Effect of lupeol on the mRNA expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver of type-2 diabetic adult male rat.

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Abstract

Lupeol is a pharmacologically active triterpenoid. It is found in latex of rubber plants and fig trees and also seen in many fruits and vegetables. Lupeol is known as phytosterols. The study is aimed to identify the effect of lupeol on the glucose-6-phosphatase and phosphoenolpyruvate carboxykinase enzyme level in the liver of type-2 diabetic adult male rats. The present study concludes that lupeol regulates glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in high fat diet induced type-2 diabetic male rats. The present study concludes that lupeol regulates glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in high fat diet induced type-2 diabetic male rats. Diabetes is the primary cause of disease such as kidney failure, cardiovascular disease includes stroke and heart attack. They also cause afflictions such as blindness and limb amputation.

Keywords: Triterpenoid, Glucose-6-phosphatase, Phosphoenolpyruvate carboxykinase, Lupeol, type-2 diabetes, innovative technology, Novel method.

Accepted on November 03, 2021

Introduction

The type-2 diabetes is becoming a complex, polygenic and heterogeneous disease that has been a leading cause of mortality and morbidity. The important factor that contributes to hyperglycemia in type-2 diabetes is our body's resistance to insulin [1] type-1 diabetes is less compared to type-2 diabetes. type-2 diabetes comprises 90% across the world. Diabetes mellitus is managed by diet, exercise and chemotherapy. Many adverse side effects are linked with conventional pharmacological treatments and also lead to high rates of secondary failure. So now-a-days there is increased demand for natural products with anti- diabetic activity with lesser side effects [2]. Metformin is a widely used antidiabetic drug that shows the effects on the carbohydrate metabolism of the liver.

Natural triterpenoids have growing interest in the field. Lupeol is known as phytosterols. The natural compound that is widespread with a number of practical importance is triterpenes. Triterpenes are stabilized by phospholipid bilayer of plant-derived components as the cholesterol does in animal cell membranes [3]. Most importantly triterpenes are plant derived natural components. The common segments of human weight control plans are linked with triterpenes. Normally 250 mg for each day of triterpenes is intaken in the west part of the country. It is consumed from grains, products of the soil and vegetable oils [4-6].

One major challenge to our health system is the burden of type-2 diabetes. A serious effort is done to improve the treatment of type-2 diabetes and develop novel therapeutic strategies [7-12]. Fasting hyperglycaemia is commonly observed in patients with type-2 diabetes with the increased hepatic glucose production caused due to underlying insulin resistance [13-15]. Metformin, known as an anti-diabetic drug increases insulin sensitivity and glucose utilisation [16-19].

Both the regulation and degradation of hepatic glucose synthesis involves the fight control of expression of respective gatekeepers, phosphoenolpyruvate carboxykinase and glucokinase as well as fine tuning of the expression and activity of intermediate enzymes catalysing the reversible reactions [20-23]. Our team has extensive knowledge and research experience that has translate into high quality publications [24-33]. The study is to estimate the effect of lupeol on the mRNA expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver of type-2 diabetic adult male rats.

Materials and Methods

Chemicals

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA. glyphosate was procured from Sigma Chemical Company St. Louis, MO, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from Genet Bio, South Korea purchased from Promega, USA.

Animals

The present experimental study was approved by the institutional animal ethics committee (IAEC no.: BRULAC/SDCH/SIMATS/IAEC/07-2019/028). Adult male wistar albino

rats, weighing 180–200 g, were obtained and maintained in clean propylene cages at the Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Saveetha dental college and hospitals, Saveetha University, India) in an air-conditioned animal house, fed with standard rat pelleted diet (Lipton India Ltd., Mumbai, India), and clean drinking water was made available ad libitum. Rats were divided into 3 groups, each consisting of 6 animals.

Induction of type-2 diabetes

Rats were subjected to 60 days of a high-fat diet containing cholesterol 3%, cholic acid 1%, coconut oil 30%, standard rat feed 66%, and 30% sucrose through drinking water. On the 58th day of treatment, after overnight fasting, blood glucose was checked and the rats that had blood glucose above 120 mg/dL were chosen as type 2 diabetic rats. Sucrose feeding through drinking water with a high-fat diet was continued until the end of the study.

