Journal of Kerman University of Medical Sciences
2022; 29(4): 341-347
10.22062/JKMU.2022.92009
Original Article
http://jkmu. kmu. ac. ir/

Dose-dependent Effect of β-caryophyllene on Glycemic Control of High-Fat Diet and Fructose-Induced Type-2 Diabetic Rats

Vadivel Mani [1*], Anandhi Danavel [2], Manikandan Balraj [3], Gayathri Venkatasan [4], Megalatha Libin [5]

1. Department of Biochemistry, Arunai Medical College and Hospital, Tiruvanamalai-606603, Tamilnadu, India
2. Department of Biochemistry, Meenakshi Ammal Dental College and Hospital, Chennai-600095, Tamilnadu, India
3. Department of Physiology, Konaseema Institute of Medical Sciences and Research Foundation, Amalapuram, East Godavari Dt-533201, Andhra Pradesh, India
4. Department of Physiology, Krishnadevaraya College of Dental Science and Hospital, Bangalore, Karnadaka, India
5. Department of Biochemistry, Zydus Medical College and Hospital, Dahod-389151, Gujarat, India

ABSTRACT

Background: Among many human diet-related disorders, Diabetes mellitus (DM) stands on the top of the table, its persistent and perdurable threat/stress response to systemic functions and endocrine control makes it's more popular. The management of diabetes and related chronic diseases has focused on the use of indigenous natural compounds, derived from plant sources that possess medicinal properties.

Methods: Wistar albino rats were fed with high-fat diet comprising 2% cholesterol, 1% cholic acid, 30% coconut oil, 67 % percent regular rat feed, and 25% fructose through drinking water for 60 days to induce type-2diabetic. After induction, type-2 diabetic rats were treated with β-Carophyllene (50, 100, 200, and 400mg/kg body weight once a day, orally) for 30 days, respectively. Fasting Blood glucose, liver and kidney function markers were analyzed.

Results: Diabetic animals showed elevated blood glucose level when compared to control. Treatment with 50 and 100 mg/kg b.wt β-Carophyllene did not reach control level. Whereas, 200 and 400 mg/kg b.wt doses effectively reduced the blood glucose levels in diabetic animals.

Conclusion: Liver function markers such as ALT, AST, and ALP and kidney function markers like urea and creatinine were also found to be elevated in diabetic animals. β-Carophyllene effectively reduced it. No toxicity was found in 200 and 400 mg/kg b.wt β-Carophyllene treated animals. Since blood glucose was restored to normal range at 200 mg dose itself, the same dose was selected as optimal dose for further study to elucidate the anti-diabetic potential.

Keywords: High-fat diet& fructose, Type-2 diabetes, β-Caryophyllene, liver function markers, kidney function markers

Citation: Mani V, Danavel A, Balraj M, Venkatasan G, Libin M. Dose-dependent effect of β-caryophyllene on Glycemic control of high-fat diet and Fructose-induced type-2 diabetic rats. Journal of Kerman University of Medical Sciences 2022; 29(4): 341-347. doi: 10.22062/JKMU.2022.92009

Received: 18. 12. 2021
Accepted: 05. 03. 2022
***Correspondence:** Vadivel Manii; Email: velvdm.vel5@gmail.com

Published by Kerman University of Medical Sciences

Introduction

The modern-day human population is at high risk of getting affected by diet-related diseases such as metabolic disorder, the main cause for the hazard was predicted to be the unregulated food selection and intake pattern. The latter includes the inclusion of rich calories, a high glycemic index diet, and exclusion/reduction of vital physical activity (1). Among many human diet-related disorders, Diabetes mellitus (DM) stands on the top of the table, its persistent and perdurable threat/stress response to systemic functions and endocrine control makes it more popular (2). Secreting ability and sensitivity of insulin in the system determines the deteriorated metabolic conditions that regulate carbohydrate, proteins, and fat content in the body of DM patients. Prolonged systemic disease symptoms and persistent endocrine disordered functional state are consistent with the propagation of type 2 Diabetes mellitus (T2DM) with respect to insulin resistance (IR) (3).

