Effect of heterologous platelet-rich plasma (PRP) on liver and modulation of glucose metabolism and Wnt signaling pathways in diabetic mice

Amin Arif
University of the Punjab
Adil Farooq
University of Okara
Muddasir Hassan Abbasi
University of Okara
Muhammad Babar Khawar
University of Narowal
Tasleem Akhter
University of Health Sciences
Humaira Allay Ali
University of Okara
Mehreen Anjum
University of Okara
Rabia Mahmood
University of the Punjab
Tayyaba Saleem
University of the Punjab
Nadeem Sheikh (nadeem.zool@pu.edu.pk)
University of the Punjab

Research Article

Keywords: Alloxan, Growth factors, Glucose metabolism, Platelet-rich plasma, Wnt

Posted Date: August 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1910439/v1

Abstract

Platelet-rich plasma (PRP) is proven a cost-effective therapeutic choice for different ailments. The current study was designed to highlight the effects of heterologous platelet-rich plasma (PRP) on deteriorated hepatic tissues and impaired glucose metabolism in alloxan-induced diabetic mice. A total of thirty (30) male mice were selected and grouped as control (CG), PRP group (PG), diabetic group (DG), treated group 1 (T1G), and treated group 2 (T2G). PG was given a subcutaneous dose of PRP (0.5 ml/kg body weight) twice a week for four weeks. DG, T1G, and T2G were first given a single dose of alloxan intraperitoneally (150 mg/kg) to induce diabetes. PRP (0.5 ml/kg body weight) was given twice a week to T1G and T2G for two and four weeks respectively. After four weeks, all the mice were sacrificed to excise liver for histological observations and gene expression analysis. Hepatic histo-morphological analysis revealed ballooning of hepatocytes, dilatation of sinusoids, and collagen deposition in the alloxan-induced diabetic group (DG) and it was significantly reduced in both T1G and T2G. Additionally, a significant change in the expression of several hepatic genes was observed. *Fbp1*, *G6pc*, and *Pklr* showed an upregulation while a downregulation was detected in the expression of *Pck1* in DG. A significant restoration was observed in *Fbp1*, and *G6pc* after PRP treatment but no change was observed in *Pklr* expression. Genes of glycolytic pathway, *Hk1* and *Gck*, were downregulated significantly in DG compared to CG and PRP treatment restore the expression in both treated groups T1G and T2G. Moreover, *Wnt2*, *Wnt4*, and *Wnt9a* genes of the Wnt signaling pathway were also upregulated in DG, conversely, downregulate in T1G and T2G. No significant change in expression of *Wnt5b* and *Wnt9b* was observed in DG compared to control. Current study revealed that PRP anticipates a reduction in glucose production and glucose consumption by ameliorating hepatic tissue and modulating the glucose metabolism. So, it may use as one of the adjunctive therapies to treat T2DM in future but further more detailed investigations are suggested.

Introduction

Platelets are natural reservoir of growth factors (GFs), viz platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and fibroblast growth factor (FGF). GFs, secreted by α-granules of activated platelets, play significant role in various cellular processes such as mitogenesis, chemotaxis, differentiation, and metabolism [1]. Platelet-rich plasma (PRP) has expanded high considerations in orthopedic and sports injuries, aesthetic surgery [2], spinal fusion [3], heart surgery [4], preventing immune reactions, and wound healing in chronic skin and soft-tissue ulcers [5].

Type 2 Diabetes Mellitus (T2DM) is a chronic disorder of metabolism characterized by hyperglycemia owing to diminished insulin secretion and reduced insulin sensitivity [6]. The latter results to develop insulin resistance and decreased uptake of glucose in extra-hepatic tissues that supplemented endogenous glucose production by the liver [7]. Moreover, elevated glycemic conditions [8] and increased lipolysis in line with a higher level of free fatty acids [9] may lead to further damage to β-cells that interferes the long-term glycemic control and dent the peripheral glucose consumption [10]. In the earlier

phase of T2DM, the β-cells of the pancreas secrete compensatory insulin to restore glucose level in plasma, but a progressive declined function of β-cells, mainly due to inherited β-cell defect(s), was observed [11].

Glucose homeostasis needs precise correspondence between the utilization and metabolic glucose synthesis and its delivery through diet. The liver is a key player in glucose metabolism and it primarily includes glucose transport, production (gluconeogenesis, glycogenolysis), and breakdown (glycolysis) [12]. Furthermore, T2DM is directly linked to impaired hepatic glucose metabolism but its role is still vague in the pathogenesis of disease [13]. So, it must be a target in the pathogenesis of T2DM. *Fbp1, Pck1, Pklr*, and *G6pc*, the main checkpoints of gluconeogenesis, in line with *Hk1* and *Gck*, the major genes of glycolysis, are some potential genes of glucose metabolism [14]. Additionally, the onset of T2DM is also contributed by obesity and feasibly the alterations in the Wnt signaling pathway could subsidize the risk of diabetes onset by affecting adipogenesis as it acts as a major modulator of adipocyte differentiation [15, 16]. Therefore, genes associated with the Wnt signaling pathway could be reflected as contestant genes to confer the susceptibility of T2DM. So, glucose metabolism and the Wnt signaling pathway in diabetic subjects are required to be further explored.

Additionally, not all diabetics may be subjected to the same treatment since their diagnostic factors vary from one patient to another. Therefore, a valuable addition as a therapy for some categories of diabetics is an absolute necessity.

Minding the critical role of GFs of PRP played in hemostasis and wound healing, cell proliferation, and inhibition of apoptosis [17]. Therefore, current study designed to reveal the hepato-protective effects of heterologous PRP on hyperglycemic hepatic tissues and modulation of glucose metabolism and Wnt signaling pathways. The efficiency of GFs of PRP is rather controversial and lacks evidence. The use of a cocktail of various GFs in plasma may prove clinically important to cure T2DM.

