




# Biochemical Investigation of Therapeutic Potentials of Plant-Based Bioactive Compounds as Stimulators of Glucagon like peptide-I Secretion

Dose-Response:  
An International Journal  
July-September 2022:1–12  
© The Author(s) 2022  
Article reuse guidelines:  
[sagepub.com/journals-permissions](https://sagepub.com/journals-permissions)  
DOI: 10.1177/115593258221114184  
[journals.sagepub.com/home/dos](https://journals.sagepub.com/home/dos)  


Areeba Javed<sup>1,‡</sup>, Ayman Muzammal<sup>1,‡</sup>, Muhammad Sajid Hamid Akash<sup>2</sup> , and Kanwal Rehman<sup>3</sup> 

## Abstract

This study was aimed to investigate the therapeutic potentials of plant-based bioactive compounds; lutein and resveratrol alone and/or in combination with DPP-4 enzyme inhibitor; sitagliptin on the secretion and bioavailability of Glucagon like peptide-I (GLP). For this, experimental rats were divided into seven groups. Group 1 was marked as control, while other six groups received streptozotocin (60 mg/kg I.P.). Later, group 2 was kept disease-control. While group 3 received 10 mg/kg/day sitagliptin (DDP-4i). Group 4 received 40 mg/kg/day lutein (LUT) and group 5 received 30 mg/kg/day resveratrol (RES). While group 6 and 7 were received combination of DPP-4i+LUT and DPP-4i+RES, respectively. Combined administration of DPP-4i+LUT or DPP-4i+RES showed expected therapeutic effects by lowering the fasting blood glucose and maintaining the serum insulin concentrations with improved glucose sensitivity and reduced insulin resistance. Further, co-administration of LUT and RES with DPP-4i revealed beneficial effects on measures of insulin resistance, circulating lipids, glycemic index, oxidative stress, and inflammatory status along with restoration of histological morphology of pancreatic cells and enterocytes that seemed to improve the level of GLP-I. Hence, substantial verdicts of this study showing therapeutic potentials of LUT and RES would surely help to recognize the potential effects in combination with DPP-4i as stimulators of GLP-I secretion.

## Keywords

glucagon like peptide-I, lutein, resveratrol, insulin resistance, oxidative stress, inflammatory responses

## Introduction

Chronic increase in blood glucose level due to impairment in insulin secretion or action leads to the development of metabolic disorders like obesity and diabetes mellitus.<sup>1</sup> Glucagon like peptide-1 (GLP-1) is a neuroendocrine hormone that is considered as an important incretin hormone, which plays a major role to maintain the level of blood glucose by promoting the insulin secretion from  $\beta$ -cells of pancreatic islets and blocking the glucagon secretion from  $\alpha$ -cells of pancreatic islets. GLP-1 also slows gastric emptying and promotes satiety. After taking a meal, the L-type enterocytes of small intestine in gastrointestinal tract produces GLP-1 that is considered responsible for the secretion of postprandial insulin.<sup>2,3</sup> Moreover, GLP-1 quickly degrades by an enzyme,

that is, dipeptidyl peptidase-4 (DPP-4). Though to overcome this, few GLP-1 analogs and DPP-4 inhibitors have been approved by FDA for the treatment of T2DM, there are some

<sup>1</sup> Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

<sup>2</sup> Department of Pharmaceutical Chemistry, Government College University, Faisalabad, Pakistan

<sup>3</sup> Department of Pharmacy, The Women University, Multan, Pakistan

Received 28 April 2022; accepted 30 June 2022

<sup>‡</sup>These authors contributed equally to this paper

## Corresponding Authors:

Kanwal Rehman, Department of Pharmacy, The Women University, Multan, Pakistan.

Email: [kanwalrehman@wum.edu.pk](mailto:kanwalrehman@wum.edu.pk)



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE

and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

shortcomings with their treatment.<sup>4</sup> DPP-4 inhibitor like sitagliptin inhibits GLP-1 degradation and thus prolongs its effects.<sup>5</sup> Sitagliptin does not directly contact with GLP-1 receptor but prevents the proteolytic breakdown of GLP-1 in blood *via* binding to DPP-4 and enhances endogenous levels of GLP-1 by decreasing glucagon level in blood, thus enhances the secretion of insulin from  $\beta$ -cell of pancreas. Though, DPP-4 inhibitors prolong the bioavailability of GLP-1 by inhibiting its metabolism, in previous years, it was recognized that they have no effect against the oxidative stress and/or inflammatory conditions. Interestingly, many recent studies have investigated the antioxidant and anti-inflammatory effects of these inhibitors.<sup>6-13</sup>

Further, many antioxidants, anti-inflammatory, and anti-infective bioactive compounds have been studied to be effective biocontrols against animal and human diseases including zoonotic conditions of intestine.<sup>3,14-18</sup> Nevertheless, antioxidants are mostly present in fruits and vegetable and possess great potential against oxidative damage.<sup>19</sup> Among these natural antioxidants, few merely focused but potentially effective are lutein and resveratrol.

Lutein (*Tagetes erecta* L.) is taken from the food like spinach, turnip, and green leafy vegetables. Recent studies on abilities of carotenoids<sup>20</sup> and tagetes species have highlighted the potential of lutein for lowering the blood glucose level. Moreover, some studies have also suggested that lutein can be beneficial not only as an anti-diabetic agent but may also exhibit the protective role against diabetes-associated complications like diabetic retinopathy.<sup>21</sup> Likewise, a potent antioxidant effect of lutein has been observed during the ischemic retina injury and provided a precise protection of retinal neuronal cell from oxidative stress.<sup>22</sup>

Recently, resveratrol has also been evaluated for its role in DM and obesity linked with insulin resistance.<sup>23</sup> Ahangarpour *et al.* have reviewed the antidiabetic potential of resveratrol assessed by many researchers using different experimental techniques.<sup>24</sup> As far as the combination treatment for DM is concerned, a previous study has showed a beneficial antidiabetic effect of co-treatment with quercetin and resveratrol in streptozotocin-induced diabetic rats.<sup>25</sup> correspondingly, both antioxidant and anti-inflammatory effects of resveratrol have been reported through numerous studies.<sup>26-30</sup> Resveratrol has shown its therapeutic potential as anticancer, anti-inflammatory, antioxidant, and cardioprotective,<sup>31</sup> whereas latest studies have also found its therapeutic potential for treating metabolic disorders such as hyperglycemia and obesity.<sup>32</sup> In addition to this, resveratrol has been observed to improve pancreatic  $\beta$ -cell mass and insulin secretion, thereby exerting antidiabetic action on body besides its antioxidant and anti-inflammatory potentials.<sup>23</sup> Studies have also supported that an understanding of gut development provides strategic approach to improve the diagnosis and management of pathological processes in a biological system.<sup>33</sup>

Correspondingly, the current work was designed to evaluate the antioxidant and anti-inflammatory effects of lutein and resveratrol alone and/or with dipeptidyl peptidase-4 (DPP-4) enzyme inhibitor; sitagliptin. It was supposed that this combination would not only help to prolong the bioavailability of GLP-1 but also contribute for controlling the oxidative stress and inflammatory conditions.

## Material and Methods

### Chemicals and Assay Kits

Colorimetric assay kits for glucose (Cat # E-BC-K234, Elabscience Biotechnology Inc.), alkaline phosphatase activity assay kit (Cat # E-BC-K091, Elabscience Biotechnology Inc.), and alanine aminotransferase activity assay kit (Cat # E-BC-K235, Elabscience Biotechnology Inc.); ELISA kits for rat insulin (Cat # E-EL-R3034, Elabscience Biotechnology Inc.), rat IL-6 (Cat # E-EL-R0015, Elabscience Biotechnology Inc.), and TNF alpha (Cat # E-EL-R0019, Elabscience Biotechnology Inc.). Creatinine (Cr) colorimetric assay kit (Cat # E-BC-K188-M, Elabscience Biotechnology Inc.), high-density lipoprotein cholesterol (HDL-C) colorimetric assay kit (Cat # E-BC-K222-S, Elabscience Biotechnology Inc.), and triglycerides colorimetric assay kit (Cat # E-BC-K261-M, Elabscience Biotechnology Inc.). GLP-1 assay kit (Cat # E-EL-R3007, Elabscience Biotechnology Inc.). Readings for ELISA kits were taken using Microplate ELISA Reader (BIOBASE-EL 10A).