Uncontrolled diabetes causes hyperglycemia, or high blood sugar, which causes significant damages to many of the body's systems, including the neurons and blood vessels, over time (1). Diabetes and its complications pose a serious healthcare problem worldwide. With the increase in the world prevalence of diabetes approximately 463 million people are affected by diabetes and this number will increase to 700 million adults by 2045. In India, more than 77 million people are affected by diabetes (4). The management of diabetes and its related chronic diseases has focused on the use of indigenous natural compounds, derived from plant sources that possess medicinal properties (5). One method for approaching to reduce the metabolic risk is administering beneficial phytochemical. Nowadays more drugs are available in the market but they have enumerated side effects and they have affected various organs for a lifetime, for this reason, we have a plan to discover the phytochemical-based drug (6).

In the present study, the anti-diabetic effect of β-caryophyllene was analyzed. β-caryophyllene is a sequiterpene, naturally present in cannabis, various herbs and spices. Black pepper, cinnamon, hops, cloves, hemp, and rosemary are rich sources (7). It has a variety of biological effects, including anti-inflammatory, anti-lipidemic, and antioxidant effects (8). However, the anti-diabetic effect of β-caryophyllene is not yet clearly elucidated. In this study, the dose-dependent effects of β-caryophyllene on blood glucose, liver and renal functions markers in high-fat diet- and fructose-induced-type 2 diabetic rats were evaluated.

Materials and Methods

Animals

In this study, 150 to 180-day-old Wistar strain healthy adult male albino rats were used. The Institutional Animal Ethics Committee (IAEC No: 007/2019, dated 04/11/2019) at Meenakshi Medical College and Research Institute, MAHER, Enathur, Kanchipuram, Tamil Nadu-631552, India, approved their treatment in line with national rules and protocols. Animals were housed at a constant temperature (21±2°C) and humidity (65±5%), with a 12/12 h light/dark cycle, and fed a normal pelleted food (Lipton India, Mumbai, India), with clean drinking water supplied ad libitum.

Induction of Type 2 Diabetes

Rats were fed a high-fat diet comprising 2% cholesterol, 1% cholic acid, 30% coconut oil, 67% standard rat pelleted food, and 25% fructose through drinking water to induce diabetes (type 2) for 60 days (9). Fasting blood glucose levels were measured after 60 days, and animals with blood glucose levels higher than 120 mg/dl were chosen for the experiment (10). The high-fat diet and sugar feeding were maintained until the study's conclusion. Normal pelleted rat feed was provided to control rats, and water was freely available.

Experimental design

Rats were randomly divided into the following groups and each groups consist 6 animals.

Group I: Control (normal rats).

Group II: Rats were induced with diabetes by a high-fat-fructose diet and 25% fructose through drinking water and kept without any treatment for 60 days.

Group III: Type-2 diabetic rats treated orally with β-caryophyllene (50 mg/kg b.wt/day) for 30 days (11).

Group IV: Type-2 diabetic rats treated orally with β-caryophyllene (100 mg/kg b.wt/day) for 30 days.

Group V: Type-2 diabetic rats treated orally with β-caryophyllene (200 mg/kg b.wt/day) for 30 days.

Group VI: Type-2 diabetic rats treated orally with β-caryophyllene (400 mg/kg b.wt/day) for 30 days.

Fasting blood glucose (FBG)

After overnight fasting, blood glucose was measured using On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA). Blood was obtained by pricking the rat's tail tip, and the results were reported in mg/dl.

Liver and kidney function markers

Liver function markers (ALT, AST, and AST) and kidney function markers (urea and creatinine) were assessed using biochemical-assay kits purchased from Spin react, Spain. Liver function markers results were expressed as IU/L and kidney function markers were expressed as mg/dl.

Statistical analysis

Using computer-based software, the data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) to determine the significance of individual differences between the control and treatment groups (GraphPad Prism version 5). The significance of DMRT was determined at $P<0.05$.

