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Antidiabetic and Antihyperlipidemic Activity of β-carotene on 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 the final manuscript.

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Original Research Article

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ABSTRACT

Objective: A vital anti-oxidant, β-carotene has the capacity to reduce reactive oxidative stress, metabolic syndrome such as Type 2 (T2) Diabetes Mellitus (DM) and prevent inflammation, obesity, alzheimer and cardiovascular diseases in human. In this study, we evaluated the efficacy of β-carotene on streptozotocin (STZ)- induced T2DM rats.
Methods: Diabetes was induced in Wister rats through the intraperitoneal administration of STZ (50 mg/kg b.w.). Antihyperlipidemic activities of β-carotene were evaluated by oral dose (10 mg/70 kg b.w.) once daily for 21 days. Metformin (12.1 mg/kg b.w.) was used as a positive control.
Results: Blood samples of rats were drawn by tail vein puncture and cardiac puncture to determine the fasting blood glucose (FBG) and serum level of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), respectively. The result of individual

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treatment of β-carotene and metformin significantly ($p<0.001$) reversed the diabetes induced increase in FBG, LDL, TC and TG, whereas pointedly increased the STZ-induced decrease in HDL, if compared to the diabetic control.

Conclusion: The monotherapy of β-carotene had important antidiabetic and antihyperlipidemic effects and provided a scientific rationale for their use in antidiabetic therapy as a potential antioxidant.

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

1. INTRODUCTION

β-carotene is most abundant carotene among all carotenoids in fruits, vegetables and human. Due to the high bioavailability of β-carotene, nearly 17% to 45% injected β-carotene remains intact in plant cells [1]. It is a vital anti-oxidant and has capacity to reduce reactive oxidative stress and can prevent metabolic syndrome such as T2DM and vascular complication in human [2-4].

Diabetes mellitus (DM) is most common chronic metabolic disorder. It has high mortality and morbidity rate due to significant effect on microvascular and macrovascular complications [5]. The disease is rapidly spread and affect at every corner in the world. T2DM is a non- insulin dependent diabetes. More than 90%–95% patient of T2DM does not produce enough insulin or cannot properly use it [6]. According to the report of World Health Organization (WHO), around 300 billion or more peoples will be affected by diabetes mellitus in the year of 2025 in all over the world. The DM-induced death reached at 28,065 or 3.61% of total deaths in Bangladesh [7-8]. While the total focus of diabetes management on hyperglycemia as much as 75% of diabetic patients ultimately dying from vascular disease [9]. T2DM showed severe lipid metabolism, abnormality persist even after optimal glycemic control has been achieved [10].

The oxidative stress also induced the pathophysiology of T2DM and vascular complication. It was found that the prooxidant activity significantly increased and antioxidant has been decreased in T2DM. Consequently, the decrease production of reactive oxygen scavenging enzyme named superoxide dismutase (SOD) and catalase have been reported in diabetic animals [11-16]. The significant increase in reactive oxygen and reduce the scavenging capacity of free radical act as trigger on lipid metabolism that were the leading causes of cardiovascular diseases [17].

"Metformin is the most common, old and widely accepted drug used for management and treatment of T2DM. It remarkably reduces oxidative stress and dicarbonyl stress in human. Still now it is safe and effective as a monotherapy than combination therapy with other antidiabetic drugs and insulin" [18].

"The antioxidant such as *N*-acetylcysteine, vitamin C, vitamin E and α-lipoic acid are effective in reducing diabetes complications. β-carotene act as a chelator of singlet oxygen and reacts with different species of free radicals (e.g., hydrogen peroxide). The conjugated double chain is responsible for eliminating singlet oxygen" [19-20]. However, our literature review shows that there has been no study on the β-carotene on STZ-induced rats to examine their antidiabetic and antihyperlipidemic activities. Hence, this study was undertaken to investigate the effect of β-carotene and metformin on antidiabetic and antihyperlipidemic activities in STZ-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Collection of Drugs

The metformin and the β-carotene were the gift from Incepta Pharmaceuticals Ltd, Savar, Dhaka, Bangladesh. STZ was purchased from Sisko Research Laboratories Pvt. Ltd., Mumbai, India. Kits for the examination of high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), triglycerides (TG) were obtained from Human, Wiesbaden, Germany. All the other chemicals and reagents were of the analytical grade.

