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Synergistic Effect of β-Carotene and Metformin on Antihyperglycemic and Antidyslipidemic Activities in Streptozotocin-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved of the final manuscript.

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ABSTRACT

Purpose: Worldwide prevalence of diabetes mellitus (DM) has become an issue of great concern in current decades. To date, a large number of biological properties have been reported from carotenoids, particularly protective effects against cancer, cardiovascular diseases, and DM, including enhancement of insulin sensitivity.In this study, we aimed to evaluate the efficacy of β-carotene as an additive agent with metformin in ameliorating Type2 (T2)DM.
Methods: In this experiment, fasting blood glucose level (BGL), low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol (TC) and triglycerides (TG) were measured in serum of Wister albino rats with streptozotocin (STZ)-induced diabetes and after treatment with metformin (850mg/70kg b.w.) and β-carotene (10 mg/70kg b.w.) administered orally once daily for three weeks.
Results: Metformin and β-carotene treatments individually resulted in significant ($p<0.001$) reversal of the diabetes induced increase in BGL, LDL, TC and TG, whereas significantly increased the STZ-induced decrease in HDL, compared to diabetic control. As compared to the monotherapy, the

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combination therapy with metformin and β-carotene showed a significant ($p<0.001$) attenuation of BGL and serum level of LDL, TC, and TG and a slight increase ($p<0.05$) in serum HDL level, as compared to the treatment with β-carotene, but not with metformin.
Conclusion: The combination therapy of β-carotene and metformin produced a significant antidiabetic and antihyperlipidemic effect than the monotherapy alone and provides a scientific rationale for their use in antidiabetic therapy as a potential antioxidant.

Keywords: Diabetes mellitus; antihyperglycemic; antihyperlipidemic; metformin; β-carotene.

1. INTRODUCTION

Diabetes mellitus (DM), the most common chronic disease characterized by chronic hyperglycemia, affects millions of people worldwide and is the leading cause of death. Currently, about half a billion people worldwide are living with diabetes and this number is projected to increase by 25% in 2030 and 51% in 2045 [1]. Carotenoids are a large class of natural antioxidants that are found in many vegetables, foods and other natural sources. β -carotene, a member of carotenoids and a lipophilic pigment, is found in colorful fruits and vegetables including carrots. There are more than 600 ubiquitous carotenoids known in nature, of which only 40 different carotenoids are present in human food and some have been identified in human serums and organs [2]. Recent studies have shown the beneficial effects of carotenoids in the prevention and management of a number of diseases, including cancer [3], cardiovascular disease [4], diabetes [5], and diabetic retinopathy (DR) [6].

Type 2 (T2) DM is the result of inadequate insulin production, insulin secretion, and the development of insulin resistance. Insulin resistance can lead to innumerable changes, such as systemic blood pressure increase, elevated triglyceride levels, and high density lipoprotein (HDL) levels decrease, which escalates the risk of macrovascular dysfunction [7]. Also, diminished insulin signaling is strongly associated with

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T2DM is the result of inadequate insulin production, insulin secretion, and the development of insulin resistance. Insulin resistance can lead to innumerable changes, such as systemic blood pressure increase, elevated triglyceride levels, and high density lipoprotein (HDL) levels decrease, which escalates the risk of macrovascular dysfunction [7]. Also, diminished insulin signaling is strongly associated with cardiovascular disease (CVD) in the setting of T2DM [8]. This relationship is strongly mediated by the reactive oxygen species (ROS) through their effects on vascular inflammation and dysfunction [9].

The hyperglycemia-induced oxidative stress in T2DM is one of the leading causes of pathogenesis of CVD. Also, accelerated atherosclerosis in DM patients may be associated with increased lipid peroxidation (LPO). Natural β-carotene dietary supplementation and β-carotene administration have been reported to normalize increased LDL oxidation in diabetic patients [10] and suppress STZ-induced lipid peroxidation in diabetic rats [11], respectively. Also, β-carotene therapy for 14 days prevented or reversed some diabetes-induced changes in the oxidative stress parameters in rats [12].

Metformin has been the most commonly prescribed drug for T2DM patients for almost 50 years. It is safe and effective for both as monotherapy and in combination with other oral antidiabetic drugs and insulin. At present, various types of drugs such as biguanides,

thiozolidinediones and sulfonylureas are used to treat diabetes. But monotherapy with these drugs is not enough to control diabetes alone with CVD and other complications [13]. As a result, combination therapy has become very popular for controlling glucose levels [14] and preventing cholesterol levels [15-16].