### Experimental Animal Model

A total number of 42 Wistar albino rats with an average body weight of 180–250 g were purchased and kept at  $25 \pm 4^\circ\text{C}$  in an animal house at University of Agriculture, Faisalabad, Pakistan. Throughout the study period, standard diet was given to all rats with water *ad Libitum*. All experimental procedures were carried out in accordance with permitted laboratory animal handling procedures. All Biosafety/Bioethics protocol of Institutional Biosafety committee (IBC) and Bioethics committee and research board were followed (No 9354/ORIC). According to study protocol, other than normal control group which was marked as control (n = 6), the other six groups (n = 6) received streptozotocin (60 mg/kg I.P.). Later, these groups were designated according to the treatment they received. Group 2 was marked as disease-control group (STZ) in which after exposure to streptozotocin, no treatment was given throughout the study time. Group 3 was marked as DPP-4i treated group, receiving sitagliptin as 10 mg/kg/day for whole study period. Groups 4 and 5 were designated as LUT and RES group, respectively, as they received lutein 40 mg/kg<sup>34</sup> and resveratrol 30 mg/kg,<sup>35</sup> respectively, for the same study period. While groups 6 and 7 received combination treatment of DPP-4i + LUT (10 mg/kg/day +40 mg/kg) and DPP-4i + RES (10 mg/kg/day +30 mg/

kg), respectively, for the defined study period. Briefly, after overnight fasting, 60 mg/kg intraperitoneal injection of freshly prepared streptozotocin was given to all rats except the control group. The fasting blood glucose level of rats were evaluated till 10 days post streptozotocin injection. The blood glucose level >270 mg/dL indicated that the STZ-exposed rats were diabetic. After that, treatment was given with lutein, resveratrol, sitagliptin either alone, or in combination considering this day as “day 1” and continued till day 30. The vehicle used was normal saline for control group. In agreement with previous studies, the dose of sitagliptin (10 mg/kg) was designated as the literature suggested dose as this dose is suitable for detecting the potential synergistic effect.<sup>36-39</sup>

### Assessment of Body Weight and Food Intake

The food intake was measured on daily basis through the study period according to the method as described previously.<sup>40</sup> Briefly, the energy consumption of each rat was estimated by multiplying the average food intake (g) of rat per day with total energy of laboratory chow diet. Similarly, for body weight, after 3 to 4 h of fasting, body weight was measured after every 7 days in whole study period.

### Biochemical Analysis

**Blood and Tissue Sampling.** For biochemical analysis of predefined biomarkers, about 1.5 ml blood samples were taken from the tail vein method from each rat at the end of treatment. The blood samples were centrifuged for 20 minutes at 3000 RPM for serum separation and stored at  $-20^{\circ}\text{C}$  until further analysis. Pancreatic and intestinal tissues from ileum part were collected for the preparation of homogenate. 0.1 M phosphate buffered saline (PBS) was taken in falcon tubes and relevant collected tissue samples were placed in falcon tubes and homogenized *via* tissue homogenizer at the speed of 3000 RPM for the analysis of GLP-1, IL-6, and TNF- $\alpha$  levels in tissues.

### Estimation of Glycemic Biomarkers

The effect of LUT and RES alone or in combination with DPP-4i treatments were assessed for their effects on insulin sensitivity, glycemia, and serum level of insulin. To estimate the effect of treatment on glycemic levels, blood glucose level was recorded with the help of glucometer at 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and 28<sup>th</sup> day of experimental study. To assess the trend of glucose tolerance in experimental animals, oral glucose tolerance test (OGTT) was performed after an overnight fasting. By oral gavage, about 2 g/kg of glucose solution was given to all experimental animals followed by the assessment of blood glucose levels at 30, 60, 90, and 120 min. We also calculated the AUC during the OGTT using GraphPad Prism 6.0 software (La Jolla, CA, USA). To analyze the effect of lutein, resveratrol, and sitagliptin on insulin sensitivity, we also

estimated the insulin resistance using homeostasis model assessment for insulin resistance (HOMA-IR) by utilizing the fasting values of insulin and glucose as follows:

$$\text{HOME} - \text{IR} = \text{Insulin}(\mu\text{U}) \times \text{Glucose}(\text{mmol/L})/22.5.$$

We measured the fasting levels of insulin and glucose prior to the administration of glucose during OGTT.

### Assessment of GLP-1 Level in Serum and Intestine

Levels of GLP-1 in serum and intestinal tissue homogenates were measured in all treated groups to assess the effect of treatment of LUT and RES alone or in combination with DPP-4i on experimental animals.

### Estimation of Lipid Peroxidation and Inflammation

To estimate the effect of LUT and RES alone or in combination with DPP-4i on oxidative stress and inflammation, we measured the serum levels of MDA and IL-6 and TNF- $\alpha$  as biomarkers of lipid peroxidation and inflammation, respectively, using their corresponding assay kits.

### Assessment of Lipid Biomarkers

To investigate the effects of LUT and RES alone or in combination with DPP-4i on dyslipidemia after streptozotocin exposure, we estimated the serum levels of cholesterol, TGs, LDL, and HDL-C during and at the end of treatment period using their corresponding assay kits.

### Estimation of Kidney and Liver Function Biomarkers

Liver function biomarkers including ALT (alanine aminotransferase) and AST (aspartate aminotransferase) and renal function biomarkers including creatinine and uric acid were assessed to evaluate the effectiveness of LUT and RES alone or in combination with DPP-4i using their corresponding assay kits.

### Histopathological Examination

For the purpose of histopathological analysis of samples collected from each group, the sections were taken from collected tissues of pancreas and ileum part of intestine. Followed by fixing them in 10% buffered formalin. These tissues were then washed using alcohol and later were dehydrated. Afterward, xylene was applied to these tissues. The clear tissue was infiltrated with melted paraffin for 2 h and embedded in paraffin wax. This was followed by slicing of tissues into thin sections (5  $\mu\text{m}$ ) *via* microtome. Sections were mounted directly on the slides. Lastly, the Hematoxylin and Eosin (H & E) stains were used to stain the mounted sections and slides were covered with glass cover slip. Slides were observed for histopathological analysis at 40X and 100X.

## Statistical Analysis

The values for all biochemical parameters were presented as mean  $\pm$  SD. The association among the biochemical markers was investigated by non-linear regression through GraphPad Prism 5. The probability value ( $P < .05$ ) was considered as statistically significant and the two-way ANOVA along with Bonferroni post-test was applied for comparison among the treated groups.

## Results

### Effect of Treatment on Body Weight and Food Intake

Among different treated groups of rats, no significant difference was observed for the intake of food when compared to the untreated diabetic group. Moreover, it was noted that the lutein or resveratrol, alone or in combination with DPP-4i did not alter the body weight of experimental animals.

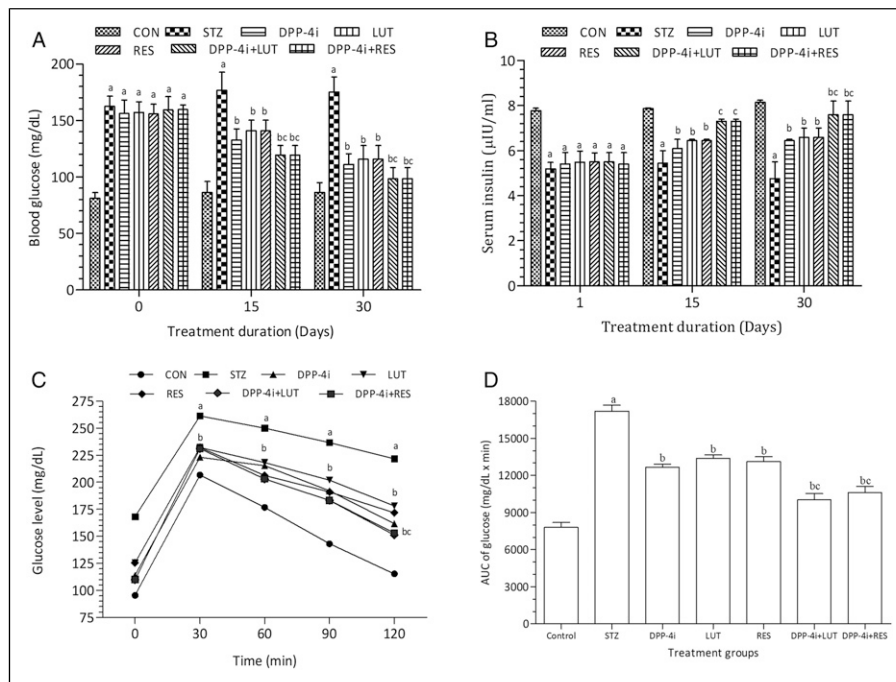
### Effect of Treatment on Glycemia and Insulin Sensitivity

It was observed that streptozotocin increased the levels of blood glucose before the start of treatment in all study groups (Figure 1A) as compared to normal control, moreover, this rise remained constant even at 15<sup>th</sup> and last day of study in STZ-group when compared to control group (Figure 1A). However, after the start of treatment, at 15th day, we found that treatment

lutein or resveratrol alone and/or with DPP-4i significantly decreased the levels of glucose when compared with that of non-treated STZ disease group. Similar pattern for the reduction of glucose level was also observed for combination groups receiving DPP-4i with lutein and DPP-4i with resveratrol, respectively. However, at the end of treatment period, both combination groups besides showing a significant decline ( $P < .05$ ) in the glucose levels as compared to STZ-group, also showed a better ( $P < .05$ ) glucose reducing ability when compared to that of DPP-4i group (Figure 1A).