Results

Dose-dependent effect of β-caryophyllene on fasting blood glucose, liver and kidney function markers in type 2 diabetic rats

Diabetic animals showed elevated blood glucose levels when compared to control animals (Figure 1). Treatment with 50 and 100 mg/kg b.wt β-Caryophyllene did not reach control level. Whereas, 200 and 400 mg/kg b.wt doses effectively reduced the blood glucose levels in diabetic animals. Liver function markers such as ALT, AST, and ALP (Figures 2A-C) and kidney function markers like urea and creatinine (Figures 3A-B) were also found to be elevated in diabetic animals. β-caryophyllene effectively reduced these markers. No toxicity was found in 200 and 400 mg β-caryophyllene-treated animals. Since blood glucose was restored to normal range at the 200 mg dose itself, the same dose was selected as the optimal dose for further study.

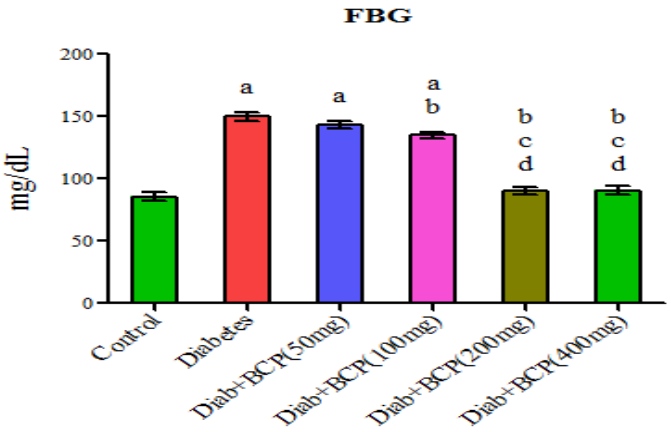

Figure 1. Dose-dependent effect of β-caryophyllene on fasting blood glucose in high-fat diet and fructose-induced type 2 diabetic rats. Each bar represents mean ± SEM of 6 animals. Significance at $P<0.05$, a- Compared with control, b- Compared with diabetic control, c- Compared with 50 mg/b.wt β-Caryophyllene, d- Compared with 100 mg/b.wt β-Caryophyllene.