Materials And Methods

Development of Animal Model

Thirty (30) adult male albino mice, 5±1-week age group and 20±5 g weight, were used in the current study. Approved guidelines of ethical and review committee of the University of the Punjab, Lahore-Pakistan (Ref/D/229/FIMS) were followed. Earlier in the trial, a group of six mice was kept per cage under controlled environmental conditions for acclimatization. Cages were then randomly tagged: Control (CG), PRP (PG), Diabetic (DG), Treated 1 (T1G), and Treated 2 (T2G). CG was given a standard chow throughout the experiment. PG was given a subcutaneous dose of PRP (0.5ml/kg BW) for consecutive four weeks. The other experimental groups (DG, T1G and T2G) were given a single intraperitoneal (IP) dose of alloxan (150mg/kg). After the confirmation of diabetes, group DG was kept untreated and PRP treatment was given to group T1G and T2G for two and four weeks, respectively. All the animal groups had *ad libitum* access to chow and water was given to all groups till the end of experiment. After four

weeks, the animals were sacrificed following a cervical dislocation to excise the liver for histological observations and gene expression analysis.

Histochemical Analysis

Liver tissues were processed for hematoxylin and eosin (H&E) and Masson's trichrome staining as described by and assigned the scores 0-4 [18].

Quantitative Real-Time PCR

100 mg snap-frozen liver sample was taken to extract total RNA of all groups by using bioZol ^{TM} -G reagent (bioPLUS, 10760055-1) following the manufacturer's specifications. DNase treatment was given to extract RNA followed by quantification. cDNA synthesis was made by using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, K1622). Samples for qPCR were prepared by using Maxima SYBR Green/ROX qPCR Master Mix (K0221, Thermo Scientific). PikoReal™ Real-Time PCR System (Thermo Scientific) was employed to analyze qPCR for each studied primer (Table 1). The potential irregularities in RNA concentration were balanced by using GAPDH to normalize all values. Effectiveness of procedure was controlled by adding a negative control (RT-) concordantly to each qPCR reaction. $2\Delta\Delta$Ct method was adopted for analysis of fold changes in expression of all studied genes.

Table 1

List and sequences of RT-PCR primer sequences.

Genes	FORWARD	REVERSE
GAPDH	GAAACCTGCCAAGTATGA	GCTGTAGCCGTATTCATT
Fbp1	GTCCATCGGAACCATTTTTG	TCCAGCATGAAGCAGTTGAC
G6pc	ATGGTCACTTCTACTCTTGC	CAAGATGACGTTCAAACAC
Pklr	TAGGAGCACCAGCATCATTG	CATCCCTGCCTTGATCATCT
Pck1	AGAAGGAGTACCCATTGAG	CTGAGGGCTTCATAGACA
Hk1	TGCCATGCGGCTCTCTGATG	CTTGACGGAGGCCGTTGGGTT
Gck	TATGAAGACCGCCAATGTGA	TTTCCGCCAATGATCTTTTC
Wnt2	AGAGTGCCAACACCAGTTCC	TACAGGAGCCACTCACACCA
Wnt5b	TCTCCGCCTCACAAAAGTCT	CACAGACACTCTCAAGCCCA
Wnt9a	CCCCTGACTATCCTCCCTCT	GATGGCGTAGAGGAAAGCAG
Wnt9b	GGGTGTGTGTGGTGACAATC	TCCAACAGGTACGAACAGCA
Wnt4	CGAGGAGTGCCAATACCAGT	GTCACAGCCACACTTCTCCA

Statistical Analysis

Statistical analysis and graphs were made by using Prism GraphPad 9 software (San Diego, CA) and *P* 0.05 was considered significant.

Results

Blood Glucose random

After the induction of diabetes, the blood glucose level of DG, T1G and T2G groups were significantly elevated as compare CG group. At the end of the 4^{th} week, the decline in blood glucose random was significant in T1G (P=0.0497) and T2G (P=0.0211) groups (Fig. 1a).

C-Peptide

C-Peptide level of the DG group was reduced significantly as compare to CG group and an elevation was observed in T1G and T2G but the increase was not significant in T1G (P=0.0781) (Fig. 1b).

Histochemical Analysis

The control group (CG) revealed a normal hepato-architecture with radial morphology of hepatocytes and normal sinusoids. Some pathological changes were observed in the diabetic group (DG), characterized as ballooning of hepatocytes (›), infiltration of inflammatory cells (→), deposition of fat globules (▲), and collagen deposition ( ). Whereas, PRP treated groups showed improvement in hepatic architecture compared to DG. Improved hepatic architecture in T1G and T2G is characterized by improved sinusoids and reduction in collagen deposition as compare to the diabetic untreated group. PRP treatment also resulted in reduced vacuolization of the hepatic tissues (Fig. 2).

Gene Expression of Gluconeogenesis pathway genes

Statistically significant variations in the expression of *Fbp1* were observed in the animal model of experimentally induced diabetes (18 folds) compared to the control and a reverse effect was observed in both treated groups T1G and T2G. Additionally, an escalation in the expression of *G6pc* (12 folds) was observed in DG as compared to CG and showed a trend similar to *Fbp1* in both T1G and T2G. Moreover, DG showed decreased expression of *Pck1*, while its expression was increased significantly in T2G compared to T1G. An escalation was observed in *Pklr* expression (24 folds) was observed in DG compared to CG but it was unchanged in T1G and T2G (Fig. 3a-d).