Similarly, to evaluate the effect of treatments on insulin levels (Figure 1B), it was observed that STZ decreased the serum level of insulin before the start of treatment in all study groups ( $P < .05$ ) as compared to that of normal control. Moreover, this reduction in the serum level of insulin remained constant even at 15<sup>th</sup> and last day of study in STZ-group ( $P < .05$ ) when compared with control group (Figure 1B). Nevertheless, similar pattern for insulin level was observed for combination groups receiving lutein with DPP-4i and resveratrol with DPP-4i with greater improvement ( $P < .05$ ) as compared to STZ-group (Figure 1B).

Correspondingly, to observe the effects of treatments on glucose tolerance, we also performed the OGTT (Figure 1C). STZ-induced diabetic rats had maximum level of glucose ( $P < .05$ ) at 30 minutes and remained persistently high at all-time points when compared with that of control-group. Conversely, a significant decline in the level of blood glucose was observed



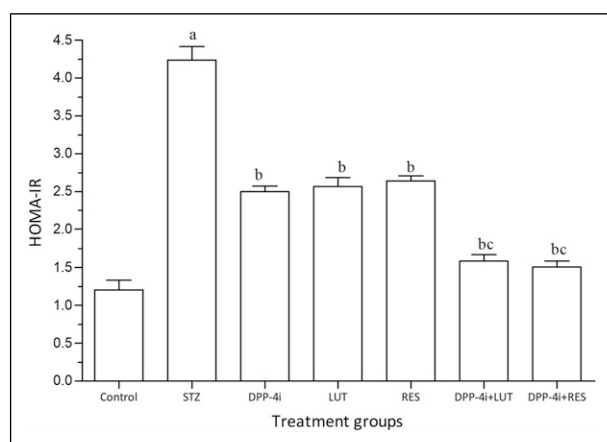
**Figure 1.** Effect of treatment on (A) glucose, (B) insulin, (C) OGTT, and (D) AUC. To estimate the effect of treatment on glycemia, we measured the serum level of glucose and insulin at 1<sup>st</sup>, 15<sup>th</sup>, and 30<sup>th</sup> day of the treatment period ( $n = 6$  rats in each group). The level of significant difference was estimated by Bonferroni post-test using two-way ANOVA and the level of significance was set at  $P < .05$ . a represents when compared with Control-group. b represents when compared with STZ-group. c represents when compared with DPP-4i alone treated group. Abbreviations| CON: control group, STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, and RES: resveratrol.

with the passage of time in experimental groups that were either receiving DPP-4i, lutein and resveratrol or combination of DPP-4i with lutein or DPP-4i with resveratrol ( $P < .05$ ) when compared with that of STZ-group (Figure 1C). Additionally, we also used the changes in the values of OGTT to calculate the AUC. STZ significantly increased the AUC when compared with that of control group but surprisingly, treatment groups significantly reduced the value of AUC when compared with that of STZ-group (Figure 1D).

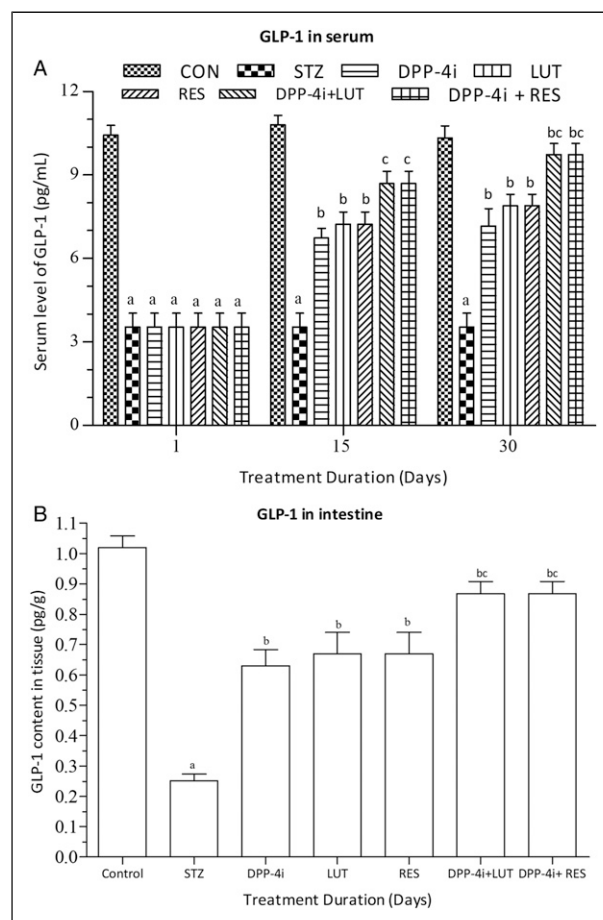
Likewise, an improvement in insulin sensitivity was also observed with combination treatments of either DPP-4i with lutein or DPP-4i with resveratrol ( $P < .001$ ) when compared to DPP-4i or lutein or resveratrol alone treated groups as calculated with the help of HOMA-IR (Figure 2).

### Effect of Treatment on GLP-1 Level

We investigated the therapeutic potential of lutein and resveratrol alone and/or in combination with DPP-4i to increase the bioavailability of GLP-1 in serum and intestine by blocking the DPP-4 enzyme that is responsible for rapid metabolism of GLP-1. Before the start of treatment, STZ significantly decreased ( $P < .05$ ) the level of GLP-1 in serum (Figure 3A) and in tissue homogenate of intestine (Figure 3B), when compared with that of control group. However, the level of GLP-1 was significantly improved ( $P < .05$ ) after DPP-4i, lutein and resveratrol treatment as compared to STZ-treated group. Interestingly, combination of lutein and resveratrol with DPP-4i further improved the levels of GLP-1 in serum and intestinal tissues ( $P < 0.05$ ) when compared with that of DPP-4i treated group (Figure 3A and B).



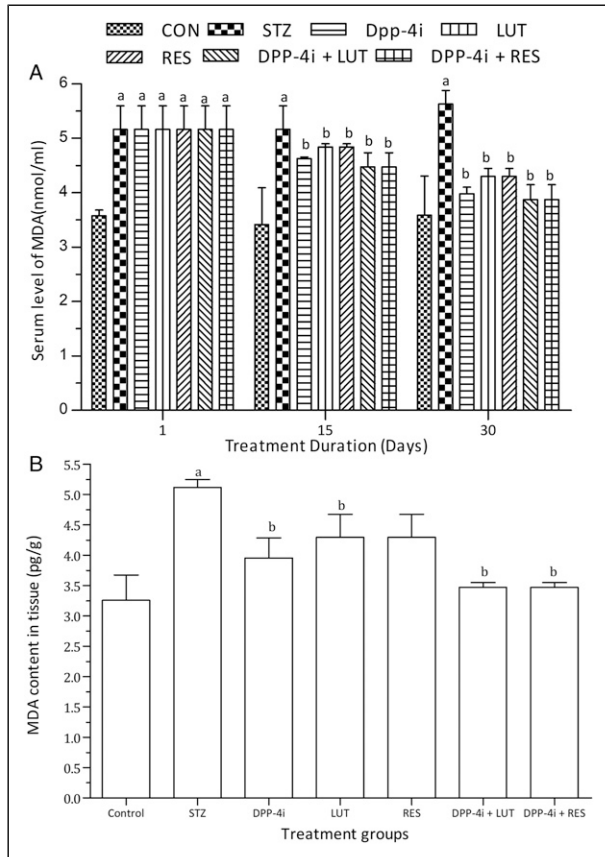
**Figure 2.** Effect of treatment on HOMA-IR. The level of significant difference was estimated by Bonferroni post-test using two-way ANOVA and the level of significance was set at  $P < .05$ . a represents when compared with Control-group. b represents when compared with STZ-group. c represents when compared with DPP-4i alone treated group. Abbreviations| STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, and RES: resveratrol.