2.2 Experimental Animals and the Lab Environment

Three months old, twenty healthy male Wister albino rats (180-220 gm) were procured from Pharmacology Research Laboratory, Department of Pharmacy, Jahangirnagar University, Bangladesh. At the beginning of the experiments, all rats were adjusted in the laboratory environmental condition for one week. The

research followed the procedure for laboratory animal care and use (NIH publication No.85-23, revised in 2004). "The rats were fed with standard pellets collected from International Centre for Diarrhoeal Disease Research (ICDDR), Bangladesh and fresh drinking water ad libitum during this period. Under normal atmospheric temperature (25 ± 2 °C), humidity and 12 hours light- dark cycles were controlled according to the animal care and welfare guidelines" [21].

2.3 Experimental Design

The male Wister albino rats (20) were randomly grouped into 4 groups (A, B, C and D) were 5 rats in each (n=5). The administration of both β-carotene and metformin was given by the oral route.

Group A (Gr. A): Non-diabetic control rats (NC) treated with physiological saline.
Group B (Gr. B): STZ-induced diabetic control rats (DC) received physiological saline.
Group C (Gr. C): Diabetic rats treated with metformin (daily administered 850 mg/70 kg b.w.) [22].
Group D (Gr. D): Diabetic rats treated with β-carotene (daily administered 10 mg/70 kg b.w.) [23].

2.4 Induction of T2DM and the Measurement of Fasting BGL

For the damage of insulin producing pancreatic islets, freshly prepared single dose of STZ (45mg/kg b.w. were dissolved in 0.01 M citrate buffer (pH: 4.5)) were injected intraperitoneally in overnight fasted rats. After the STZ injection, rats were given drinking water supplemented with sucrose (15 g/L) for 48 h to limit early mortality [23]. Diabetes was confirmed in rats after 3 days of STZ injection. The hypoglycemic condition was measured by the fasting serum blood glucose level using one-touch glucometer (Glucox TD-4183, Germany). The blood glucose levels >200 mg/dL were considered as diabetes and selected for the study. After three weeks (day 21) treatment with metformin and β-carotene in STZ-induced diabetic rats, the rats were fasted overnight and sacrificed. Blood samples of rats were drawn by tail vein puncture to determine the fasting blood glucose and cardiac puncture for lipid profile.

2.5 Biochemical Analysis

At the end of the experiment, the overnight fasted rats were anesthetized with chloroform. 3 mL to 5 mL 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 low density lipoprotein (LDL)-cholesterol were determined in the serum using commercial kit reagents (Human, Wiesbaden, Germany), following the company's instruction and 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 metformin and β- carotene on the parameters of blood glucose level and lipid profile (TC, TG, LDL, HDL) were performed on STZ-induced diabetic rats.

3.1 Hypoglycemic Effects of Metformin and β-carotene in STZ- Induced Diabetic Rats

The effect of metformin and β-carotene were separately determined on FBG level in STZ-induced diabetic rats groups at day 0 and day 21 were shown in Fig. 1. At the day 0, the FBG level of STZ - induced diabetic (DC) rats was significantly ($p<0.05$) higher (20.42±0.816mmol/L) than those of non-diabetic (NC) rats (5.68± 0.159mmol/L). At the day 21, the FBG of DC group remained higher and significant (18.66±0.51mmol/L) compared to NC rat groups. After the treatment of β-carotene, FBG level became reduced (13.88±0.816 mmol/L) remarkably in the same manner as that with the standard drug metformin (7.48±0.11 mmol/L).