There have been some reports on the effect of different antioxidants on antihyperglycemic and antidyslipidemic activities in animal models. However, our literature review shows that there has been no study on the combined effect of metformin and β-carotene on STZ-induced rats to examine their antidiabetic and antidyslipidemic activities. Hence, this study was undertaken to investigate the synergistic effect of β-carotene with metformin on antidiabetic and antidyslipidemic activities in STZ- induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Collection of Drugs

The antidiabetic drug, metformin and the antioxidant β-carotene were the generous gift from Incepta Pharmaceuticals Ltd, Savar, Dhaka, Bangladesh. STZ was purchased from Sisko Research Laboratories Pvt. Ltd., Mumbai, India.

2.2 Experimental Animals and the Lab Environment

A total number of 25 Wister male rats weighing about 180-220 gm (3 months old) were purchased from Pharmacology Research Laboratory, Department of Pharmacy, Jahangirnagar University, Bangladesh. Prior to commencement of the experiments, all rats were acclimatized to the new environmental condition in our lab for a period of one week. The rats were fed with standard pellets collected from International Centre for Diarrhoeal Disease Research (ICDDR), Bangladesh and fresh drinking water ad libitum.The ambient temperature, humidity and 12 hours day-night cycles were maintained according to the animal care and welfare guidelines [17].

2.3 Experimental Design

25 male Wister rats were randomly assigned into 5 groups (A, B, C, D and E), 5 rats in each group (n=5). The administration of both β-carotene and metformin was given by the oral route. The scheme maintained in our experiments is as follows:

Group A	Non-diabetic control rats (NC) treated with physiological saline.
Group B	STZ-induced diabetic control rats (DC) received physiological saline.
Group C	Diabetic rats treated with metformin (daily administered 850 mg/70 kg b.w.) [18].
Group D	Diabetic rats treated with β-carotene (daily administered 10 mg/70 kg b.w.) [16].
Group E	Diabetic rats treated with metformin (425 mg/70 kg b.w.) and β-carotene (5 mg/70kgb.w.).

2.4 Induction of T2DM and the Measurement of Fasting BGL

For the induction of diabetes, overnight fasted rats were injected intraperitoneally with a freshly prepared single dose of STZ (45mg/kg b.w.) dissolved in 0.01 M citrate buffer (pH: 4.5). Following the STZ injection, rats were given drinking water supplemented with sucrose (15 g/L) for 48 h to limit early mortality, as stores of insulin are released from damaged pancreatic islets [16]. Diabetes was confirmed 3 days later by measuring the fasting serum blood glucose level using one-touch glucometer (Glucox TD-4183, Germany). The blood sample was collected from the tail vein and the rats with blood glucose levels >200 mg/dL were considered as diabetic and selected for the study.

For the experiment, the drugs metformin and β-carotene were administered orally daily for three weeks in STZ-induced diabetic rats. After three weeks of treatment, the fasting BGL was determined by the glucometer.

2.5 Biochemical Analysis

At the end of the experiment, the overnight fasted animals were anesthetized with chloroform. 3-5 milliliters of fasting blood sample was collected from thoracic artery by a heparinized syringe. After centrifugation at 4000 RPM for 10 minutes, the serum was separated for lipid measurement. Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL)-cholesterol and lowdensity lipoprotein (LDL)-cholesterol were determined in the serum using commercial kit reagents (Human, Wiesbaden, Germany), following the company's instructionand the absorbance was read using a UV-Vis spectrophotometer (Shimadzu UV-1280, Kyoto, Japan).

2.6 Statistical Analysis

The average of five samples was reported as the measured value with standard deviation. The difference among the groups was calculated by one-way analysis of variance (ANOVA) with multiple comparison tests, where applicable. The difference between two groups was measured by the student t-test. A p- value at <0.05 was considered to be statistically significant.

3. RESULTS

The effects of the treatment alone with metformin or β-carotene and their combination (metformin + β- carotene) on the parameters of fasting BGL and lipid profile (TC, TG, LDL, HDL) were performed on STZ- induced diabetic rats.

3.1 Effect of Metformin and β-carotene on Fasting BGL in STZ-induced Diabetic Rats

The effect of metformin, β-carotene and combination of metformin with β-carotene were tested on the fasting BGL in non-diabetic and diabetic rats on Day 0 and Day 21, and are shown in Figure 1. Intraperitoneal injection of STZ in rats significantly ($p<0.001$) increased the fasting BGL (20.42 ± 1.81 mmol/L), when compared to NC rats (5.68 ± 0.36 mmol/L) on Day 0, and the fasting BGL still remained high (18.66± 0.639 mmol/L) and significant ($p<0.001$) compared to non-diabetic rats after three weeks.