Gene Expression of Glycolysis pathway genes

A concordant statistically significant decline in expression of *Hk1* was found in DG while a significant escalation was observed in both T1G and T2G. In line with it, *Gck*, in DG, showed decreased expression as compared to CG and it significantly increase in T1G and T2G compared to DG (Fig. 4a-b).

Gene Expression of Wnt pathway genes

Expression of the Wnt signaling pathway was observed by taking a few important genes such as *Wnt2*, *Wnt 4*, *Wnt5b*, *Wnt9a*, and *Wnt9b* as a reference. Statistically noticeable variations were detected in the expression of *Wnt2* (42 folds), *Wnt4* (16 folds), and *Wnt9a* (12 folds) genes among all groups as it was increased in DG as compared to CG. The expression was decreased significantly in both T1G and T2G. *Wnt5b* and *Wnt9b* showed no significant change in DG as compared to CG (Fig. 5a-e).

Discussion

Diabetes Mellitus (DM), a chronic disorder, requires a long-term remedy to control glucose in the blood, as both hyperglycemia and hypoglycemia prompt severe health issues. DM affect about 390 million people worldwide and the number is expected to grow up by 500 million by 2030 [19]. An alloxan induced diabetic mice model was developed to mimic the human pathogenesis of DM by a single intraperitoneal dose [20] and PRP treatment was employed to diabetic mice. Several studies have suggested the antidiabetic potential and glycemic control capability of PRP in animals [21]. Previous studies revealed the ability of autologous PRP to recover insulin secretion of pancreatic islets as well as to improve oxidative stress and regulation of insulin and glucose levels in plasma in diabetic rats [22]. Current study was therefore conceded to highlight the therapeutic outcome of heterologous platelet-rich plasma (PRP) on diabetic liver injury by regulating glucose metabolism in a mice model of T2DM.

Analysis of blood revealed a higher level of glucose and C-peptide in diabetic group and PRP treatment reversed the effect significantly in T2G but it was insignificant in T1G (Fig. 1a,b). C- Peptide, as a result of pro-insulin cleavage, is produced in equimolar concentration to insulin and it is used to detect endogenous insulin secretion by β-cells of pancreatic islets as elevated level of C-Peptide in hyperglycaemia indicates an inappropriately low level of insulin and consequent higher glucose in blood.

Histochemical analysis revealed a noteworthy increase in collagen protein in alloxan-induced diabetic mice (Fig. 2b). Hepatic fibrosis, the increased deposition of extracellular matrix (ECM) protein, is a major cause of chronic liver injury [23] and T2DM potentiates liver inflammation and ultimate severe liver failure in addition to the prevalence of bacterial infections [24]. The inflammation along with oxidative stress causes chronic hyperglycemic injuries and lipid disorder in hepatic tissues [25]. Persistent hepatic fibrosis can lead to liver cirrhosis and ultimately to organ failure and death.

Moreover, ballooning of hepatocytes, infiltration of inflammatory cells and deposition of fat globules with damaged sinusoidal spaces (Fig. 2a) was also observed in DG which is consistent with the previous studies [19] and it was significantly reduced in both the treated groups T1G and T2G. PRP may ameliorate hepatic microarchetecture and reduce the accumulation of ECM and inflammation in hepatic tissues by moderating the activation of hepatic stellate cells (HSCs) as they have a critical role in hepatic fibrogenesis [26].

The liver, as a central organ, is a key player of glucose homeostasis. To deepen the role of the liver in glucose homeostasis, some indicators of glucose metabolism and the Wnt signaling pathway, connected to impaired homeostasis of glucose in T2DM, were also investigated in current study. The expression of some key gluconeogenesis genes, *Fbp1*, *G6pc*, and *Pklr*, was upregulated which is consistent with the previous reports but we found reduced expression of *Pck*, another gluconeogenesis gene, in DG compared to CG that contradict the previous studies [27]. The increased expression of *Fbp1*, *Pklr*, and G6pc perceived in the current study is associated with the fact that mice liver might be able to boost the rate of gluconeogenesis at a relatively earlier stage of diabetes to elevate glucose level in diabetic mice.

In the glycolytic pathway, it is reported that the activity of both *Hk1* and *Gck* is decreased in the diabetic liver which is in line with the previous study [28, 29]. The decreased expression of *Hk1* and *Gck* detected in the current study is also connected to the fact that mouse liver might be able to downregulate glycolysis to elevate glucose level in diabetic mice. Taken together, our data implicate that downregulation of glycolysis to decrease consumption of glucose and upregulation of gluconeogenesis were augmented to yield higher glucose level (Fig. 1a). Increased gluconeogenesis accounted for about 90% elevation in hepatic glucose production and the remaining about 10% rise is probably contributed by slightly elevated glycogenolysis in T2DM subjects [14]. PRP treatment restore the expression of *Fbp1*and *G6pc*, which was increased in DM, to nondiabetic level but no significant restoration in expression of *Pklr* was observed. *Pck1* expression tended to decrease in DM, and PRP therapy reversed the effect. Moreover, the expression of *Hk1* and *Gck* is also observed to be restored after the treatment of PRP.

The current study also showed an increased expression of *Wnt2*, *Wnt4*, and *Wnt9a* genes in DG as compared to CG, and a noteworthy reduction in expression was observed in both T1G and T2G. DG showed no significant change in expression of *Wnt5b* and *Wnt9b* as compared to CG (Fig. 3). The Wnt signaling pathway is critically important to modulate the differentiation of adipocytes [30]. *Wnt2*, *Wnt4*, and *Wnt5b* have been studied previously and an escalation in expression was observed in experimentally induced T2DM [31] but no direct evidence is available for the link of *Wnt9a* and *Wnt9b* to T2DM. Expression of *Wnt* genes in preadipocytes inhibit the canonical Wnt signaling and increase adipogenesis and expression of adipokine genes. The adipokines, synthesized and secreted by adipocytes, were thought to be intricate in systemic insulin sensitivity. Thus, adipogenesis could be altered by the change in *Wnt* gene expression and, consequently, it affects the risk of the onset of diabetes.