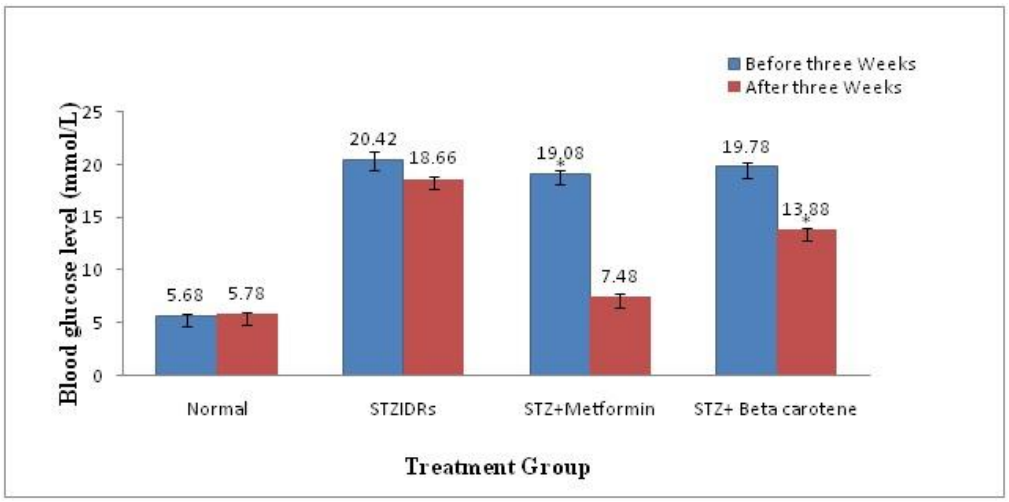

Fig. 1. The hypoglycemic effect of metformin and β-carotene were determined on FBG level in STZ- induced diabetic rat groups and compared with diabetic control (Gr. B) and normal control group (Gr. A) at day 0 and day 21. n=5, the values are expressed as mean ± SEM. P < 0.05 compared with normal control group (Gr. A). *P < 0.05 compared with diabetic control group (Gr. B)

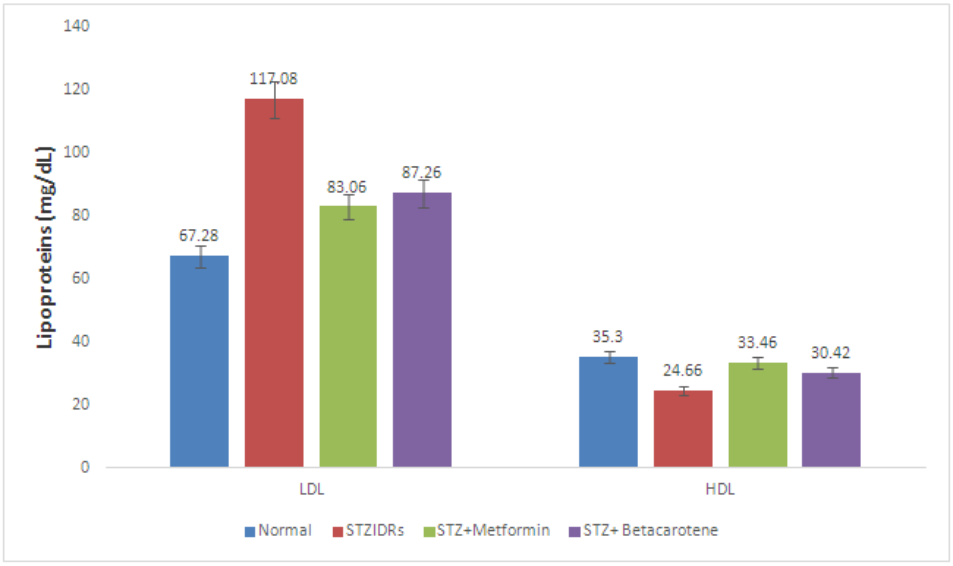

Fig. 2. Effect of metformin and β-carotene on Lipoproteins (LDL and HDL) in normal (Gr. A) and STZ-induced diabetic (Gr. B) rats after three weeks of treatment. n=5, the values are expressed as mean ± SEM. †P < 0.05 compared with normal control (Gr. A) group. *P < 0.05 compared with diabetic control (Gr. B) group.