However, as measured by one-way ANOVA with multiple comparison test, Gr-C through Gr-E exhibited a significant hypoglycemic effect tested on Day 21, when compared to diabetic control (Gr-C: 7.48 ± 0.69 mmol/L, Gr-D: 13.88 ± 0.33 mmol/L, Gr-E: 5.82 ± 0.31 mmol/L vs. Gr-B: 18.66 ± 0.64 mmol/L, $p<0.001$). Additionally, the combination therapy reduced the fasting blood glucose level significantly ($p<0.001$) than their monotherapy (Figure 1). Also, an intragroup analysis as measured by t-test between Day 0 and Day 21, the fasting BGL revealed a significant ($p<0.001$) difference in Gr-B to Gr-E (Fig. 1).

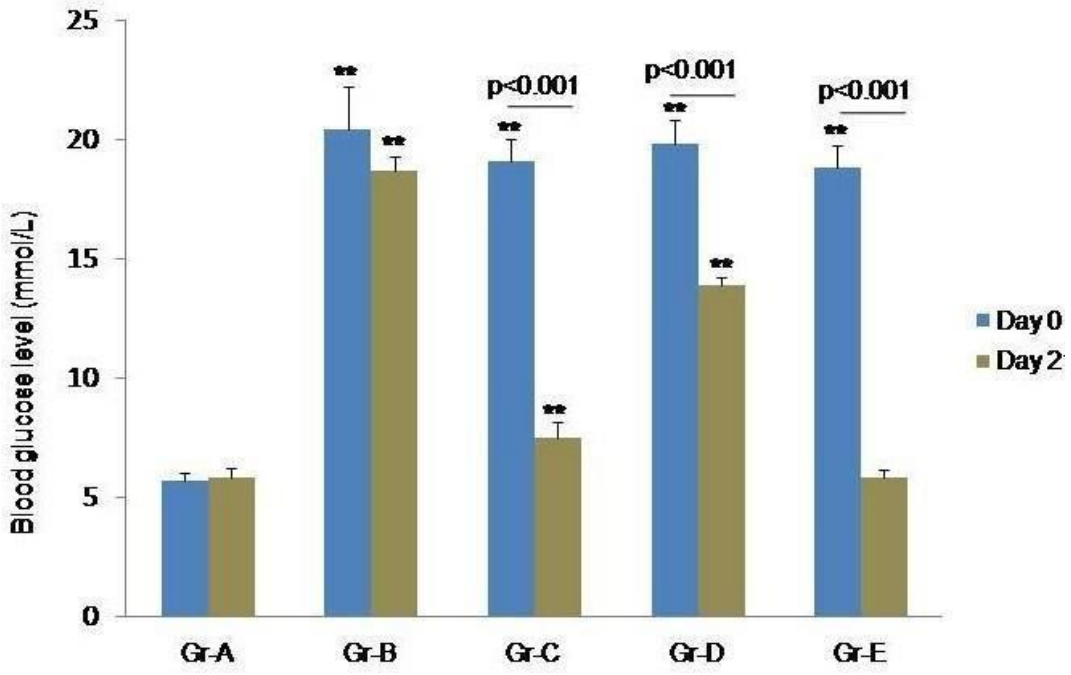

Fig. 1. Effect of metformin and β-carotene on fasting blood glucose in normal and STZ-induced diabetic rats

*n=5; Data are means ± SD. The difference among the groups on day 21 was analyzed by one-way ANOVA followed by multiple comparison tests and the difference between Day 0 and Day 21 of the same group was analyzed by Student t-test. **p<0.001 vs. respective NC. Gr-A: NC — Normal control; Gr-B: DC - Diabetic control; Gr-C: Diabetic rats+Metformin; Gr-D: Diabetic rats+β-carotene; Gr-E: Diabetic rats+Met+β-carotene*

3.2 Effect of Combined Therapy on Lipid Profile in STZ-Induced Diabetic Rats

The effect of combined treatment of metformin and β-carotene on lipid profile in diabetic rats, as compared to their monotherapy, was shown (Figure 2 and 3). The STZ-induced diabetic control (DC, Gr- B) significantly ($p<0.001$) elevated the serum level of LDL (117.08 ± 3.22 mg/dl), TC (227.14 ± 3.03 mg/dl), and TG (181.86 ± 1.87 mg/dl) (Figure 2, 3), but significantly reduced the level of HDL (24.66± 1.47 mg/dl), as compared to NC (Gr-A, Figure 2). However, both the treatment with metformin and β-carotene significantly reduced the levels of LDL ($p<0.001$), TC ($p<0.001$), and TG ($p<0.001$), but significantly ($p<0.001$) increased the level of HDL, when compared with their respective diabetic control, Gr-B (Figure 2, 3). Additionally, the combined treatment with metformin + β-carotene significantly reduced the level of LDL, TC, TG, when compared to their monotherapy. On the other hand, the combined therapy markedly increased HDL value compared to β-carotene treatment alone ($p=0.004$), but failed to increase the level of HDL compared to metformin (Figure 2, 3). Also shown in Figure 2 and 3, the combination therapy with metformin and β-carotene showed a significant ($p<0.001$) decrease in TC and TG level, when compared to both the monotherapy.