Taken together, the findings of the current study provide new insights to the control of hepatic glucose metabolism and the development of T2DM and revealed that the heterologous PRP anticipates a reduction in hepatic glucose production as well as glucose consumption. So, it may use as one of the adjunctive therapies to treat T2DM in the future but further studies are warranted.

Abbreviations

PRP Platelet-rich plasma

T2DM Type 2 Diabetes Mellitus

GFs Growth factors

DG Diabetic group

T1G Treated group 1

T2G Treated group 2

Declarations

Conflict of interest

The authors declare no conflict of interests.

Author contribution

AA and AF performed the major part of this research work, did statistical analysis, interpretation of results, and initial draft. MBK revised the figures, improved the initial draft, and helped in lab work. TA, RM and TS, HAA and MA helped in lab work. MHA and NS conceived the idea, supervised the work, revised, modified, and approved the final draft.

Acknowledgements

The authors are highly thankful to the Vice-Chancellor, University of the Punjab, Lahore, Pakistan for his support for accomplishing this research work.

References

1. Sills ES, Wood SH (2019) Autologous activated platelet-rich plasma injection into adult human ovary tissue: molecular mechanism, analysis, and discussion of reproductive response. Biosci Rep 39: https://doi.org/10.1042/BSR20190805
2. Everts P, Onishi K, Jayaram P, Lana JF, Mautner K (2020) Platelet-rich plasma: new performance understandings and therapeutic considerations in 2020. Int J Mol Sci 21:7794. https://doi.org/10.3390/ijms21207794
3. Hsu WK, Mishra A, Rodeo SR, et al (2013) Platelet-rich plasma in orthopaedic applications: evidence-based recommendations for treatment. *J* Am Acad Orthop Surg 21:739-748. https://doi: 10.5435/JAAOS-22-08-469
4. Patel AN, Selzman CH, Kumpati GS, McKellar SH, Bull DA (2016) Evaluation of autologous platelet rich plasma for cardiac surgery: outcome analysis of 2000 patients. J Cardiothorac Surg 11:1-6. https://doi: 10.1186/s13019-016-0452-9
5. Gentile P, Garcovich S (2021) Systematic review: adipose-derived mesenchymal stem cells, platelet-rich plasma and biomaterials as new regenerative strategies in chronic skin wounds and soft tissue

defects. *Int J Mol* Sci 22:1538. https://doi.org/10.3390/ijms22041538

6. Chaudhury A, Duvoor C, Reddy Dendi VS, et al (2017) Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. Front Endocrinol 8:6. https://doi.org/10.3389/fendo.2017.00006
7. Sparling DP, Yu J, Kim K, et al (2016) Adipocyte-specific blockade of gamma-secretase, but not inhibition of Notch activity, reduces adipose insulin sensitivity. Mol Metab 5:113-121. https://doi.org/10.1016/j.molmet.2015.11.006
8. Kumar S, Patial V, Soni S, et al (2017) Picrorhiza kurroa enhances β-Cell mass proliferation and insulin secretion in streptozotocin evoked β-Cell damage in rats. Front pharmacol 8:537. https://doi.org/10.3389/fphar.2017.00537
9. Prentki M, Madiraju SM (2012) Glycerolipid/free fatty acid cycle and islet β-cell function in health, obesity and diabetes. Mol Cell Endocrinol MOL 353:88-100. https://doi.org/10.1016/j.mce.2011.11.004
10. Scott SN, Anderson L, Morton JP, Wagenmakers AJ, Riddell MC (2019) Carbohydrate restriction in type 1 diabetes: a realistic therapy for improved glycaemic control and athletic performance? Nutrients 11:1022. https://doi.org/10.3390/nu11051022
11. Ashcroft FM, Rorsman P (2012) Diabetes mellitus and the β cell: the last ten years. Cell 148:1160-1171. https://doi.org/10.1016/j.cell.2012.02.010
12. Adeva-Andany MM, Pérez-Felpete N, Fernández-Fernández C, Donapetry-García C, Pazos-García C (2016) Liver glucose metabolism in humans. Biosci Rep 36. https://doi.org/10.1042/BSR20160385
13. Jiang S, Young JL, Wang K, Qian Y, Cai L (2020) Diabetic-induced alterations in hepatic glucose and lipid metabolism: The role of type 1 and type 2 diabetes mellitus. Mol Med Rep 22:603-611. https://doi.org/10.3892/mmr.2020.11175
14. Zhang F, Xu X, Zhang Y, Zhou B, He Z, Zhai Q (2013) Gene expression profile analysis of type 2 diabetic mouse liver. PloS one 8:e57766. https://doi.org/10.1371/journal.pone.0057766
15. Ashrafizadeh M, Ahmadi Z, Mohamamdinejad R, et al (2020) Curcumin therapeutic modulation of the wnt signaling pathway. Curr Pharm Biotechnol 21:1006-1015. https://doi.org/10.2174/1389201021666200305115101
16. Nie X, Wei X, Ma H, Fan L, Chen WD (2021) The complex role of Wnt ligands in type 2 diabetes mellitus and related complications. J Cell Mol Med 25:6479-6495. https://doi.org/10.1111/jcmm.16663
17. Moussa M, Lajeunesse D, Hilal G, et al (2017) Platelet rich plasma (PRP) induces chondroprotection via increasing autophagy, anti-inflammatory markers, and decreasing apoptosis in human osteoarthritic cartilage. Exp Cell Res 352:146-156. https://doi.org/10.1016/j.yexcr.2017.02.012
18. Abbasi MH, Fatima S, Khawar MB, Jahan S, Sheikh N (2018) An In Vivo Study on intoxicating effects of Nerium oleander Water based extract on multiorgans of Wistar rat. Can J Gastroenterol Hepatol 2018. https://doi.org/10.1155/2018/4739637