3.2 The Effect of Metformin and β-carotene on Lipoprotein (LDL and HDL) in STZ- Induced Diabetic Rats

Measurement the effect of metformin and β-carotene of serum lipoprotein (LDL and HDL) on STZ- induced diabetic rats is shown in Fig. 2. The serum concentration of LDL levels increased and HDL levels were decreased as compared with diabetic controlled rat groups (Gr.B). Repeated treatment by metformin and β-carotene were able to decrease ($P < 0.05$) the serum concentration of LDL (87.26±0.021 mg/dL) whereas increase ($P < 0.05$) the level of HDL (30.42± 0.033 mg/dL) as compared with STZ-induced diabetic control (24.66± 0.03 mg/dL) rats.

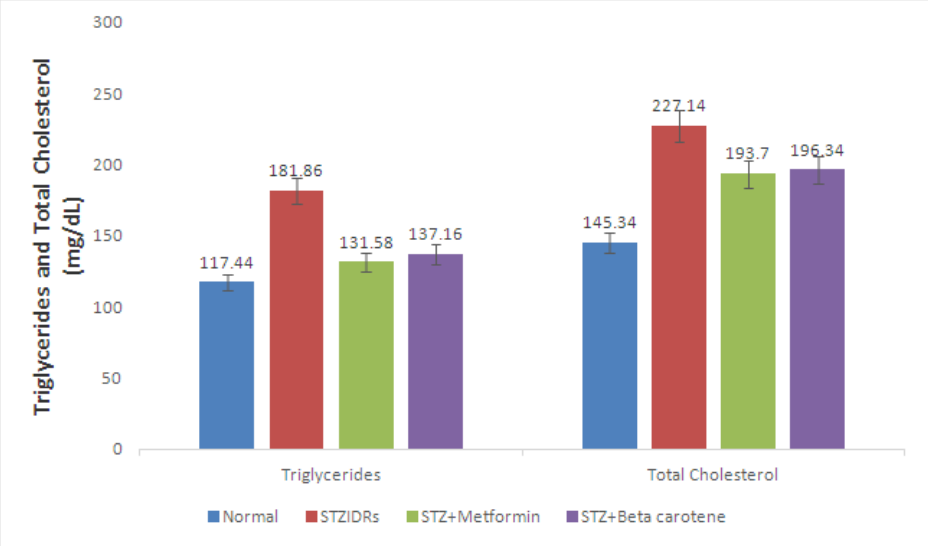

Fig. 3. Effect of metformin and β-carotene on triglyceride (TG) and total cholesterol (TC) in normal and STZ- induced diabetic rats after three weeks of treatment. n=5, the values are expressed as mean ± SEM. †P < 0.05 compared with normal control group (Gr. A) *P < 0.05 compared with diabetic control group (Gr. B)

3.3 The Effect of Metformin and β-carotene on the Concentration of Serum TC and TG in STZ- Induced Diabetic Rats

Determination the effect of metformin and β-carotene on the concentration of serum TC and TG level was determined on STZ- induced diabetic rats and is found in Fig. 3. The serum concentration of TC and TG levels were decreased as compared with diabetic controlled rats group (Gr. B). Repeated treatment by metformin and β-carotene had ability to decrease ($P < 0.05$) the serum concentration of TG (137.16 ±0.027 mg/dL) and TC (196.34 ±0.025 mg/dL) level as compared with the TG and TC level on STZ- induced diabetic control (Gr. B) rats.

4. DISCUSSION

Diabetes is an abundant metabolic disorder found all over the world. Insulin resistance and hyperglycemia could associate with vascular complication. Non-insulin dependent T2DM leads to the change the plasma lipoprotein concentration [24]. We investigated the antidiabetic and antihyperlipidimic activity of β-carotene on STZ- induced diabetic rats.