**Figure 3.** Effect of treatment on (A) GLP-1 in serum and (B) GLP-1 in intestinal tissue. To estimate and measure the effect of treatment on GLP-1 level in serum at 1<sup>st</sup>, 15<sup>th</sup>, and 30<sup>th</sup> day of the treatment period ( $n = 6$  rats in each group). The level of significant difference was estimated by Bonferroni post-test using two-way ANOVA and the level of significance was set at  $P < .05$ . a represents when compared with Control-group. b represents when compared with STZ-group. c represents when compared with DPP-4i alone treated group. Abbreviations| CON: control group, STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, and RES: resveratrol.

### Effect of Treatments on Lipid Peroxidation and Inflammation

To estimate the influence of treatment on lipid peroxidation and inflammatory responses, we measured the levels of MDA (Figure 4), IL-6 (Figure 5A and B), and TNF- $\alpha$  (Figure 5C and D) in the serum and tissue homogenates of intestine. Before the start of treatment, STZ significantly increased ( $P < .05$ ) the serum levels of MDA (Figure 4A), IL-6 (Figure 5A), and TNF- $\alpha$  (Figure 5C) as compared to that of normal group. However, after the start of treatment, at 15<sup>th</sup> day, we found that treatment with DPP-4i or lutein or resveratrol alone significantly decreased ( $P < .05$ ) the levels of MDA, IL-6, and TNF- $\alpha$  when compared with that of STZ-group. A more pronounced decline in the levels of MDA, IL-6, and TNF- $\alpha$  was observed



**Figure 4.** Effect of treatment on (A) MDA in serum and (B) MDA in intestinal tissue. To estimate and measure the effect of treatment on MDA level in serum at 1st, 15th, and 30th day of the treatment period ( $n = 6$  rats in each group). The level of significant difference was estimated by Bonferroni post-test using two-way ANOVA and the level of significance was set at  $P < .05$ . a represents when compared with Control-group. b represents when compared with STZ-group. Abbreviations| CON: control group, STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, and RES: resveratrol.

in combination groups receiving DPP-4i with lutein or DPP-4i with resveratrol as compared to that of DPP-4i treated group ( $P < .05$ ).

### Effect of Treatments on Lipid Profile

We also assessed the therapeutic effects of DPP-4i, lutein, resveratrol alone and as well as the combination of DPP-4i with lutein, or resveratrol on lipidemia by measuring the serum levels of cholesterol, TGs, LDL, and HDL (Figure 6). STZ caused a significant disturbance ( $P < .05$ ) in the levels of lipid profile biomarkers as compared to that of control group (Figure 6). Nevertheless, improvement in STZ mediated altered levels of cholesterol, TGs, HDL, and LDL was observed *via* significantly increased serum level of HDL (Figure 6C) and decreased levels of cholesterol (Figure 6A), TGs (Figure

6B) and LDL (Figure 6D) when treated with either DPP-4i, lutein, or resveratrol and/or in combination.

### Effect of Treatments on Liver and Kidney Functions

We observed that STZ significantly elevated the serum levels of liver (Figure 7) and kidney (Figure 8) function biomarkers when compared with that of control group. A decline in the serum levels of AST (Figure 7A), ALT (Figure 7B), creatinine (Figure 8A), and urea (Figure 8B) were observed when experimental rats treated with DPP-4i, lutein or resveratrol either alone and/or in combination of DPP-4i with lutein and/or resveratrol.

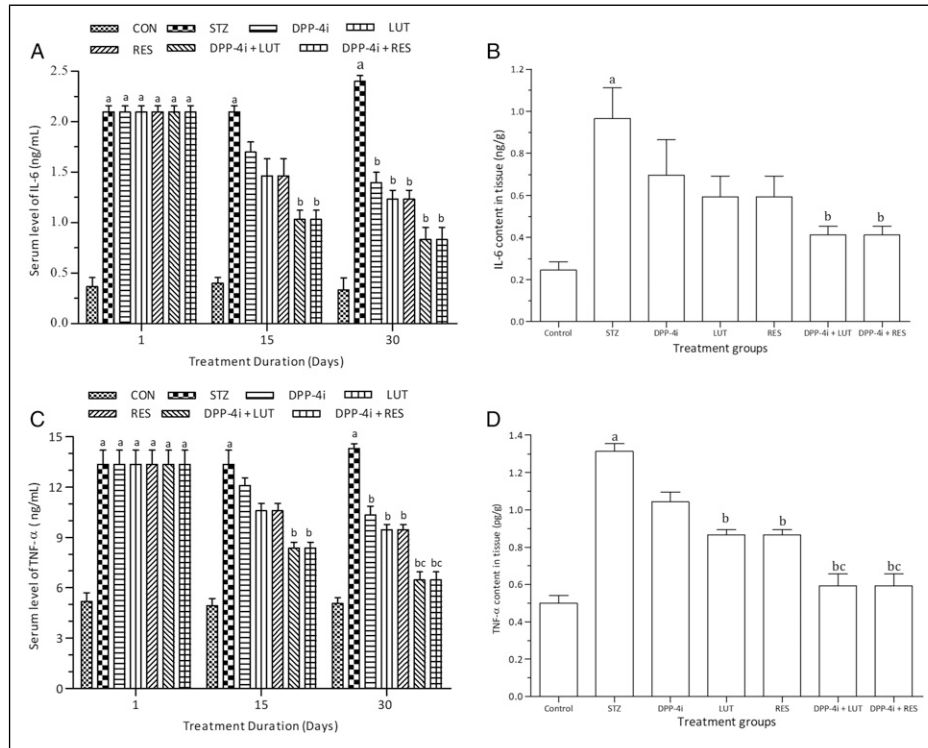
### Effect of Treatment on Histopathology of Pancreas and Intestine

The morphological and histopathological analysis of control group showed usual percentage and construction of islets of Langerhans with typical histological manner. The acinar cells were finely organized with noticeable nuclei. Nevertheless, the rats exposed to STZ, presented the destructive effects to pancreatic  $\beta$ -cells counting the islets and acini with the presence of vacuoles. The treatment with DPP-4i, lutein, or resveratrol alone and/or the combination of DPP-4i either with lutein or resveratrol reduced the injury in  $\beta$ -cell with or without fractional repair (Figure 9A-G).

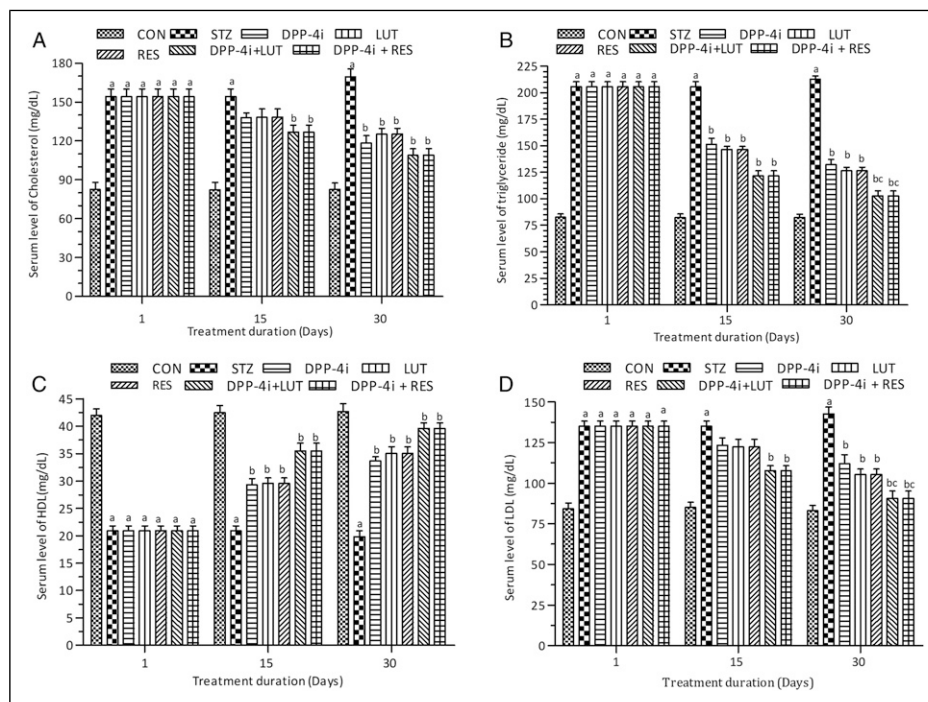
At the end of treatment period, we also inspected the histology of intestine. In control group, the intestinal cells were found in typical state, however, the rats exposed to STZ, presented the destructive effects on intestinal cells in relation to histological design and arrangement. The treatment with DPP-4i or lutein or resveratrol and/or the combination of DPP-4i either with lutein or resveratrol, reduced the disruptions in villi epithelium and increased the blood vessels which showed the improvement in morphology and histology of intestine as compared to that of streptozotocin exposed group (Figure 9H-N).