19. Dubey S, Yadav C, Bajpeyee A, Singh MP (2020) Effect of Pleurotus fossulatus aqueous extract on biochemical properties of liver and kidney in streptozotocin-induced diabetic rat. Diabetes Metab Syndr Obes 13:3035-3046. https://doi.org/10.2147%2FDMSO.S265798
20. Dey P, Saha MR, Chowdhuri SR, Sen A, Sarkar MP, Haldar B, Chaudhuri TK (2015) Assessment of anti-diabetic activity of an ethnopharmacological plant Nerium oleander through alloxan induced diabetes in mice. J Ethnopharmacol 161:128-37. http://dx.doi.org/10.1016/j.jep.2014.12.012
21. Nemati M, Karbalaei N, Mokarram P, Dehghani F (2020) Effects of platelet-rich plasma on the pancreatic islet survival and function, islet transplantation outcome and pancreatic pdx1 and insulin gene expression in streptozotocin-induced diabetic rats. Growth Factors 38:137-151. https://doi.org/10.1080/08977194.2021.1881502
22. Zarin M, Karbalaei N, Keshtgar S, Nemati M (2019) Platelet-rich plasma improves impaired glucose hemostasis, disrupted insulin secretion, and pancreatic oxidative stress in streptozotocin-induced diabetic rat. Growth Factors 37:226-237. https://doi.org/10.1080/08977194.2020.1735382
23. Arriazu E, Ruiz de Galarreta M, Cubero FJ, et al (2014) Extracellular matrix and liver disease. Antioxid Redox Signal 21:1078-1097. https://doi.org/10.1089/ars.2013.5697
24. Elsahar M, Elwan NM, El-Nakeep S, et al (2018) Managing diabetes and liver disease association. Arab J Gastroenterol 19:166-179. https://doi.org/10.1016/j.ajg.2018.08.003
25. Rosenblatt R, Atteberry P, Tafesh Z, et al (2021) Uncontrolled diabetes mellitus increases risk of infection in patients with advanced cirrhosis. Dig Liver Dis 53:445-451. https://doi.org/10.1016/j.dld.2020.10.022
26. Saito Y, Morine Y, Shimada M (2017) Mechanism of impairment on liver regeneration in elderly patients: role of hepatic stellate cell function. Hepatol Res 47:505-513. https://doi.org/10.1111/hepr.12872
27. Hatting M, Tavares CD, Sharabi K, Rines AK, Puigserver P (2018) Insulin regulation of gluconeogenesis. Ann N Y Acad Sci 1411:21-35. https://doi.org/10.1111/nyas.13435
28. Lu G, Teng X, Zheng Z, et al (2016) Overexpression of a glucokinase point mutant in the treatment of diabetes mellitus. Gene therapy 23:323-329. https://doi:10.1038/gt.2016.1
29. Li M, Dang Y, Li Q, et al (2019) Berberine alleviates hyperglycemia by targeting hepatic glucokinase in diabetic db/db mice. Sci Rep 9:1 12. https://doi.org/10.1038/s41598-019-48075-7
30. Moreno-Navarrete JM, Fernández-Real JM (2017) Adipocyte differentiation. Adipose tissue biology: Springer :69-90. https://doi.org/10.1007/978-3-319-52031-5_3
31. Welters HJ, Kulkarni RN (2008) Wnt signaling: relevance to β-cell biology and diabetes. Trends Endocrinol Metab 19:349-355. https://doi.org/10.1016/j.tem.2008.08.004