From our short term study confirmed that, the effect of the oral intake of β-carotene in a single dose (10 mg/70 kg b.w) in STZ- induced diabetic rats was significantly reduced the FBG level. The values of FBG after 21 days became turned down (13.88±0.816 mmol/L) remarkably in the same manner as happened with the standard drug metformin (7.48±0.11 mmol/L). Still now, metfornin is an old and magical first line of oral drug used worldwide for the treatment of T2DM. It can improve the endothelial dysfunction, oxidative stress, insulin resistance, lipid profiles, fat redistribution and increased peripheral glucose uptake and utilization [25], reduced intestinal absorption of glucose [26] and translocation of glucose transporter GLUT4 from the circulation [27]. Also, Gemfibrozil with metformin can decrease MMP-9, increase IL-10 and adiponectin acting as anti-atherogenic, anti-inflammatory and immunomodulatory drug in IR. T2DM [28]. Beside this, long term treatment of metformin have some side effects also [29]. To avoid the complication of metformin researcher hunt natural or herbal product or those substance which has ability to have antihyperglycemic and antihyperlipidemic activity. In this sense, the therapies based on an antioxidant β-carotene had the ability to improve the efficacy of glycemic control promoted by metformin to avoid the transition of insulin therapy is of great interest [30]. Also, it was found that the mono therapy of antioxidant; like as *N*-acetylcysteine, vitamin C, and α-lipoic acid had capability to reduced diabetes complications [31]. It also found that combination therapy such as alcoholic extracts of *Annona squamosa* fruit peel and Glibenclamide

showed synergistic effects on hyperglycemic and hyperlipidaemic activities [32]. Both root extract of *Piper chaba* and seed extract of *Cucurbita maxima* resulted in a significant reduction of FBG and lipid profile parameter [33,34]. In this consequence, the novel therapies based on single dose of β-carotene have an ability to control hyperglycemia might be used as antihyperglycemic therapy.

After day 21, we found that monotherapy of β-carotene reduced the serum FBG with significant decrease in the serum lipid profiles, such as LDL, TC and TG level. However, monotherapy of β-carotene slightly increased the level of serum HDL against the diabetes control (Gr. B). "The similar results also found that β-carotene can reduce, low-density lipoprotein cholesterol (LDL-c), and very-low-density lipoprotein cholesterol (VLDL-c); and an increase in high-density lipoprotein cholesterol (HDL-c). Moreover, it can improve insulin resistance and insulin binding receptor protein on cell wall in human [35-38], also noted that the bioavailability of β-carotene is low through the gut, though rodents (rats and mice) can readily convert β-carotene to vitamin A" [39]. Therefore, administration of β-carotene at higher doses required needs more attention in our future research.

"High level of glucose concentration in blood stimulates ROS production that leads to increased oxidative stress. The antioxidants play vital role against ROS through the antioxidant defense system of the body. Also, diabetes and the increased oxidative stress related protein are the major risk factors for the development of cardiovascular disease" [40]. "The β-carotene is reported to reduce oxidative stress and increase antioxidant activity, mainly glutathione-related defense systems in lead-exposed worker" [41]. "Plasma triglyceride and total cholesterol was increased by diabetes, but it was reduced by β-carotene. It has been reported that the higher plasma concentration of β-carotene could improved insulin resistance and help to reduction of body adiposity" [42,43]. "Also that the administration of β-carotene suppresses the elevation of lipid peroxidation (LPO) and reduces the symptoms of DM in the STZ-induced diabetic rats" [44].

"In our experiment, we have selected β-carotene as a mono therapy, as β-carotene was reported to be defensive against diabetes via improving insulin sensitivity and also shielding against DR [45] and cardiovascular diseases" [46]. "Justifying it with the findings [47,48], our results showed that β-carotene markedly reduced serum triglycerides, total cholesterol and other lipid indices of diabetes risk in diabetic rats and thus suggest antihyperglycemic and antidyslipidaemic potentials of β-carotene in reducing the incidence of diabetes vascular complications through the normalization of lipid metabolism in diabetes condition. More work is required to truly understand how β-carotene works at the molecular level in individuals with T2DM".