### Discussion

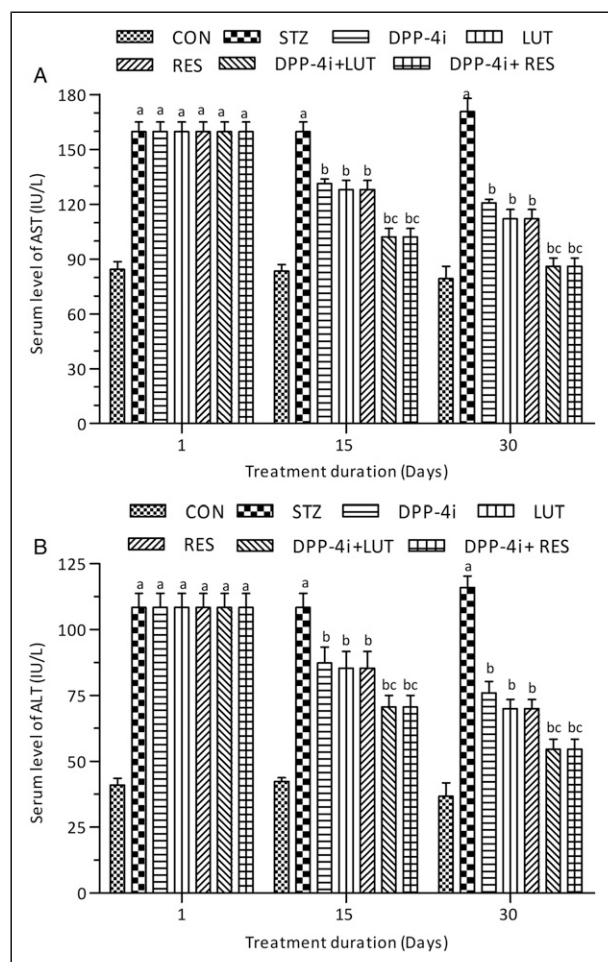
The inhibitors of DPP-4 enzymes can avert the prompt cleavage of GLP-1 and consequently intensify the action of GLP-1 by increasing its active levels; subsequently there occurs an increase in the insulin secretion with a decline in glucagon secretion. This not only reduces the glucose level, but may also decrease the percentage of glycosylated hemoglobin.<sup>41</sup> Many DPP-4 inhibitors like linagliptin has also shown beneficial role in improving GLP-1 and glycemic profile alone and/or in combination with other approved antidiabetics like metformin and/or thiazolidinediones.<sup>42</sup> As far as the therapeutic effects of these inhibitors are concerned, it is not only related to blockade of GLP-1 metabolism through DPP-4 inhibition but has also been suggested to have an important role in potentiating the intestinal secretion of GLP-1



**Figure 5.** Effect of treatment on (A) IL-6 in serum, (B) IL-6 in intestinal tissue, (C) TNF- $\alpha$  in serum and (D) TNF- $\alpha$  in intestinal tissue. To estimate and measure the effect of treatment on IL-6 and TNF- $\alpha$  level in serum at 1<sup>st</sup>, 15<sup>th</sup>, and 30<sup>th</sup> day of the treatment period (n = 6 rats in each group). The level of significant difference was estimated by Bonferroni post-test using two-way ANOVA and the level of significance was set at P < 0.05. a represents when compared with Control-group. b represents when compared with STZ-group. c represents when compared with DPP-4i alone treated group. Abbreviations| CON: control group, STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, and RES: resveratrol.



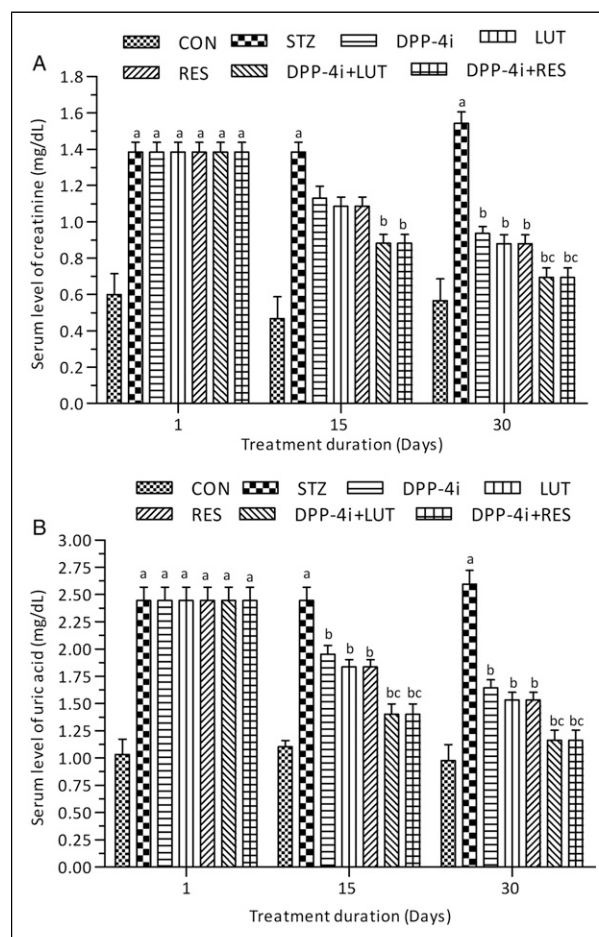
**Figure 6.** Effect of treatment on (A) cholesterol, (B) triglyceride, (C) HDL, and (C) LDL. To estimate the effect of treatment on lipid profile, we measured the serum level of cholesterol, triglyceride, HDL and LDL at 1<sup>st</sup>, 15<sup>th</sup>, and 30<sup>th</sup> day of the treatment period (n = 6 rats in each group). The level of significant difference was estimated by Bonferroni post-test using two-way ANOVA and the level of significance was set at P < .05. a represent when compared with Control-group. b represents when compared with STZ-group. c represents when compared with DPP-4i alone treated group. Abbreviations| CON: control group, STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, RES: resveratrol.



**Figure 7.** Effect of treatment on (A) AST and (B) ALT. To estimate the effect of treatment on liver function biomarkers, we measured the serum level of AST and ALT at 1<sup>st</sup>, 15<sup>th</sup>, and 30<sup>th</sup> day of the treatment period (n = 6 rats in each group). The level of significant difference was estimated by Bonferroni post-test using two-way ANOVA and the level of significance was set at P < .05. a represents when compared with Control-group. b represents when compared with STZ-group. c represents when compared with DPP-4i alone treated group. Abbreviations| CON: control group, STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, RES: resveratrol.

which helps to increase its plasma bioavailability and concentration.

We found that the levels of GLP-1 improved in serum as well as in intestinal tissues after exposure to DPP-4i; sitagliptin and a more pronounced increase in GLP-1 level was also observed when sitagliptin was used in combination with lutein or resveratrol. This was all accompanied with a positively amended glycemic profile controlling blood glucose levels along with better secretion of insulin and improved insulin sensitivity. Previously, many of the trace elements,<sup>43</sup> plant-oriented bioactive compounds, and essential oils<sup>44</sup> have also been reported to show the protective effects against many diseased conditions in animal studies<sup>45</sup> including intestinal

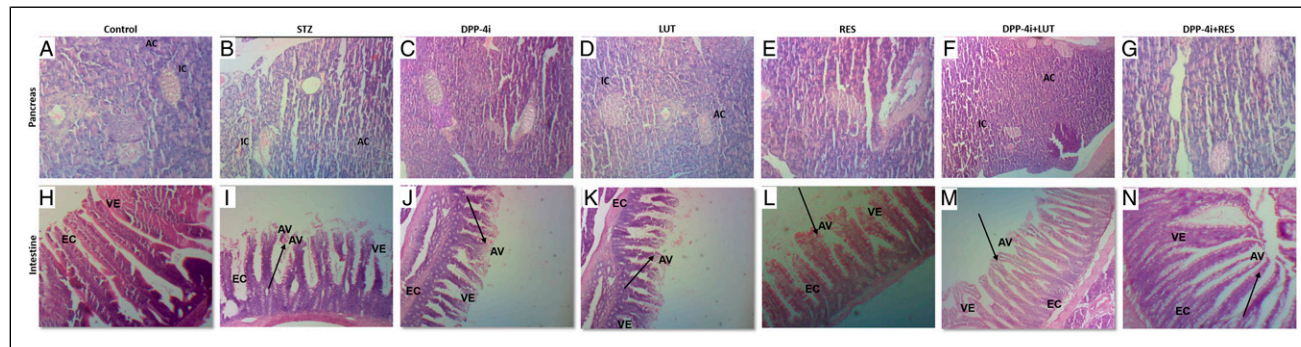


**Figure 8.** Effect of treatment on (A) creatinine and (B) uric acid. To estimate the effect of treatment on kidney function biomarkers, we measured the serum level of urea and creatinine at 1<sup>st</sup>, 15<sup>th</sup>, and 30<sup>th</sup> day of the treatment period (n = 6 rats in each group). The level of significant difference was estimated by Bonferroni post-test using two-way ANOVA and the level of significance was set at P < .05. a represents when compared with Control-group. b represents when compared with STZ-group. c represents when compared with DPP-4i alone treated group. Abbreviations| CON: control group, STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, RES: resveratrol.