Figures

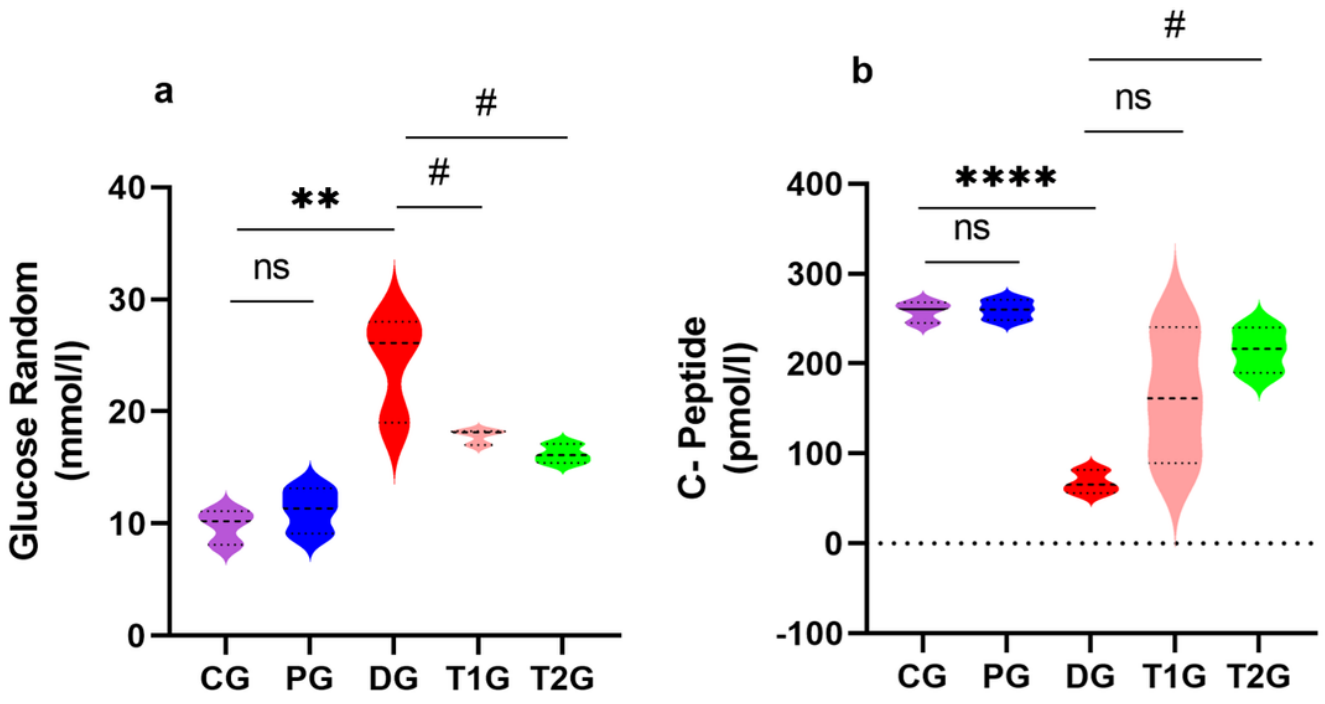

Figure 1

Comparison of Glucose random (a) and C-Peptide (b) among groups. Statistically significant difference among groups is manifested in graph. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

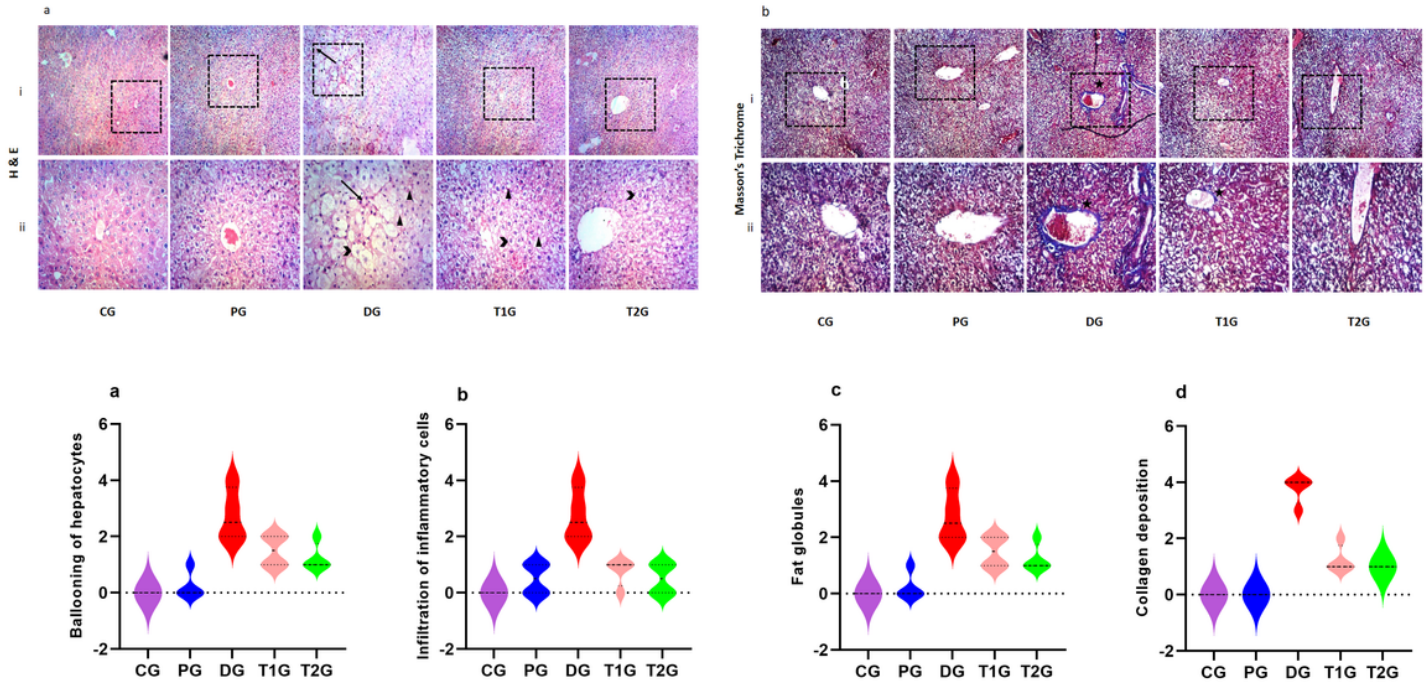

Fig. 2 Microphotographs of H & E (a) and Masson's Trichrome (b) stained sections of liver tissues. Ballooning of hepatocytes (❯), Infiltration of inflammatory cells (⟶), Presence of fat globules (▲) and Collagen deposition (★) were marked and score was calculated. Statistically significant difference among groups is manifested in graph. a. Ballooning of hepatocytes; b. Infiltration of inflammatory cells; c. Presence of fat globules; d. Collagen deposition. Magnification; (i) 40×; (ii) 100×

Figure 2

See image above for figure legend.