5. CONCLUSION

β-carotene is an anti-oxidant and it had an effect on oxidative stress in diabetes rats. From our experiment it may be concluded that β-carotene had anti-glycemic activity on STZ- induced diabetes rats as well as antihyperlipidemic activity by reducing the glycooxidative stress in blood. Therefore, the β-carotene it is very promising antidiabetic and antihyperlipidemic drug like as traditional antidiabetic drugs metformin. Further studies are needed to know how β-carotene works at molecular level.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shete V, Quadro L. Mammalian metabolism of β-carotene: gaps in knowledge. Nutrients. 2013;5(12):4849-68. DOI: 10.3390/nu5124849

2. Mounien L, Tourniaire F, Landrier JF. Anti-Obesity Effect of Carotenoids: Direct Impact on Adipose Tissue and Adipose Tissue-Driven Indirect Effects. Nutrients. 2019;11 (7):1562.
DOI: 10.3390/nu11071562
3. Rodriguez-Concepcion M, Avalos J, Bonet ML, Boronat A, Gomez-Gomez L, Hornero-Mendez D, Limon MC, Meléndez-Martínez AJ, Olmedilla-Alonso B, Palou A, Ribot J, Rodrigo MJ, Zacarias L, Zhu C. A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. Prog Lipid Res. 2018;70:62-93.
DOI: 10.1016/j.plipres.2018.04.004
4. Conrad O. Perera, Gan Mei Yen, Functional Properties of Carotenoids in Human Health, International Journal of Food Properties. 2007; 10:2, 201- 230.
DOI: 10.1080/10942910601045271
5. Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and in vitro antioxidant potential of Hybanthus enneaspermus (Linn) F. Muell in streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed. 2011;1(4):316-22.
DOI: 10.1016/S2221-1691(11)60051-8
6. Li WL, Zheng HC, Bukuru J,De Kimpe N. Natural medicines used in traditional Chinese medical system for therapy of diabetic mellitus. J. Ethnopharmacol. 2004;92:1-21.
7. Sy GY, Cissé A, Nongonierma RB, Sarr M, Mbodj NA, Faye B. Hypoglycaemic and antidiabetic activity of acetonic extract of Vernonia colorata leaves in normoglycaemic and alloxan-induced diabetic rats. J Ethnopharmacol. 2005; 98(1-2):171-5.
8. Web World Health Rankings. Bangladesh: Diabetes Mellitus.
Available:https://www.worldlifeexpectancy.com/ bangladesh-diabetes-mellitus
[Last accessed on 10 Apr 2021]
9. Basha B, Samuel SM, Triggle CR, and Ding H. Endothelial Dysfunction in Diabetes Mellitus: Possible Involvement of Endoplasmic Reticulum Stress?. Exp Diabetes Res. 2012; 2012:481840.
10. O'Brien T, Nguyen TT, Zimmerman BR. Hyperlipidemia and diabetes mellitus. Mayo Clin Proc. 1998;73(10): 969-76.
DOI: 10.4065/73.10.969
11. Beladi Mousavi SS, Nasri H, Rafieian-Kopaei M, Tamadon MR. Metformin improves diabetic kidney disease. J Nephropharmacol. 2012;1(1):1-2.
12. Ayodhya S, Kusum S, Saxena A. Hypoglycaemic activity of different extracts of various herbal plants. Int J Res Ayurveda Pharm. 2010;1:212.
13. Abdollahi M, Farshchi A, Nikfar S, Seyedifar M. Effect of chromium on glucose and lipid profiles in patients with type 2 diabetes; a meta-analysis review of randomized trials. J Pharm Pharm Sci. 2013;16(1):99-114.
DOI: 10.18433/j3g022
14. Rafieian-Kopaei M, Nasri H. Ginger and diabetic nephropathy. J Renal Inj Prev. 2013;2(1):9-10.
DOI: 10.12861/jrip.2013.05
15. Nasri H, Rafieian-Kopaei M. Tubular Kidney Protection by Antioxidants. Iran J Public Health. 2013;42(10):1194-6.
16. Ardalan MR, Sanadgol H, Nasri H, Baradaran A, Tamadon MR, Rafieian-Kopaei R. Vitamin D therapy in diabetic kidney disease; current knowledge on a public health problem. J Parathyr Dis. 2014;2:15-7.
17. Basha B, Samuel SM, Triggle CR, Ding H. Endothelial dysfunction in diabetes mellitus: possible involvement of endoplasmic reticulum stress?. Exp Diabetes Res. 2012;481840.
DOI: 10.1155/2012/481840
18. Rojas LB, Gomes MB. Metformin: an old but still the best treatment for type 2 diabetes. Diabetol Metab Syndr. 2013; 5(1):6.
DOI: 10.1186/1758-5996-5-6
19. Namitha KK, Negi PS. Chemistry and biotechnology of carotenoids. Crit Rev Food Sci Nutr. 2010;50(8):728-60.
DOI: 10.1080/10408398.2010.499811
20. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 2014;6(2): 466-88.
DOI: 10.3390/nu6020466
21. Roy P, Islam M, Islam MS, Rashid M. Beneficial Effects of Combination Therapy of Sitagliptin and β-carotene Drugs on Streptozotocin-induced Diabetic Rats. Bangladesh Pharm J. 2020;23:87-95.
22. Guide for the Care and Use of Laboratory Animals. 8th Edition. Washington DC: The National Academies Press; 2011.
23. Zannah S, Islam M, Ali Y, Asaduzzaman M, Sarwar MS, Al Bari AA, Rashid M. Antidiabetics in Combination with