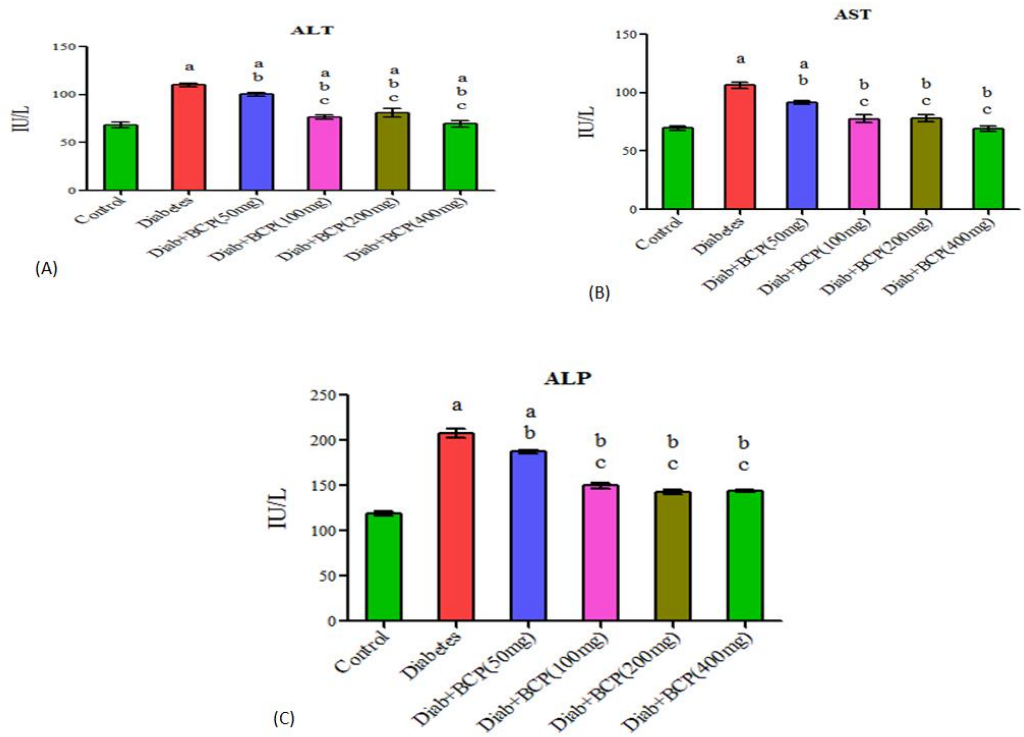

Figure 2. Dose-dependent effect of β-caryophyllene on liver function markers in high-fat diet and fructose-induced type 2 diabetic rats. Each bar represents mean ± SEM of 6 animals. Significance at P<0.05, a-Compared with control, b- Compared with diabetic control, c- Compared with 50 mg/b.wt β-Caryophyllene.

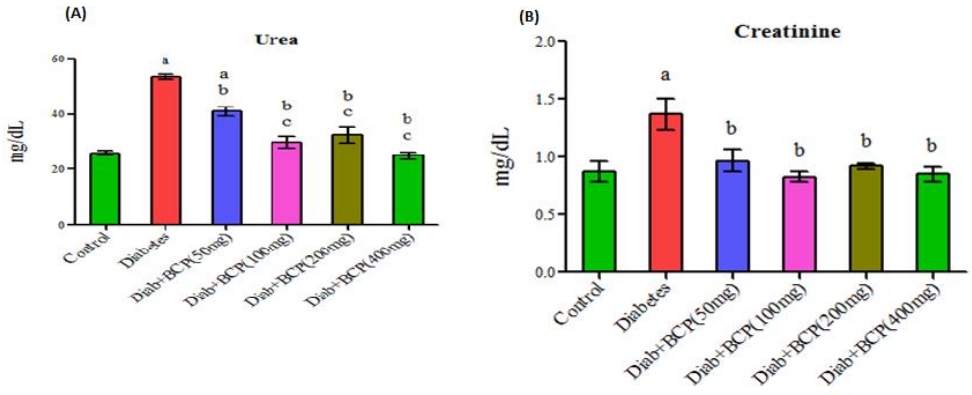

Figure 3. Dose-dependent effect of β-caryophyllene on urea and creatinine in high-fat diet and fructose-induced type 2 diabetic rats. Each bar represents mean ± SEM of 6 animals. Significance at P<0.05, a-Compared with control, b-Compared with diabetic control, c- Compared with 50 mg/b.wt β-Caryophyllene.

Discussion

Type 2 diabetes is a complicated, diverse, and polygenic disease that rapidly increases morbidity and death. One of the major contributors to hyperglycemia in type 2 diabetes is the body's insulin resistance. Many studies have shown that in rats fed a high-fat diet (HFD), fructose develops insulin resistance which is similar to the clinical symptoms and pathophysiology of human type 2 diabetes mellitus (12, 13). As a result, a high-fat diet was employed to induce type 2 diabetes in rats in the present study. This model provides a perfect platform for screening antidiabetic agents, as interest in the investigation of natural remedies is at its peak due to the crooked features of the regular therapies for diabetes (14). The present study investigated the dose-dependent effects of β-caryophyllene by assessing fasting blood glucose, liver and kidney function markers.

Blood glucose control is an important tool in preventing or delaying the complications of diabetes. It was observed a significant increase in blood glucose levels as a result of insulin resistance in rats with type 2 diabetes induced by the high-fat diet. Treatment with low-dose β-caryophyllene (50 and 100 mg/kg b.wt) did not reach the control level whereas, supplementation of 200 and 400 mg/kg b.wt β-caryophyllene to diabetic rats resulted in reduced blood glucose levels, which may be due to its antidiabetic potential as reported by Basha and Sankaranarayanan (15).

Liver and kidney function markers are frequently used in clinical practice to screen liver damages, monitor the progression of diabetes, and analyze the effects of drugs. In both fasting and postprandial stages, the liver aids in maintaining normal blood glucose content. Glycogenolysis and hepatic glucose synthesis rise when the effect of insulin on the liver is lost. Triglyceride accumulation and lipolysis abnormalities and elevations of transaminase enzymes in insulin-sensitive organs, such as the liver are an early indication of insulin resistance and can be detected before fasting hyperglycemia (16). Hyperglycemia causes an increase in the production of free radicals due to the auto-oxidation of glucose, which can cause cell damage. Uncontrolled diabetes mellitus has been linked to increased liver enzymes (17).