The difference among the groups was analyzed by one-way ANOVA followed by multiple comparison test. Gr-A: NC – Normal control; Gr-B: DC - Diabetic control; Gr-C: Diabetic rats+Metformin; Gr-D: Diabetic rats+β-carotene; Gr-E: Diabetic rats+Met+β-carotene.

4. DISCUSSION

Metformin is still a first line oral drug used worldwide to treat T2DM. The molecular mechanism of metformin action is not completely understood. However, the hypoglycemic effect of metformin is thought to be mediated through the suppression of hepatic gluconeogenesis [19], enhancement of insulin sensitivity via increased peripheral glucose uptake and utilization [20] reduced intestinal absorption of glucose [21] and translocation of glucose transporter GLUT4, a major mediator of glucose removal from the circulation [22]. Also in combination with metformin, the most commonly prescribed second-line therapies used are dipeptidyl peptidase-4 inhibitor or sulfonylurea [23]. However, to avoid long-term unreasonable drug use and their adverse side effects in human body, a combination therapy of metformin with natural products has become popular for the treatment of T2DM. Moreover, many combinations of metformin with natural preparations have shown additive effects, mainly related to the antihyperglycemic potentials. In this sense, the search for novel combined therapies based on an antioxidant β-carotene with the ability to improve the efficacy of glycemic control promoted by metformin and to delay or even avoid the transition to insulin therapy is of great interest. Hence, in our study, we evaluated the therapeutic potential of β-carotene in combination with metformin to improve glycemia and dyslipidemia in STZ-induced diabetic rats.

After three weeks of treatment, our results showed that β-carotene combined with metformin had a more beneficial promotion than their monotherapy treatment for reducing the serum fasting blood glucose with a concomitant significant decrease in the serum lipid profiles, such as LDL, TC and TG in STZ-induced diabetic rats, which indicates the synergistic nature of their interaction in lowering the level of metabolic parameters tested in diabetic condition. However, the combination therapy of β-carotene with metformin in our experiments slightly increased the level of serum HDL, but failed to elevate the serum HDL level in comparison to monotherapy treatments. The inability of not achieving the expected level of serum HDL is possibly due to the reduction of β-carotene dose in combination therapy by two folds (from 850 mg/70 kg bw and 10 mg/70 kg bw to 425 mg/70 kg bw and 5 mg/70 kg bw, respectively), and thus lack of enough synergistic action of the drugs, though β-carotene as a monotherapy significantly increased the level of HDL against the diabetic control showing the antioxidant power of β-carotene per se. Moreover, the bioavailability of β-carotene is low in case of absorption through the gut, though rodents (rats and mice) can readily convert β-carotene to vitamin A [24]. Therefore, administration of β-carotene at higher doses both in mono- and combined therapy than physiologically required needs more attention in our future research.

Hyperglycemia induces ROS production that leads to increased oxidative stress. It is known that antioxidants play a very important role in the antioxidant defense system of the body against ROS. Also, diabetes and the increased oxidative stress associated with it are major risk factors for

the development of cardiovascular disorders [25]. The administration β-carotene is reported to reduce oxidative stress and increase antioxidant activity, mainly glutathione-related, of defense systems in workers chronically exposed to lead [25]. Also, oxidative stress in DR is involved in apoptosis by activating the retinal caspase-3 in endothelial cells and pericytes, as studied in the retina of alloxan-induced diabetic rats, but β-carotene and other antioxidants were found to inhibit diabetes-mediated activation of Caspase-3 [26]. Also, it has been reported that the administration of β-carotene suppresses the elevation of LPO and reduces the symptoms of DM in the STZ-induced diabetic rats [11].