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Experimental design

Adult male albino rats of Wistar 150–180 days old with 180–200 g body weight (b.wt) were randomly divided into five groups of six rats each;

Group I: Control (vehicle treated).

Group II: Type-2 diabetic rats.

Group III: Type-2 diabetic rats treated with lupeol (25 mg/kg, b.wt/day) orally for 30 days.

Group IV: Type-2 diabetic rats treated with metformin (50 mg/kg, b.wt/day orally for 30 days.

Two days before sacrifice, control and experimental animals were subjected to Oral Glucose Tolerance (OGT) test and insulin tolerance test. At the end of the treatment, animals were anesthetized with sodium thiopentone (40 mg/kg b.wt), blood was collected through cardiac puncture, sera were separated and stored at −80°C, and 20 ml of isotonic sodium chloride solution was perfused through the left ventricle to clear blood from the organs. Liver tissues from control and experimental animals were immediately dissected out and used for assessing the various parameters.

mRNA expression analysis

Total RNA isolation, cDNA conversion and real-time PCR Using a Total RNA Isolation Reagent Invitrogen kit (TRIR), total RNA was isolated from control and experimental samples. In brief, to 100 mg fresh tissue, 1 ml of TRIR was added and homogenized. The content was transferred to a microcentrifuge tube instantly and 0.2 ml of chloroform was added, vortexed for 1 min then kept at 4°C for 5 min. Later, the contents were centrifuged at 12,000 xg for 15 min at 4°C. The aqueous phase (upper layer) was carefully transferred to a fresh microfuge tube and an equal volume of isopropanol was added, vortexed for 15 s and placed on ice for 10 min. After centrifugation of the content at 12000 xg for 10 min at 4°C, the supernatant was discarded and RNA pellet was washed with 1 ml of 75% ethanol by the vortex. The isolated RNA was estimated spectrometrically by the method of Fourney et al. The RNA concentration was expressed in microgram (µg). By using the reverse transcriptase kit from Eurogentec (Seraing, Belgium), complementary DNA (cDNA) was synthesized from 2 µg of total RNA as stated in the manufacturer's protocol. To perform real-time PCR, the reaction mixture containing 2x reaction buffer (Takara SyBr green master mix). Forward and reverse primers of the target gene and house-keeping gene, water and β-actin in total volume of 45 µl expect the cDNA was made, mixed intensively and spun down. In individual PCR vials, about 5 µl of control DNA for positive control, 5 µl of water for negative control and 5 µl of template cDNA for samples were taken and reaction mixture (45 µl) were added. 40 cycles (95°C for 5 min, 95°C for 5 s, 60°C for 20 s and 72°C for 40 s) was set up for the reaction and obtained results were plotted by the PCR machine (CFX96 Touch Real-Time PCR Detection System) on a graph. Relative quantification was calculated from the melt and amplification curves analysis.

PEPCK mRNA primer

5-AGCCTCGACAGCCTGCCCCAGG-3

5- CCAGTTGTTGACCAAAGGCTTTT-3

Glucose 6 phosphatase primer

5’-TAGAATTCAAGGATGGAGGAAGGAATGAAC-3

5”-TACTGCAGTGCCTTACAAAGACTTCTTGTG-3’

Statistical analysis

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ± standard deviation. Results were analysed statistically by a one - way Analysis of Variance (ANOVA) and significant differences between the mean values were measured using Duncan’s multiple range test using Graph Pad Prism version 5. The results with the $p<0.05$ level are considered to be statistically significant

Results

Effect of lupeol on mRNA expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver of type-2 diabetic adult male rats. In the present study using diabetic rats the glucose-6-phosphatase is significantly increased. Oral administration of lupeol of type-2 diabetic rats effectively decreased enzyme near to that of the control level,

stating that lupeol regulates glucose-6-phosphatase in the liver (Figure 1).