health.<sup>46</sup> Where resveratrol has been reported to reverse the hyperglycemic status and improves the insulin sensitivity.<sup>47,48</sup> Moreover, resveratrol has been observed to improve the pancreatic-cell mass and insulin secretion.<sup>23</sup> Similarly lutein has proven to act against the disturbed glucose homeostasis in STZ-induced hyperglycemic experimental model.<sup>49</sup>

Correspondingly, treatment with lutein and/or resveratrol alone or in combination with sitagliptin increased the plasma and tissue concentration of GLP-1 indicating that besides having their well-known antioxidant and anti-inflammatory therapeutic profile, lutein, and resveratrol can improve GLP-1 in STZ-induced diabetic rats. Previously, lutein has not been focused to show any direct effect on enterocyte improving secretion of GLP-1; however, it may be considered from





**Figure 9.** Effect of treatment of histopathology of pancreas and intestine: In the histopathology of pancreas (A–G), Control group has regular  $\beta$ -islet cells (IC) and acinar cells (AC) with normal presentation and staining. STZ-group; presence of IC is irregular with deformed morphology. DPP-4i-group; AC have irregular appearance with congestion at few areas. LUT- and RES-alone groups; IC and AC have regular appearance with normal distribution. LUT- and RES- + DPP4-i-group; IC and AC have better appearance and numbers as compared to other groups except control group (see text for detail). The histopathological examination of intestine (H–N), Control group; the villi epithelium (VE) and enterocytes (EC) have normal appearance having integral epithelium. STZ-group; the morphology of enterocytes (EC) seems disrupted with insignificant length of villi representing short apical villi (AV). DPP-4i-group; EC are regular, but villi epithelium (VE) seems to be disrupted with regular length (AV). LUT- and RES-alone group; apical villi have regular length (AV) with good condition of EC having intact epithelium (IE). LUT- and RES- + DPP4-i-group; enterocytes (EC) are normal, and villi are full in length (AV) and have intact epithelium. Abbreviations| STZ: streptozotocin group, DPP-4i: sitagliptin, LUT: lutein, RES: resveratrol.

current results that the anti-inflammatory and/or antioxidant ability may have contributed for helping to maintain the serum levels of GLP-1. Nevertheless, some other naturally occurring flavones have shown to promote cell mass and function *via* GLP-1 stimulation.<sup>2</sup> Similarly, resveratrol has also shown its effect for improving GLP-1 secretion and showed anti-hyperglycemic effects in experimental animal model of diabetes.<sup>50</sup> Moreover, resveratrol has been shown to be effective in type 1 diabetes and/or in vitro or in vivo studies as summarized by Arumugam et al.<sup>51</sup> But in our current work, we have seen that both lutein and resveratrol when combined with sitagliptin had better therapeutic effects for controlling glycemic profile and GLP-1 levels in serum and intestine. Correspondingly, a recent study has suggested that lutein can spontaneously interact with active side of GLP-1 protein that can contribute for its effects to improve insulin secretion.<sup>52</sup> This in part shows the improved levels of GLP-1 observed by lutein and sitagliptin in present work where sitagliptin blocked the effect of DPP-4 that further enhanced the potential of lutein. This combinatorial effect of lutein and resveratrol with DPP-4 inhibitor was not only effective in regulating glycemic profile, improving insulin resistance and GLP-1 levels, but providentially the finest of these combinatorial groups were also seen in modulating oxidative stress and inflammatory response by controlling the elevated levels of MDA, IL-6, and TNF $\alpha$  in serum and intestinal tissues. Previous studies also reported the similar effects for lutein supplementation that controlled the production of inflammatory cytokine and antioxidant status in experimental animals.<sup>53-56</sup> Recently, lutein has shown protective effect on hyperglycemia-mediated alteration in oxidative stress along with antioxidant capacity in vitro.<sup>57</sup> Further, lutein treatment significantly inhibited the high glucose-triggered production of reactive oxygen

species.<sup>58</sup> Further, this goes in agreement with previously published reports documenting the potential of resveratrol against oxidative stress and inflammatory responses during stressed and diseased conditions.<sup>59,60</sup> Whereas, grape seed extract, a great source of resveratrol has been also declared to possess the antioxidant and anti-inflammatory effects.<sup>61</sup>

This evidently relates the fact that how these natural compounds can help to ameliorate the metabolic impairment which are the key factors associated with prevalence and worsening of metabolic disorders.<sup>62</sup> Additionally, the outcomes of histological inspection of pancreas and intestine in our study were found to be in accordance with biochemical effects observed during the study period. STZ made a noticeable deterioration of pancreatic islets and acinar cells as depicted by the histological study, whereas lutein and resveratrol both succeeded in protecting and restoring the islets cells from the damaging effects of streptozotocin. Likewise, protective response was observed on intestinal L-enterocytes.

In short, the substantial verdicts of this study would surely help to recognize the therapeutic potential of lutein and resveratrol in combination with DPP-4i as stimulators of GLP-1 secretion *via* downregulating inflammatory responses and protecting/restoring the damaged pancreatic and intestinal morphology. This study may provide a substantial recommendation for impending the drug designing of such types of combinatorial therapeutic approach.

## Conclusion

Combined administration of DPP-4i+LUT or DPP-4i+RES showed the expected therapeutic effects, lowering blood glucose, and maintaining the serum insulin concentrations with improved glucose sensitivity and reduced insulin

resistance. Whereas delivery of either DPP-4i, LUT, or RES alone had low therapeutic effects while the co-administration of LUT and RES with DPP-4i revealed the potential beneficial effects on insulin resistance, circulating lipids, glycemic index, oxidative stress, and inflammatory status along with restoration of histological morphology of pancreatic cells and enterocytes that seemed to facilitate GLP-1 level in serum and intestine. This depicts that lutein and resveratrol alone and/or in combination with DPP-4i reduced the STZ-induced oxidative stress and inflammation in enteroendocrine L Cells restoring histopathology that resulted in fractional restoration of GLP-1 expression and associated insulin sensitivity.

### Study Limitations

It would be worth mentioning that our study has some limitations as we did not opt for *in vitro* and/or *in silico* studies, and hence, we could not explore the particular protein or receptor interaction and binding affinity and/or energy of lutein and resveratrol. Moreover, immunohistochemistry (IHC) of insulin antibody within the pancreas to explore the degeneration of pancreatic-cells and GLP-1 antibody in intestinal tissues could not be carried out. In addition, we also suggest future work on pharmacokinetic parameters of lutein and resveratrol.