In the present study, the liver enzymes (AST, ALT, and ALP), which serve as pathophysiological markers of metabolic syndrome, tissue damage and predictors of diabetes were examined. A significant increase in the activities of serum transaminase was observed in high-fat diet and fructose-induced type-2 diabetic rats as a result of perturbed homeostasis in the metabolic organs (18). A significant increase in the level of ALT and AST, a gluconeogenic enzyme in diabetic rats as an indicator of impaired insulin signaling was observed. ALP activity is also increased in the liver of diabetic rats due to an increase in the oxidative insult of high-fat diet-induced diabetes resulting in hepatic damage (19). Administration of 50 mg/kg b.wt β-caryophyllene showed no significant changes whereas treatment with 100, 200, and 400 mg/kg b.wt β-caryophyllene significantly restored the altered levels of these markers to normal level in diabetic rats, suggesting that it can repair the high-fat diet-induced liver damage and enhances glucose and insulin metabolism.

Serum urea and creatinine levels are crucial biomarkers of renal function in human and animal studies. Data from the present study revealed that high-fat diet-induced type 2 diabetes caused a significant increase in urea and creatinine levels that may be due to decreased glomerular filtration rate (GFR), which is an indication of loss of renal function. This may attribute to oxidative stress-induced cellular damage induced by high-fat diet (20). Treatment with β-caryophyllene (200 and 400 mg/kg b.wt) significantly decreased the altered levels of urea and creatinine, which may be the result of renal tissue damage repair mediated by β-caryophyllene as potential antioxidant. There was no significant changes between these doses, therefore, 200 mg of β-caryophyllene was selected as the optimal dosage and will be used in further studies to elucidate the anti-diabetic potential of β-caryophyllene and its effect on insulin resistance.

Conclusion

Supplementation of β-caryophyllene with various concentrations significantly decreases the elevated levels of fasting blood glucose, liver (ALP, AST, and ALT) and kidney (urea and creatinine) function markers in high-fat diet-induced type 2 diabetic rats. From the present findings, 100 mg β-caryophyllene restore only liver function marker alone, whereas 200 mg and 400 mg β-caryophyllene restore fasting blood glucose, liver and kidney function markers. According to the results of the present study, it is concluded that 200 mg of β-caryophyllene shows potential protective effect against high-fat diet-induced type 2 diabetes. Hence, further studies are needed to elucidate the anti-diabetic potential of β-Caryophyllene.

Conflict of interests

The authors declare that there is no conflict of interests.