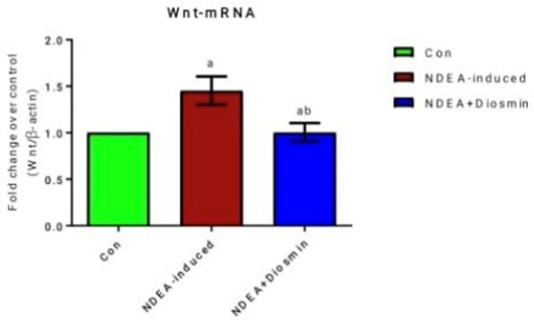

Figure 1. *The bar graph depicts the effect of lupeol on glucose-6-phosphatase mRNA expression in the liver of type-2 diabetic rats. The X axis represents the control group and the experimental group and the Y axis represents fold change over control of glucose 6 phosphatase. Green colour denotes control group, orange colour denotes diabetic group.Blue colour denotes the lupeol treated group and purple colour denotes metformin treated group. Expression of glucose 6 phosphatase significantly increased in diabetic experimental rats compared to the control group and was found to significantly decrease when treated with lupeol and metformin. Each bar represents mean ± SEM (n=6). Significance at p<0.05, a: Significantly different from control group, ab: Significantly different from diabetic group, bc: Significantly different from lupeol treated group.*

In the present study, type-2 diabetic rats showed significant increase in levels of phosphoenolpyruvate carboxykinase enzyme when compared to control. Oral administration of lupeol to type-2 diabetic rats was effectively reduced to mRNA expression near to that of the control level (Figure 2).

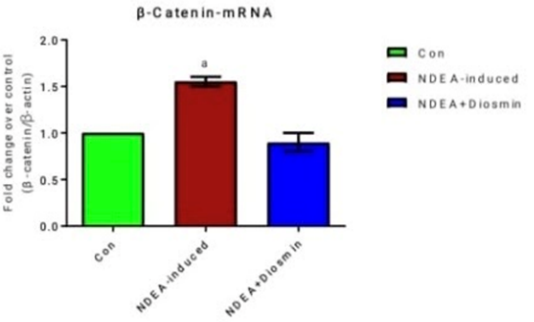

Figure 2. *The bar graph depicts the effect of lupeol on phosphoenolpyruvate carboxykinase mRNA expression in the liver of type-2 diabetic rats. The X axis represents the control group and the experimental group and the Y axis represents fold change over control of phosphoenolpyruvate carboxykinase. Dark green colour denotes control group. Red colour denotes diabetic group. Light green colour denotes the lupeol treated group and pink colour denotes metformin treated group. Expression of phosphoenolpyruvate carboxykinase significantly increased in diabetic experimental rats compared to the control group and the phosphoenolpyruvate carboxykinase expression was found to significantly decrease when treated with lupeol and metformin. Each bar represents mean ± SEM (n=6). Significance at p<0.05, a: Significantly different from control group, b: Significantly different from diabetic group, b: Significantly different from lupeol treated group.*

Discussion

The study shows the effect of lupeol subjecting to glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver of type-2 diabetes induced male rats.

In the present study lupeol restored the altered levels of carbohydrate metabolic enzymes such as Glucose-6-phosphatase and Phosphoenolpyruvate carboxykinase activity which were induced by a high fat diet and sucrose. Increased level of fat accumulation due to high fat diet feed results in the detrimental changes in the enzyme activity in the liver. In accordance with the present findings, other plant sterol compounds have been reported to reduce diabetic risk in controlling carbohydrate metabolic enzyme activity.

Diabetes is the primary cause of disease such as kidney failure; cardiovascular disease includes stroke and heart attack. They also cause afflictions such as blindness and limb amputation **(World Health Organization, 2009)**. Rats induced with high fat diet seem to develop insulin resistance and pathogenesis of human type-2 diabetes mellitus is reported in many experimental studies. Similar to this study effect of lupeol on glucose transporter 2 and insulin receptor was done in same high fat induced type-2 diabetic rats in that increase in free fatty acid formed at time of high fat diet treatment inhibits the IR gene expression and shows decreased amount of IR protein in the insulin target cells. Accordingly, the lupeol was chosen and metformin was used to compare. The addition of an excess amount of lupeol causes damage to tissues. Apoptosis and growth inhibition also takes place in excess of lupeol addition. Cardio protective activity was found in lupeol because rats used for research were HFD-induced rats. Finally lupeol is found to have anti-diabetic activity and also called multitarget agent that performs different activities.