### Author Contributions

Conceptualization: Conceived and designed the experiments: Kanwal Rehman and Muhammad Sajid Hamid Akash. Analyzed the data: Areeba Javed, Ayman Muzammal, and Muhammad Sajid Hamid Akash. Wrote the manuscript: Areeba Javed and Ayman Muzammal. Editing of the Manuscript: Muhammad Sajid Hamid Akash. Critically reviewed the article: Kanwal Rehman

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

### ORCID iD

Muhammad Sajid Hamid Akash  <https://orcid.org/0000-0002-9446-5233>

Kanwal Rehman  <https://orcid.org/0000-0001-7873-6681>

### References

1. Wondmkun YT. Obesity, insulin resistance, and type 2 diabetes: associations and therapeutic implications. *Diabetes Metab Syndr Obes.* 2020;13:3611-3616. doi:10.2147/DMSO.S275898
2. Wang Y, Wang A, Alkhalidy H, et al. Flavone hispidulin stimulates glucagon-Like peptide-1 secretion and ameliorates hyperglycemia in streptozotocin-induced diabetic mice. *Mol Nutr Food Res.* 2020; 64:1900978. doi:10.1002/mnfr.201900978.
3. Rehman K, Ali MB, Akash MSH. Genistein enhances the secretion of glucagon-like peptide-1 (GLP-1) via downregulation of inflammatory responses. *Biomed Pharmacother.* 2019;112: 108670. doi:10.1016/j.biopha.2019.108670.
4. Lalitha N, Sadashivaiah B, Ramaprasad TR, Singh SA. Anti-hyperglycemic activity of myricetin, through inhibition of DPP-4 and enhanced GLP-1 levels, is attenuated by co-ingestion with lectin-rich protein. *PLoS one.* 2020;15:e0231543. doi:10.1371/journal.pone.0231543.
5. Tremblay A, Lamarche B, Deacon CF, Weisnagel S, Couture P. Effect of sitagliptin therapy on postprandial lipoprotein levels in patients with type 2 diabetes. *Diabetes Obes Metab.* 2011;13: 366-373. DOI:10.1111/j.1463-1326.2011.01362.x.
6. Abbasi F, Adatorwovor R, Davarpanah MA, et al. A randomized trial of sitagliptin and spironolactone with combination therapy in hospitalized adults With COVID-19. *J Endocr Soc.* 2022;6: bvac017. doi:10.1210/jendso/bvac017.
7. He X, Kuang G, Zuo Y, et al. The Role of Non-coding RNAs in Diabetic Nephropathy-Related Oxidative Stress. *Front Med.* 2021;8:626423. doi:10.3389/fmed.2021.626423.
8. Li Y, Lv X, Jiang M, Jin Z. Sitagliptin ameliorates hypoxia-induced damages in endometrial stromal cells: an implication in endometriosis. *Bioengineered.* 2022;13:800-809. doi:10.1080/21655979.2021.2012950.
9. Patel R, Parmar N, Rathwa N, et al. A novel therapeutic combination of sitagliptin and melatonin regenerates pancreatic  $\beta$ -cells in mouse and human islets. *Biochim Biophys Acta Mol Cell Res.* 2022; 1869:119263. doi:10.1016/j.bbamcr.2022.119263.
10. Ren J, Wang X, Yee C, Gorrell MD, McLennan SV, Twigg SM. Sitagliptin is more effective than gliclazide in preventing profibrotic and pro-inflammatory changes in a rodent model of diet-induced non-alcoholic fatty liver disease. *Molecules* 2022;27: 727. doi:10.3390/molecules27030727.
11. Safaeian A, Izadi M, Zare Mehrjerdi F, Yadegari M, Rezvani ME. The effect of sitagliptin on inflammatory mediators in the ovary of rat with polycystic ovary syndrome. *International Journal of Medical Laboratory.* 2022;9:55-64. doi:10.18502/ijml.v9i1.9085.
12. Wadie W, Ahmed GS, Shafik AN, El-Sayed M. Effects of insulin and sitagliptin on early cardiac dysfunction in diabetic rats. *Life Sci.* 2022;299:120542. doi:10.1016/j.lfs.2022.120542.
13. Xu L, Shao F. Sitagliptin protects renal glomerular endothelial cells against high glucose-induced dysfunction and injury. *Bioengineered.* 2022;13:655-666. doi:10.1080/21655979.2021.2012550.
14. Abdullah MAK, Ahmad W, Adhikari A, et al. Exploration of hepatoprotective potential and phytochemicals of *Ziziphos oxyphylla* Edgew. *Pak Vet J.* 2020;40:431-436. doi:10.29261/pakvetj/2020.054.
15. Zhang K, Li X, Na C, Abbas A, Abbas RZ, Zaman MA. Anticoccidial effects of *Camellia sinensis* (green tea) extract and its

- effect on blood and serum chemistry of broiler chickens. *Pak Vet J.* 2020;40:77-80. doi:10.29261/pakvetj/2019.119.
16. Rasheed S, Rehman K, Shahid M, Suhail S, Akash MSH. Therapeutic potentials of genistein: New insights and perspectives. *J Food Biochem.* 2022:e14228. doi:10.1111/jfbc.14228.
  17. Rehman K, Chohan TA, Waheed I, Gilani Z, Akash MSH. Taxifolin prevents postprandial hyperglycemia by regulating the activity of  $\alpha$ -amylase: Evidence from an in vivo and in silico studies. *J Cell Biochem.* 2019;120:425-438. doi:10.1002/jcb.27398.
  18. Rehman K, Rashid U, Jabeen K, Akash MSH. Morin attenuates L-arginine induced acute pancreatitis in rats by downregulating myeloperoxidase and lipid peroxidation. *Asian Pac J Trop Biomed.* 2021;11:148-154. doi:10.4103/2221-1691.310201.
  19. Ijaz MU, Aziz S, Faheem M, et al. Orientin attenuates cisplatin-induced renal toxicity by reducing oxidative stress and inflammation. *Pak Vet J.* 2021;41:574-578. doi:10.29261/pakvetj/2021.076.
  20. Sahu R, Dewanjee S. Carotenoids as Antidiabetic Agents. In: Zia-Ul-Haq M, Dewanjee S, Riaz M, eds. *Carotenoids: Structure and Function in the Human Body.* Cham: Springer International Publishing; 2021:513-532.
  21. Kang MK, Lee EJ, Kim YH, et al. Chrysin ameliorates malfunction of retinoid visual cycle through blocking activation of AGE-RAGE-ER stress in glucose-stimulated retinal pigment epithelial cells and diabetic eyes. *Nutrients.* 2018;10:1046. DOI: 10.3390/nu10081046.
  22. Fu Z, Meng SS, Burmim SB, Smith LE, Lo AC. Lutein facilitates physiological revascularization in a mouse model of retinopathy of prematurity. *Clin Experiment Ophthalmol.* 2017;45:529-538. doi:10.1111/ceo.12908.
  23. Hoca M, Becer E, Vatansver HS. The role of resveratrol in diabetes and obesity associated with insulin resistance. *Arch Physiol Biochem.* 2021;1-7. doi:10.1080/13813455.2021.1893338.
  24. Ahangarpour A, Sayahi M, Sayahi M. The antidiabetic and antioxidant properties of some phenolic phytochemicals: A review study. *Diabetes Metab Syndr.* 2019;13:854-857. doi:10.1016/j.dsx.2018.11.051.
  25. Yang DK, Kang HS. Anti-diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats. *Biomol Ther (Seoul).* 2018;26:130-138. doi:10.4062/biomolther.2017.254.
  26. Oh WY, Gao Y, Shahidi F. Stilbenoids: Chemistry, occurrence, bioavailability and health effects—a review. *J Food Bioact.* 2021;13:20-31. doi:10.31665/JFB.2020.13256.
  27. Khalid MF, Rehman K, Irshad K, Chohan TA, Akash MSH. Biochemical Investigation of Inhibitory Activities of Plant-Derived Bioactive Compounds Against Carbohydrate and Glucagon-Like Peptide-1 Metabolizing Enzymes. *Dose Response.* 2022;20:16. doi:10.1177/15593258221093275.
  28. Irshad K, Rehman K, Akash MSH, Hussain I. Biochemical Investigation of Therapeutic Potential of Resveratrol Against Arsenic Intoxication. *Dose Response.* 2021;19:13. doi:10.1177/15593258211060941.
  29. Qader A, Rehman K, Akash MSH. Biochemical profiling of lead-intoxicated impaired lipid metabolism and its amelioration using plant-based bioactive compound. *Environ Sci Pollut Res Int.* 2022;12. doi:10.1007/s11356-022-20069-5.
  30. Akash MSH, Sharif H, Rehman K, Rasheed S, Kamal S. Biochemical profiling of arsenic trioxide-induced impaired carbohydrate metabolism and its therapeutic intervention via modulation of metabolic pathways. *Pak J Zoo.* 2022;10. DOI: 10.17582/journal.pjz/20220204050235.
  31. Sanches-Silva A, Testai L, Nabavi SF, et al. Therapeutic potential of polyphenols in cardiovascular diseases: Regulation of mTOR signaling pathway. *Pharmacol Res.* 2020;152:104626. DOI:10.1016/j.phrs.2019.104626.
  32. Szkudelska K, Szkudelski T. Resveratrol, obesity and diabetes. *Eur J Pharmacol.* 2010;635:1-8. doi:10.1016/j.ejphar.2010.02.054.
  33. Umar Z, Qureshi AS, Shahid RU, Deeba F. Macroscopic, microscopic and histomorphometric analysis of intestine, liver and pancreas of ostrich (*Struthio camelus*) with advancement of age and sex. *Pak Vet J.* 2021;41:313-320. doi:10.29261/pakvetj/2020.029.
  34. Kusmiati CW, Afiati F, Hutabarat R, eds. Effect lutein of marigold flower (*Tagetes erecta* L.) on decreasing glucose and malondialdehyde levels in Alloxan-induced blood mice. In: AIP Conference Proceedings. Malang, Indonesia, 03 July 2019.
  35. Yarmolinskaya M, Bulgakova O, Abashova E, Borodina V, Tral T. The effectiveness of resveratrol in treatment of PCOS on the basis of experimental model in rats. *Gynecol Endocrinol.* 2021; 37:54-57. doi:10.1080/09513590.2021.2014665.
  36. Mega C, Vala H, Teixeira de Lemos E, et al. Biochemical and histological effects of sitagliptin on Zucker Diabetic Fatty rat pancreas. *Virchows Arch.* 2010;457:153. doi:10.1007/s00428-010-0947-z.
  37. Mohamed NA, Zaitone SA, Moustafa YM. Effect of sitagliptin in combination with glimepiride on glycemic control and islet cell diameter/proliferation in a model of type 2 diabetic rats. *IOSR J Pharm.* 2013;3:72-80. doi:10.9790/3013-0309-72-80.
  38. Saad MI, Kamel MA, Hanafi MY. Modulation of Adipocytokines Production and Serum NEFA Level by Metformin, Glimepiride, and Sitagliptin in HFD/STZ Diabetic Rats. *Biochem Res Int.* 2015;2015:138134-138137. doi:10.1155/2015/138134.
  39. Shawky LM, Morsi AA, El Bana E, Hanafy SM. The Biological Impacts of Sitagliptin on the Pancreas of a Rat Model of Type 2 Diabetes Mellitus: Drug Interactions with Metformin. *Biology.* 2019;9:6. doi:10.3390/biology9010006.
  40. Halenova T, Zlatskiy I, Syroeshkin A, Maximova T, Pleteneva T. Deuterium-depleted water as adjuvant therapeutic agent for treatment of diet-induced obesity in rats. *Molecules.* 2019;25:23. doi:10.3390/molecules25010023.
  41. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet.* 2006;368:1696-1705. doi:10.1016/S0140-6736(06)69705-5.
  42. Gomis R, Espadero RM, Jones R, Woerle H, Dugi K. Efficacy and safety of initial combination therapy with linagliptin and pioglitazone in patients with inadequately controlled type 2