Hydroxychloroquine Improve Antioxidant and Hepatoprotective Activities in Alloxan-Induced Diabetic Rats. Bangladesh pharm J. 2015;18:72-77.

24. Ira J. Goldberg. Diabetic Dyslipidemia: Causes and Consequences. J. Clin. Endocrinol. Metab. 2001;86(1):965–971. Available:https://doi.org/10.1210/jcem.86.3.7304.
25. Viollet B, Guigas B, Garcia NS, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond). 2012;122: 253-70.
26. Minamii T, Nogami M, Ogawa W. Mechanisms of metformin action: In and out of the gut. J Diabetes Investig. 2018;9:701-703.
27. Yang J, Holman GD. Long-term metformin treatment stimulates cardiomyocyte glucose transport through an AMP-activated protein kinase-dependent reduction in GLUT4 endocytosis. Endocrinology. 2006;147:2728-36.
28. Sharma AK, Raikwar SK, Kurmi MK, Srinivasan BP. Gemfibrozil and its combination with metformin on pleiotropic effect on IL-10 and adiponectin and anti-atherogenic treatment in insulin resistant type 2 diabetes mellitus rats. Inflammopharmacology. 2013;21(2): 137-45. DOI: 10.1007/s10787-012-0154-4
29. Ghosal S and Ghosal S. The Side Effects of Metformin - A Review. J Diabetes Metab Disord. 2019; 6 030. DOI: 10.24966/DMD-201X/100030.
30. Khanam K, Rajia S, Yeasmin M, Morshed M, and Haque R. Synergistic Effect of β-Carotene and Metformin on Antihyperglycemic and Antidyslipidemic Activities in Streptozotocin-Induced Diabetic Rats. J. Pharm. Res. Int. 2021; 33(51A):79-87. DOI: 10.9734/jpri/2021/v33i51A33471
31. Ruhe RC, McDonald RB. Use of Antioxidant Nutrients in the Prevention and Treatment of Type 2 Diabetes. J Am Coll Nutr. 2001;20:363S-9.
32. Sharma A, Chand T, Khardiya M, Yadav KC, Mangal R, Sharma AK, Antidiabetic and Antihyperlipidemic Activity of Annona Squamosa Fruit Peel in Streptozotocin Induced Diabetic Rats. IJPTR. 2013; 5(1):15-21.
33. Rajia S, Khanam K, Farhana U, Rahman S, Haque R. *Piper Chaba* Extracts With Antibiofilm Activity Influence Antihyperglycemic And Antihyperlipidemic Responses In Diabetic Wister Rats. Int J Pharm Pharm Sci [Internet]. 2021;13(9): 44-9.
34. Sharma A, Sharma AK, Chand T, Khardiya M, Yadav KC. Antidiabetic and Antihyperlipidemic Activity of *Cucurbita maxima* Duchense (Pumpkin) Seeds on Streptozotocin Induced Diabetic Rats. RJPP. 2013;1(6):108-116.
35. Amengual J, Gouranton E, Helden YGJ, Hessel S, Ribot J, Kramer E, Kiec-Wilk B, Razny U, Lietz A, Wyss A, et al. Beta-carotene reduces body adiposity of mice via BCMO1. PLoS ONE. 2011;6:e20644.
36. Beydoun MA, Chen X, Jha K, Beydoun HA, Zonderman AB, Canas JA. Carotenoids, vitamin A, and their association with the metabolic syndrome: A systematic review and meta-analysis. Nutr. Rev. 2019;77:32–45.