Conclusion

Our present findings of the first time reports that lupeol regulates glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in high fat diet induced type-2 diabetic model and hence, the natural plant sterol may be considered as therapeutic drug for the management of diabetes. However, further studies need to be carried out in order to show the better efficiency of the drug.

Acknowledgement

The authors would like to thank Saveetha dental college and hospitals, Saveetha institute of medical and technical Sciences, Saveetha University for providing research laboratory facilities to carry out the study.

Source of Funding

The present study was supported by the following agencies: Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha dental college, Saveetha University, Lions Clubs International District 324 A2.

Statement of Conflict of Interest

The author declares that there is no conflict of interest in the present study.

References

1. Balogh J, Victor D, Asham EH, et al. Hepatocellular carcinoma: a review. J Hepatocell Carcinoma. 2016;3:41–53.
2. Acharya SK. Epidemiology of hepatocellular carcinoma in India. J Clin Exp Hepatol. 2014;4:S27–33.
3. Wu MC. Clinical research advances in primary liver cancer. World J Gastroenterol. 1998;4(6):471–474.
4. Liu CY, Chen KF, Chen PJ. Treatment of Liver Cancer. Cold Spring Harb Perspect Med. 2015;5(9):021535.
5. Li P, Cao Y, Li Y, et al. Expression of Wnt-5a and β-catenin in primary hepatocellular carcinoma. Int J Clin Exp Pathol. 2014;7(6):3190–3195.
6. Wang Z, Sheng YY, Gao XM, et al. β-catenin mutation is correlated with a favorable prognosis in patients with hepatocellular carcinoma. Mol Clin Oncol. 2015;3(4):936–940.
7. Lee JM, Yang J, Newell P, et al. β-Catenin signaling in hepatocellular cancer: Implications in inflammation, fibrosis, and proliferation. Cancer Lett. 2014;343(1):90–97.
8. Friemel J, Rechsteiner M, Frick L, et al. Intratumor heterogeneity in hepatocellular carcinoma. Clin Cancer Res. 2015;21(8):1951–1961.
9. Wang H, Zhang J, Feng W, et al. PIN1 gene overexpression and beta-catenin gene mutation/expression in hepatocellular carcinoma and their significance. J Huazhong Univ Sci Technolog Med Sci. 2007;27(1):54–57.
10. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009;17:9–26.
11. Seifert JRK, Mlodzik M. Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. Nat Rev Genet. 2007;8(2):126–138.
12. Clevers H. Wnt/β-Catenin Signaling in Development and Disease. Cell. 2006;127:469–480.
13. Khalaf AM, Fuentes D, Morshid AI, et al. Role of Wnt/β-catenin signaling in hepatocellular carcinoma, pathogenesis, and clinical significance. J Hepatocell Carcinoma. 2018;5:61–73.
14. Siddiqui M, Vincent Rajkumar S. The high cost of cancer drugs and what we can do about it. Mayo Clin Proc. 2012;87:935–943.
15. Lewinska A, Siwak J, Rzeszutek I, et al. Diosmin induces genotoxicity and apoptosis in DU145 prostate cancer cell line. Toxicol *InVitro*. 2015;29:417–425.
16. Perumal S, Langeswaran K. Diosmin anti-tumour efficacious against Hepatocellular Carcinoma. J Pharm Tech. 2020;13:1707.
17. Perumal S, Langeshwaran K, Selvaraj J, et al. Effect of diosmin on apoptotic signaling molecules in N-nitrosodiethylamine-induced hepatocellular carcinoma in experimental rats. Mol Cell Biochem. 2018;449(1-2):27–37.
18. Srinivasan S, Pari L. Ameliorative effect of diosmin, a citrus flavonoid against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. Chem Biol Interact. 2012;195:43–51.
19. Queenthy SS, John B. Diosmin exhibits anti-hyperlipidemic effects in isoproterenol induced myocardial infarcted rats. Euro J Pharm. 2013;718:213–218.
20. Wang Y, Fang X, Ye L, et al. A randomized controlled trial evaluating the effects of diosmin in the treatment of radicular pain. Biomed Res Int. 2017;2017:1–7.
21. Abotaleb M, Samuel SM, Varghese E, et al. Flavonoids in cancer and apoptosis. Cancers. 2018;11(1).
22. Veeramuthu D, Raja WRT, Al-Dhabi NA, et al. Flavonoids: anticancer properties. flavonoids: From Biosynthesis to Human Health. 2017.
23. Wu F, Zhu J, Li G, et al. Biologically synthesized green gold nanoparticles from *Siberian ginseng* induce growth-inhibitory effect on melanoma cells (B16). Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3297–305.
24. Chen F, Tang Y, Sun Y, et al. 6-shogaol, a active constiuents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells). J Photochem Photobiol B. 2019;197:111518.
25. Li Z, Veeraraghavan VP, Mohan SK, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells *via* alleviating the MMP signaling pathway. J Photochem Photobiol B. 2020;203:111773.
26. Babu S, Jayaraman S. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomed Pharmacother. 2020;131:110702.
27. Malaikolundhan H, Mookkan G, Krishnamoorthi G, et al. Anticarcinogenic effect of gold nanoparticles synthesized from *Albizia lebbeck* on HCT-116 colon cancer cell lines. Artif Cells Nanomed Biotechnol. 2020;48(1):1206–1213.
28. Han X, Jiang X, Guo L, et al. Anticarcinogenic potential of gold nanoparticles synthesized from *Trichosanthes kirilowii* in colon cancer cells through the induction of apoptotic pathway. Artif Cells Nanomed Biotechnol. 2019;47(1): 3577–3584.
29. Gothai S, Muniandy K, Gnanaraj C, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. Biomed Pharmacother. 2018;107:1514–1522.