- diabetes: a randomized, double-blind, placebo-controlled study. *Diabetes Obes Metab.* 2011;13:653-661. doi:10.1111/j.1463-1326.2011.01391.x.
43. Ashraf S, Bhatti SA, Nawaz H, Khan MS. Assessment of dietary selenium sources in commercial male broiler breeders: effects on semen quality, antioxidant status and immune responses. *Pak Vet J.* 2020;39:13-18. doi:10.29261/pakvetj/2019.081.
  44. Yasmin S, Nawaz M, Anjum AA, et al. Phytochemical analysis and in vitro activity of essential oils of selected plants against *Salmonella enteritidis* and *Salmonella gallinarum* of poultry origin. *Pak Vet J.* 2019;40:139-144. doi:10.29261/pakvetj/2019.110.
  45. Asrar B, Khan I, Homberg JR, Haleem DJ. Nigella sativa oil ameliorates chronic ethanol induced anxiety and impaired spatial memory by modulating noradrenaline levels. *Pak Vet J.* 2020;40:350-354. doi:10.29261/pakvetj/2020.029.
  46. Masood A, Qureshi AS, Shahid RU, Jamil H. Effects of oral administration of essential oil (Mix Oil®) on growth performance and intestinal morphometry of Japanese Quails (*Coturnix coturnix japonica*). *Pak Vet J.* 2020;40:385-389. doi:10.29261/pakvetj/2020.018.
  47. Movahed A, Nabipour I, Lieben Louis X, et al. Anti-hyperglycemic effects of short term resveratrol supplementation in type 2 diabetic patients. *Evid Based Complement Alternat Med.* 2013;2013:851267-851311. doi:10.1155/2013/851267.
  48. Zhang H, Zhang J, Ungvari Z, Zhang C. Resveratrol improves endothelial function: role of TNF {alpha} and vascular oxidative stress. *Arterioscler Thromb Vasc Biol.* 2009;29:1164-1171. doi:10.1161/atvbaha.109.187146.
  49. Sharavana G, Joseph GS, Baskaran V. Lutein attenuates oxidative stress markers and ameliorates glucose homeostasis through polyol pathway in heart and kidney of STZ-induced hyperglycemic rat model. *Eur J Nutr.* 2017;56:2475-2485. doi:10.1007/s00394-016-1283-0.
  50. Dao T-MA, Waget A, Klopp P, et al. Resveratrol increases glucose induced GLP-1 secretion in mice: a mechanism which contributes to the glycemic control. *PLoS One.* 2011;6:e20700. doi:10.1371/journal.pone.0020700.
  51. Arumugam S, Thandavarayan RA, Arozal W, et al. Quercetin offers cardioprotection against progression of experimental autoimmune myocarditis by suppression of oxidative and endoplasmic reticulum stress via endothelin-1/MAPK signalling. *Free Radic Res.* 2012;46:154-163. doi:10.3109/10715762.2011.647010.
  52. Hartati FK, Djauhari AB, Viol Dhea K. Evaluation of pharmacokinetic properties, toxicity, and bioactive cytotoxic activity of black rice (*Oryza sativa* L.) as candidates for diabetes mellitus drugs by in silico. *Biointerface Res Appl Chem.* 2021;11:12301-12311. DOI:10.33263/BRIAC114.1230112311.
  53. Shanmugasundaram R, Selvaraj R. Lutein supplementation alters inflammatory cytokine production and antioxidant status in F-line turkeys. *Poult Sci.* 2011;90:971-976. doi:10.3382/ps.2010-01150.
  54. Meriwether L, Humphrey B, Peterson D, Klasing K, Koutsos E. Lutein exposure, in ovo or in the diet, reduces parameters of inflammation in the liver and spleen laying-type chicks (*Gallus gallus domesticus*). *J Anim Physiol Anim Nutr.* 2010;94:e115-e122. doi:10.1111/j.1439-0396.2010.00990.x.
  55. Rehman K, Akash MSH, Liaqat A, et al. Role of Interleukin-6 in Development of Insulin Resistance and Type 2 Diabetes Mellitus. *Crit Rev Eukaryot Gene Expr.* 2017;27:229-236. DOI:10.1615/CritRevEukaryotGeneExpr.2017019712.
  56. Akash MSH, Rehman K, Liaqat A. Tumor necrosis factor-alpha: Role in development of insulin resistance and pathogenesis of type 2 diabetes mellitus. *J Cell Biochem.* 2018;119:105-110. doi:10.1002/jcb.26174.
  57. Shivarudrappa AH, Ponesakki G. Lutein reverses hyperglycemia-mediated blockage of Nrf2 translocation by modulating the activation of intracellular protein kinases in retinal pigment epithelial (ARPE-19) cells. *J Cell Commun Signal.* 2020;14:207-221. doi:10.1007/s12079-019-00539-1.
  58. Hwang JS, Han SG, Lee C-H, Seo HG. Lutein suppresses hyperglycemia-induced premature senescence of retinal pigment epithelial cells by upregulating SIRT1. *J Food Biochem.* 2018;42:e12495. doi:10.1111/jfbc.12495.
  59. Bishayee A, Barnes KF, Bhatia D, Darvesh AS, Carroll RT. Resveratrol suppresses oxidative stress and inflammatory response in diethylnitrosamine-initiated rat hepatocarcinogenesis. *Cancer Prev Res.* 2010;3:753-763. doi:10.1158/1940-6207.CAPR-09-0171.
  60. Rehman K, Saeed K, Munawar SM, Akash MSH. Resveratrol regulates hyperglycemia-induced modulations in experimental diabetic animal model. *Biomed Pharmacother.* 2018;102:140-146. doi:10.1016/j.biopha.2018.03.050.
  61. Qamar H, Li A, Zeng Z, et al. Effect of grape seed extract on tibial dyschondroplasia incidence, liver weight, and tibial angiogenesis in chickens. *Pak Vet J.* 2020;40:187-219. doi:10.29261/pakvetj/2019.109.
  62. Marselli L, Piron A, Suleiman M, et al. Persistent or transient human  $\beta$  cell dysfunction induced by metabolic stress: specific signatures and shared gene expression with type 2 diabetes. *Cell Rep.* 2020;33:108466. doi:10.1016/j.celrep.2020.108466.