37. Canas JA, Lochrie A, McGowan AG, Hossain J, Schettino C, Balagopal PB. Effects of mixed carotenoids on adipokines and abdominal adiposity in children: A pilot study. J. Clin. Endocrinol. Metab. 2017;102:1983–1990.
38. Asemi Z, Alizadeh SA, Ahmad K, Goli M, Esmailzadeh A. Effects of beta-carotene fortified symbiotic food on metabolic control of patients with type 2 diabetes mellitus: A double-blind randomized cross-over controlled clinical trial. Clin. Nutr. 2016;35:819–825.
39. Szuszkiewicz-Garcia MM, Davidson JA. Cardiovascular disease in diabetes mellitus: Risk factors and medical therapy. Endocrinol Metab Clin of N Am. 2014;43:25-40.
40. Braunwald E. Biomarkers in heart failure. N Engl J Med. 2008;358(20):2148-59. DOI: 10.1056/NEJMra0800239
41. Kasperczyk S,Dobrakowski M, Kasperczyk J, Ostałowska A, Zalejska-Fiolka J, Birkner E. Beta-carotene reduces oxidative stress, improves glutathione metabolism and modifies antioxidant defense systems in lead-exposed workers. Toxicology and Applied Pharmacology. 2014;280:36- 41.
42. Canas JA, Lochrie A, McGowan AG, Hossain J, Schettino C, Balagopal PB. Effects of mixed carotenoids on adipokines and abdominal adiposity in children: A pilot study. J. Clin. Endocrinol. Metab. 2017; 102:1983–1990. DOI: 10.1210/jc.2017-00185

43. Canas JC, Damaso L, Altamare A, Killen K, Hossain J, Balagopal PB. Insulin resistance and adiposity in relation to serum β-carotene levels. J. Pediatr. 2012; 161:58–64.
DOI: 10.1016/j.jpeds.2012.01.030
44. Furusho T, Kataoka E, Yasuhara T, Wada M, Innami S. Administration of betacarotene suppresses lipid peroxidation in tissues and improves the glucose tolerance ability of streptozotocin-induced diabetic rats. Int J Vitam Nutr Res. 2002;72:71-6.
45. Roohbakhsh A, Karimi G, Iranshahi M. Carotenoids in the treatment of diabetes mellitus and its complications: a mechanistic review. Biomed Pharmacother. 2017;91:31-42.
46. Fathalipour M, Fathalipour H, Safa O, Nowrouzi-Sohrabi P, Mirkhani H, Hassanipour S. The Therapeutic Role of Carotenoids in Diabetic Retinopathy: A Systematic Review. Diabetes Metab Syndr Obes. 2020;13:2347-2358.
DOI: 10.2147/DMSO.S255783
47. Gammone MA, Pluchinotta FR, Bergante S, Tettamanti G, D'Orazio N. Prevention of cardiovascular diseases with carotenoids. Front Biosci. 2017;9: 165-71.
48. Seo JS, Lee KS, Jang HJ, Quan Z, Yang KM, Burri BJ. The effect of dietary supplementation of β-carotene on lipid metabolism in streptozotocin-induced diabetic rats. Nutr Res. 2004;24: 1011- 21.

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