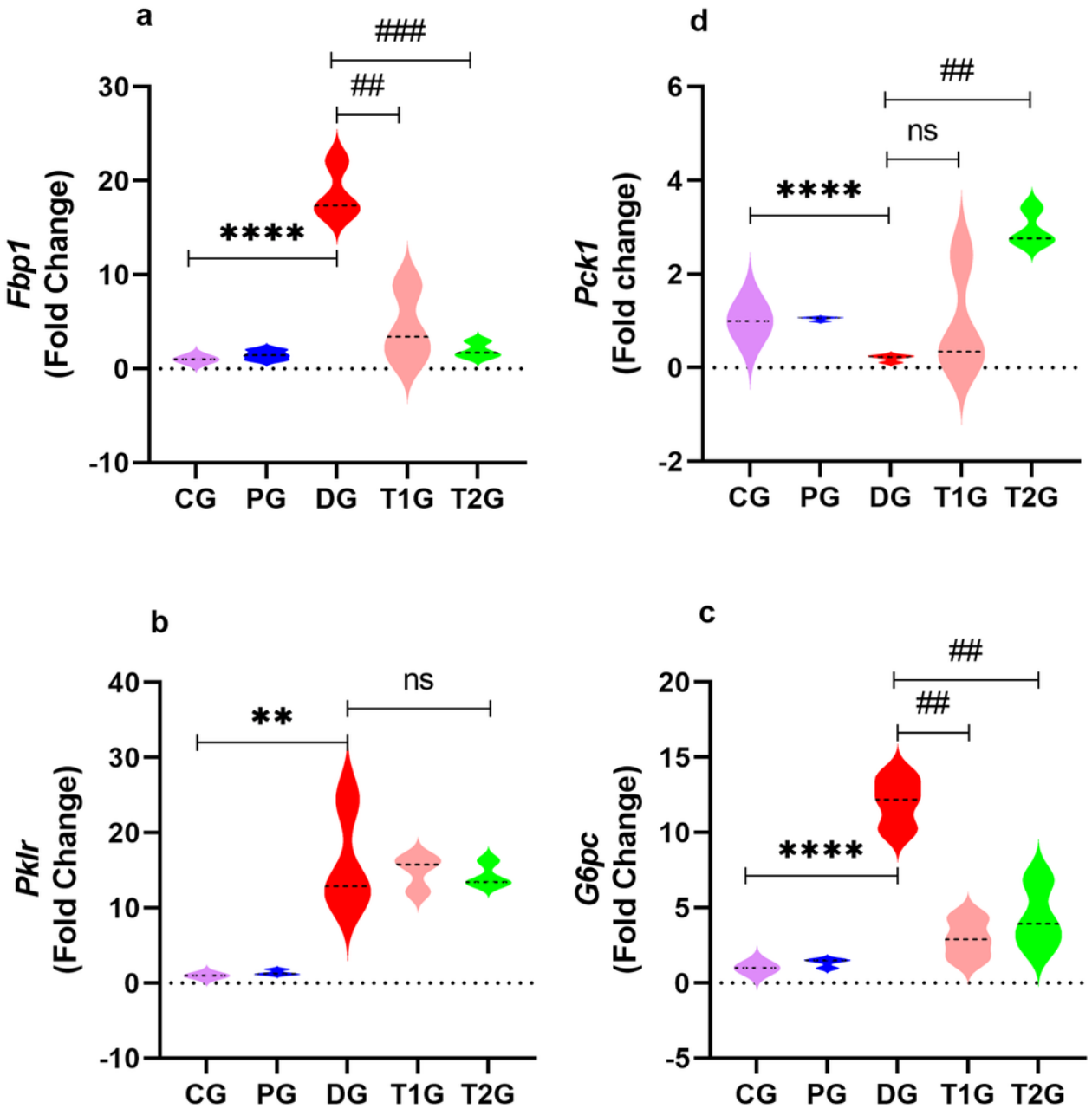

Figure 3

Quantified mRNA expression of gluconeogenesis genes. (a) *Fbp1* (b) *Pklr* (c) *G6pc* and (d) *Pck1*. The qRT-PCR data showed fold change in mRNA expression after normalization with GAPDH and statistical significance in mRNA expression are manifested in graph. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; Mean±S.E.M