References

1. Onuekwuzu IM, Chidinma IC, Chigozie IJ. Anti-diabetic effect of a flavonoid and sitosterol - rich aqueous extract of pleurotus tuberregium sclerotia in alloxan-induced diabetic rabbits. Endocr Metab Immune Disord Drug Targets. 2019; 19(8):1148-56. doi: 10.2174/1871530319666190206213843.
2. Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, et al. Global, regional, and national burden and trend of diabetes in 195 countries and territories: An analysis from 1990 to 2025. Sci Rep. 2020; 10(1):14790. doi: 10.1038/s41598-020-71908-9.
3. Guex CG, Reginato FZ, de Jesus PR, Brondani JC, Lopes GHH, Bauermann LF. Antidiabetic effects of Olea Europaea L. leaves in diabetic rats induced by high-fat diet and low-dose streptozotocin. J Ethnopharmacol. 2019; 235:1-7. doi: 10.1016/j.jep.2019.02.001.
4. Atlas D. International diabetes federation: IDF Diabetes Atlas. Brussels, Belgium: International Diabetes Federation; 2015.
5. Nabi SA, Kasetti RB, Sirasanagandla S, Tilak TK, Kumar MV, Rao CA. Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats. BMC Complement Altern Med. 2013; 13:37. doi: 10.1186/1472-6882-13-37.
6. Ambika S, Saravanan R. Effect of bergenin on hepatic glucose metabolism and insulin signaling in C57BL/6J mice with high fat-diet induced type 2 diabetes. J Appl Biomed. 2016: 14(3):221-7. doi: 10.1016/j.jab.2016.04.002.
7. Jha NK, Sharma C, Hashiesh HM, Arunachalam S, Meeran MN, Javed H, et al. β-caryophyllene, a natural dietary cb2 receptor selective cannabinoid can be a candidate to target the trinity of infection, immunity, and inflammation in COVID-19. Front Pharmacol. 2021; 12:590201. doi: 10.3389/fphar.2021.590201.
8. Chen L, Lu X, El Seedi H, Teng H. Recent advances in the development of sesquiterpenoids in the treatment of type 2 diabetes. Trends Food Sci Technol. 2019: 88(4):46-56. doi: 10.1016/j.tifs.2019.02.003.
9. Nampurath GK, Mathew SP, Khanna V, Zachariah RT, Kanji S, Chamallamudi MR. Assessment of hypolipidaemic activity of three thiazolidin-4-ones in mice given high-fat diet and fructose. Chem Biol Interact. 2008; 171(3):363-8. doi: 10.1016/j.cbi.2007.10.006.
10. Augustine AW, Narasimhan A, Viswanathan M, Karundevi B. Evaluation of antidiabetic property of Andrographis paniculata powder in high fat and sucrose-induced type-2 diabetic adult male rat. Asian Pac J Trop Dis. 2014; 4(Suppl 1):140-7. doi: 10.1016/S2222-1808(14)60429-1.
11. Harb AA, Bustanji YK, Abdalla SS. Hypocholesterolemic effect of β-caryophyllene in rats fed cholesterol and fat enriched diet. J Clin Biochem Nutr. 2018; 62(3):230-7. doi: 10.3164/jcbn.17-3.
12. Sears B, Perry M. The role of fatty acids in insulin resistance. Lipids Health Dis. 2015; 14:121. doi: 10.1186/s12944-015-0123-1.
13. Softic S, Stanhope KL, Boucher J, Divanovic S, Lanaspa MA, Johnson RJ, et al. Fructose and hepatic insulin resistance. Crit Rev Clin Lab Sci. 2020; 57(5):308-22. doi: 10.1080/10408363.2019.1711360.
14. Makinde EA, Radenahmad N, Adekoya AE, Olatunji OJ. Tiliacora triandra extract possesses antidiabetic effects in high fat diet/streptozotocin-induced diabetes in rats. Food Biochem. 2020; 44(6):e13239. doi: 10.1111/jfbc.13239.
15. asha RH, Sankaranarayanan C. β-Caryophyllene, a natural sesquiterpene lactone attenuates hyperglycemia mediated oxidative and inflammatory stress in experimental diabetic rats. Chem Biol Interact. 2016; 245:50-8. doi: 10.1016/j.cbi.2015.12.019.
16. Sears B, Perry M. The role of fatty acids in insulin resistance. Lipids Health Dis. 2015; 14:121. doi: 10.1186/s12944-015-0123-1.
17. fortmueller CA, Wiemann C, Funk GC, Leichtle AB, Fiedler GM, Exadaktylos AK, et al. Hypoglycemia is associated with increased mortality in patients with acute decompensated liver cirrhosis. J Crit Care. 2014; 29(2):316.e7-12. doi: 10.1016/j.jcrc.2013.11.002.
18. Lasker S, Rahman MM, Parvez F, Zamila M, Miah P, Nahar K, et al. High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. Sci Rep. 2019; 9(1):20026. doi: 10.1038/s41598-019-56538-0.
19. Mandal A, Bhattarai B, Kafle P, Khalid M, Jonnadula SK, Lamicchane J, et al. Elevated liver enzymes in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease.

Cureus. 2018; 10(11):e3626. doi: 10.7759/cureus.3626.

20. Nisha R, Srinivasa Kannan SR, Thanga Mariappan K, Jagatha P. Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis. J Clin path Lab Med. 2017; 1(2):1-5.