourgh NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951;193:265-75.
12. Nieshus WG, Samuelsson B. Formation of MDA from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem 1986;6:126-30.
13. Racker E. Glutathione reductase from bakers' yeast and beef liver. J Biol Chem 1955;217:855-66.
14. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959;82:70-7.
15. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;2:130-2.

16. Aebi H. Methods in enzymatic analysis. 2nd edition. Academic press: New York; 1974. p. 673-84.
17. Christos Kalofoutis, Christina Piperi, Anastasios Kalofoutis, Fred, Harris, David Phoenix, *et al*. Type II diabetes mellitus and cardiovascular risk factors: Current therapeutic approaches. Exp Clin Cardiol 2007;12:17–28.
18. Parthasarathy R, Ilavarasan R, Karrunakaran CM. Antidiabetic activity of Thespesia Populnea bark and leaf extracts against streptozotocin induced diabetic rats. Int J PharmTech Res 2009;1:1062-71.
19. Szkudelski T. The mechanism of alloxan and streptozocin action in β cell of the rats pancreas. Physiol Res 2001;50:536-46.
20. Kamchouing P, Sokeng DS, Moundipa FP, Watcho P, Jatsa BH Lontsi D. Protective role of anacardium occidentale extract against streptozocin-induced in rats. J Ethnopharmacol 1998;62:55-99.
21. Kako M, Miura T, Nishiyama Y, Ichimaru M, Moriyasu Kato M. Hypoglycemic activity of some triterpenoid glycosides. J Nat Prod 1997;60:604-5.