30. Veeraraghavan VP, Hussain S, Balakrishna JP, et al. A comprehensive and critical review on ethnopharmacological importance of desert truffles: *Terfezia claveryi*, *Terfezia boudieri*, and *Tirmania nivea*. Food Rev Int. 2010;1–20.
31. Sathya S, Ragul V, Veeraraghavan VP, et al. An *in vitro* study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. Environ Nanotechnol Monit. 2020;14:100333.
32. Yang Z, Pu M, Dong X, et al. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway *via* attenuating the development of gastric carcinoma: *In vitro* and *in vivo* studies. Arab J Chem. 2020;13(5):5501–5516.
33. Rajendran P, Alzahrani AM, Rengarajan T, et al. Consumption of reused vegetable oil intensifies BRCA1 mutations. Crit Rev Food Sci Nutr. 2020;1–8.
34. Barma, M D, Muthupandiyan I, Samuel SR, et al. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021;126:105132.
35. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021;31(2):285–286.
36. Samuel SR, Kuduruthullah S, Khair AMB, et al. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021;31(3):436–441.
37. Tang Y, Rajendran P, Veeraraghavan VP, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from *Scutellaria baicalensis* on human osteoblast-like MG-63 cells. Mater Sci Eng C. 2021;119(3): 111656.
38. Yin Z, Yang Y, Guo T, et al. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. Environ Toxicol. 2021;36(6):1011–1020.
39. Veeraraghavan VP, Periadurai ND, Karunakaran T, et al. Green synthesis of silver nanoparticles from aqueous extract of *Scutellaria barbata* and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). Saudi J Biol Sci. 2021;28(7):3633–3640.
40. Mickymaray S, Alfaiz FA, Paramasivam A, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. Saudi J Biol Sci. 2021;28(7):3641–3649.
41. Teja KV, Ramesh S. Is a filled lateral canal-A sign of superiority? J Dent Sci. 2020;15(4):562–563.
42. Kadanakuppe S, Hiremath S. Social and behavioural factors associated with dental caries experience among adolescent school children in Bengaluru city, India. J Adv Med. 2016;14(1):1–10.
43. Silvestro L, Tarcomnicu I, Dulea C, et al. Confirmation of diosmetin 3-O-glucuronide as major metabolite of diosmin in humans, using micro-liquid-chromatography-mass spectrometry and ion mobility mass spectrometry. Anal Bioanal Chem. 2013;405(25):8295–8310.
44. Wang W, Smits R, Hao H, et al. Wnt/β-catenin signaling in liver cancers. Cancers 2019;11(7):926.

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