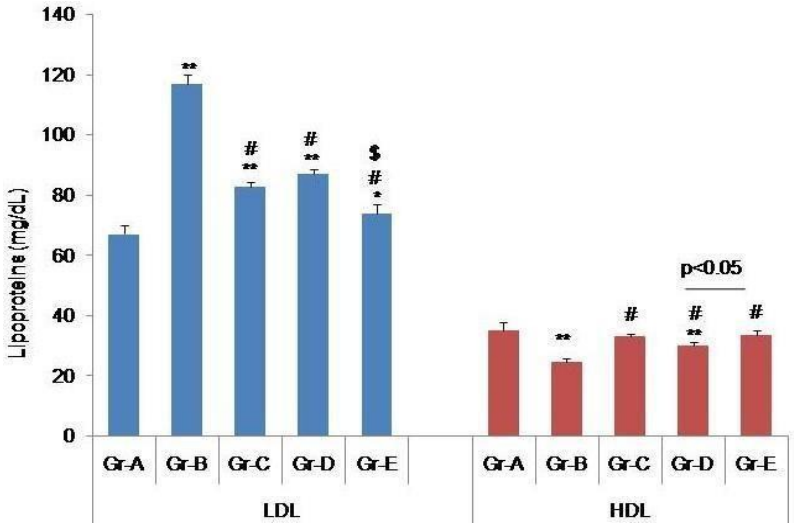

Fig. 2. Effect of metformin and β-carotene on Lipoproteins (LDL and HDL) in normal and STZ-induced diabetic rats after three weeks of treatment

*n = 5; Data are means ± SD. *p<0.05, **p<0.001, vs. respective control (Gr-A); #p<0.001, vs. respective diabetic control (Gr-B); $p<0.001, vs. Gr-C and Gr-D. The difference in lipid profile among the groups was analyzed by one-way ANOVA followed by multiple comparison test. Gr-A: NC — Normal control; Gr-B: DC - Diabetic control; Gr-C: Diabetic rats+Metformin; Gr-D: Diabetic rats+β-carotene; Gr-E: Diabetic rats+Met+β-carotene*

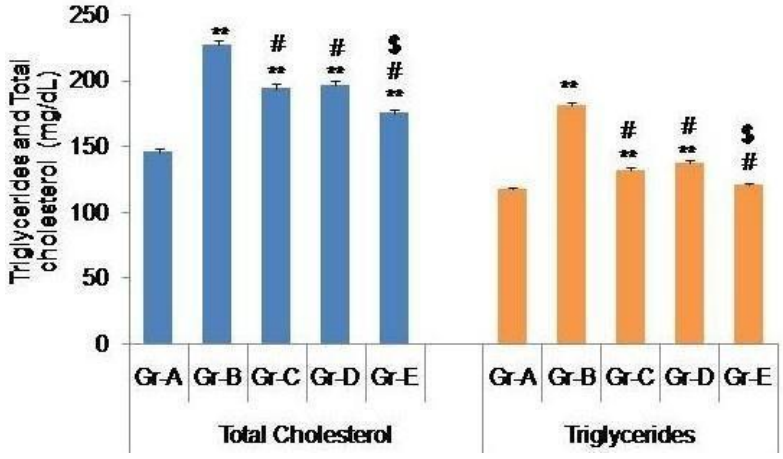

Fig. 3. Effect of metformin and β-carotene on total cholesterol and triglycerides in normal and STZ-induced diabetic rats after three weeks of treatment

*Data are means ± SD. n=5; **p<0.001, vs. respective control (Gr-A); #p<0.001, vs. respective diabetic control (Gr-B); $p<0.001, vs. Gr-C and Gr-D*

In our experiment, we have selected β-carotene in combination therapy, as β-carotene was reported to be protective against diabetes via enhancing insulin sensitivity [5] and also protective against DR [27] and cardiovascular diseases [4]. Corroborating with the findings [28,29], our results showed that β-carotene markedly reduced serum triglycerides, total cholesterol and other lipid indices of diabetic risk in diabetic rats and thus suggest antihyperglycemic and antidyslipidemic potentials of β-carotene in reducing the incidence of diabetic vascular complications through the normalization of lipid metabolism in diabetic condition. Therefore, Food sources supplemented with β┼carotene or even molecular therapies seem to be the future [30]. Additionally, other studies have confirmed the benefits of medicinal plants with hypoglycemic effects and may delay the development of diabetic complications and correct the metabolic abnormalities [31]. More work is required to truly understand how β-carotene works at the molecular level in individuals with T2DM.

5. CONCLUSION

The synergistic effect of an antioxidant β-carotene combined with the leading antidiabetic drug, metformin, showed the additive effect on decreasing glycemia and dyslipidemia possibly by reducing glycooxidative stress in diabetic rats. Therefore, combining current antibiotic drugs with β-carotene with beneficial effects for diabetic disorders may be a promising therapeutic strategy. However, further research is needed to understand how this combination therapy works at the molecular level.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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