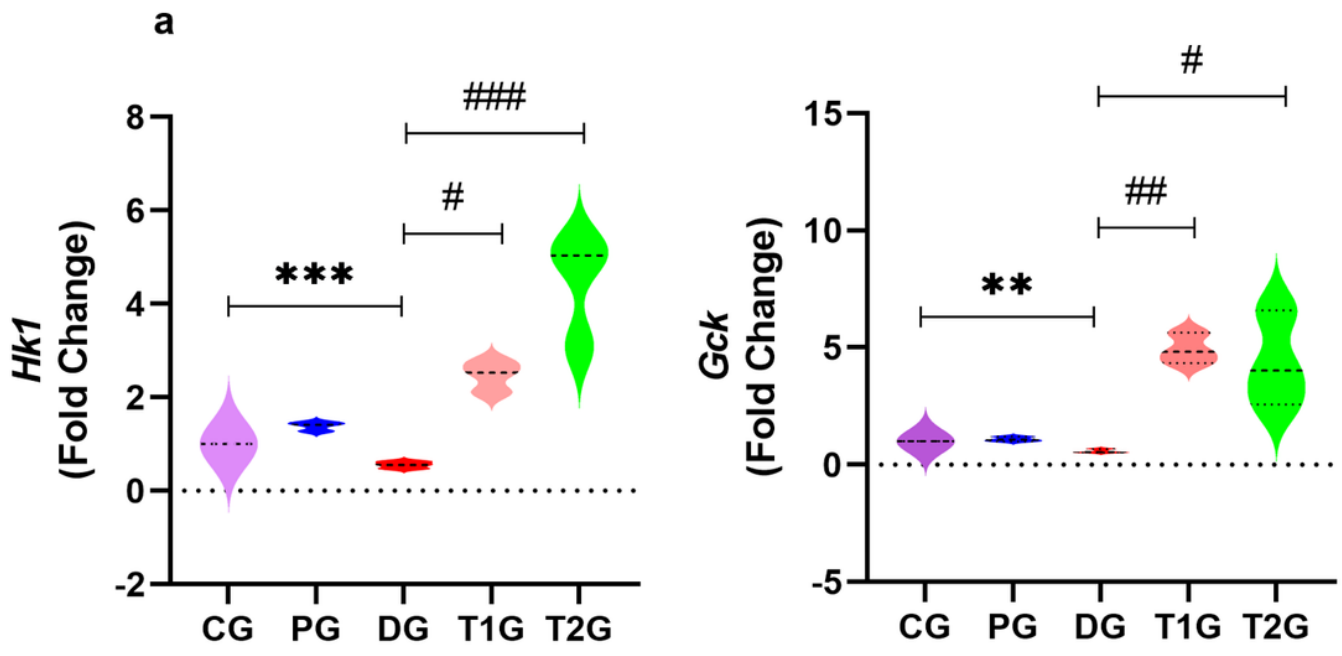

Figure 4

Quantified mRNA expression of glycolysis genes. (a) *Hk1* (b) *Gck*. The qRT-PCR data showed fold change in mRNA expression after normalization with GAPDH and statistical significance in mRNA expression are manifested in graph. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; Mean±S.E.M

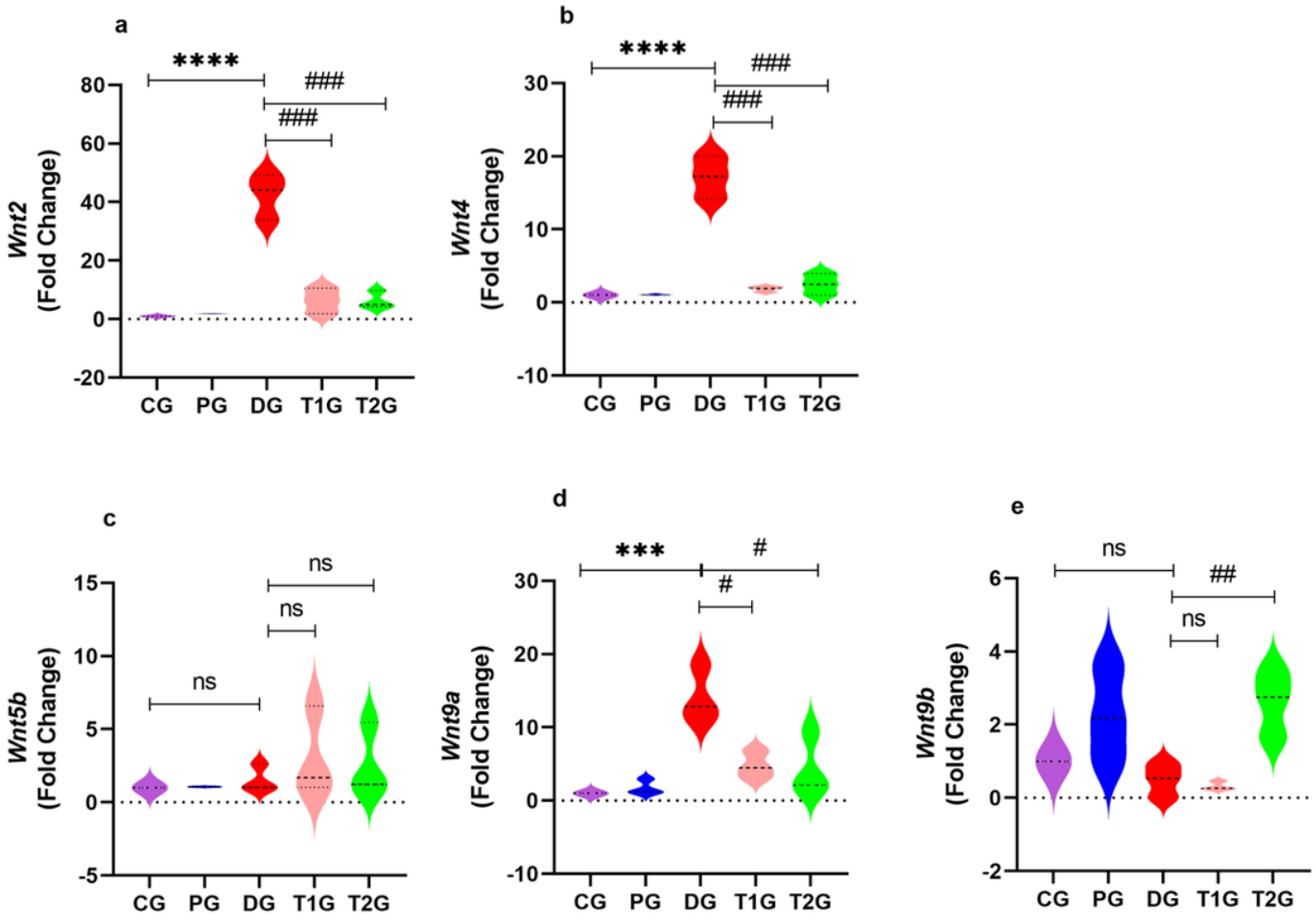

Figure 5

Quantified mRNA expression of Wnt signaling pathway genes. (a) *Wnt2* (b) *Wnt4* (c) *Wnt5b* (d) *Wnt9a* and (e) *Wnt9b*. The qRT-PCR data showed fold change in mRNA expression after normalization with GAPDH and statistical significance in mRNA expression are manifested in graph. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; Mean±S.E.M