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Beyond Monotherapy: Synergistic Antioxidant and Antidiabetic Potential of para-Coumaric Acid Combined with Vitamin D

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Abstract

Oxidative stress plays a central role in the progression of diabetes mellitus and its associated complications. Although the therapeutic potential of individual phytochemicals has been widely explored, their synergistic effects with micronutrients remain largely unknown. In this study, we investigated the antioxidant and antidiabetic potential of para-coumaric acid (p--CA) in combination with vitamin D and riboflavin. Antioxidant activity was assessed using NBT, DPPH, and FRAP assays, revealing superior efficacy of the p-CA–vitamin D combination. In H_2O_2-induced oxidative stress in lymphocytes, the equimolar p-CA–vitamin D combination (P50+D50) restored superoxide dismutase and catalase activities, reduced malondialdehyde levels and intracellular reactive oxygen species generation, and preserved normal cellular morphology. Furthermore, P50+D50 exhibited enhanced α-amylase and α-glucosidase inhibitory activities. These findings suggest that combining p-CA with vitamin D exerts synergistic antioxidant and antidiabetic effects and may represent a promising strategy for mitigating diabetes-associated oxidativestress and its complications.

Keywords: Para-coumaric acid; Vitamin D and Riboflavin; Synergistic approach; Antidiabetic; Antioxidative, SEM

Abbreviation

SEM-scanning electron microscope

NBT- Nitro Blue Tetrazolium

DCFH-DA- Dichlorofluorescein Diacetate

FRAP- ferric reducing antioxidant power

MDA-malondialdehyde

1. Introduction

Diabetes and Oxidative Stress Intensify Each Other. The studies carried out on the relationship of oxidative stress with diabetes have played the role of a missing piece of the puzzle in the understanding of the mechanism by which diabetes and its associated complications occur. Free radicals are essential in cellular signaling, synaptic plasticity, memory formation, cell-cell interactions, autophagy, cell growth, apoptotic events, and aging [1,2]. However, oxidative stress develops when the rate at which the free radicals are generated exceeds the cell's antioxidant capacity. The various antioxidant mechanisms the cell possesses include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPR), and glutathione reductase (GR), as well as Glutathione as a reducing buffer, which protects the cell against oxidative damage to a certain extent [3,4]. These free radicals when generated in excess/ exceeds the antioxidants' capacity can cause oxidative damage to proteins, lipids, and nucleic acids, producing toxic byproducts and resulting in tissue dysfunction. Oxidative stress is one of the major causes of developing of various complications associated with diabetes and the development of insulin resistance [5,6]. There are at least five major molecular mechanisms through which it can reduce peripheral insulin sensitivity. These include β-cell dysfunction, inflammatory responses, down regulation and localization of GLUT-4, mitochondrial dysfunction, and impaired insulin signaling pathways [7].

On the other hand, as a reducing sugar, glucose is suggested to have a role in the catalysis of the oxidative modification and crosslinking of proteins. Glycation induced by oxidative stress also plays a role in the enhanced production of free radicals, ultimately leading to the fragmentation of proteins as well as the oxidation of the associated lipids. It is initiated with the oxidation of an aldose or a ketose in the presence of metal ions to a more reactive dicarbonyl sugar and the generation of reactive oxygen species like superoxide, the hydroxy radical, and hydrogen peroxide. The dicarbonyl sugar reacts with protein, whereas the byproducts cause oxidative damage to the neighboring molecules. Autoxidative Glycation increases with an increase in blood glucose concentration [8,9]. Early Glycation products formed as a result of autoxidation Glycation undergo further chemical rearrangement by free radical-mediated oxidation, leading to the formation of irreversible, advanced Glycation end products (AGEs). These AGEs tend to accumulate with time in structural proteins such as collagens, or they may induce covalent crosslinking, resulting in the hardening of the vessel walls and a loss in their elasticity and permeability [10,11].

Numerous studies published in the past have indicated positive outcomes when using or treating patients with natural and herbal remedies [3,13].4 Hydroxycinnamic acid, p-coumaric acid (p-CA), is a phenolic acid found in fruits, vegetables, and mushrooms [12]. Its biosynthesis depends on the conversion of tyrosine into p-CA by tyrosine ammonia lyase. From there, p-CA is transformed into phenolic acids like caffeic acid, ferulic acid, sinapic acid, and other secondary metabolites like lignin and its precursors [14]. Studies conducted have shown that p CA has a variety of bioactivities, including antioxidant, anti-inflammatory, anti-platelet aggregation, analgesic, anticancer, and neuroprotective activity [12]. One recent study in our lab showed the potential antioxidant and antiglycation activity of p-CA among three isomers (ortho, Meta and para coumaric acid) [33]. Another study in our lab suggested that the anti-glycation antioxidant and ant-fibrillation effects of p-CA were found to increased when used with vitamin-D [32]. Due to biological benefits, research is being conducted to see whether this compound can lessen, prevent, or cure diabetes. On the other hand, vitamin D is a prohormone and a fat-soluble vitamin generated from 7-dehydrocholesterol in the skin under the influence of UV light. Regardless of the source from which it is derived, vitamin D needs to get hydroxylated twice to be biologically active. Vitamin D deficiency can lead to severe outcomes such as low bone density, increased risk of hip fractures, non-vertebral fractures, etc. Vitamin D deficiency was linked to insulin sensitivity or abnormal glucose metabolism and diabetes [16]. Over the years, diabetes has been linked to vitamin D deficiency. In the 1980s, it was reported that insulin secretion was inhibited in rodents and rabbits due to vitamin D deficiency. Later, with the discovery of VDR (Vitamin D Receptor) and DPB (vitamin D Binding Protein) in the pancreatic tissue (insulin-producing β cells), it was proposed as a possible therapeutic agent in the prevention and treatment of T1D and T2D [17]. The active form of vitamin D (1, 25-dihydroxy vitamin D) stimulates islets of the Langerhans in the pancreas to secrete insulin by increasing the cytosolic concentration

of Ca2+ ions, essential for its exocytosis from βcells. Recent animal and human studies have suggested that vitamin D can reduce diabetes risk. This may be possible by several pathways, like direct stimulation of the beta cells through the VDR for insulin secretion, the expression of the 1-α-hydroxylase enzyme in the βcells, and activation of human insulin gene transcription, which may further improve insulin secretion. It was also reported that vitamin D could regulate the synthesis and secretion of insulin by the cleavage of pro-insulin to insulin which is catalysed by a βcell calcium-dependent endopeptidase [18]. Another important molecule is Riboflavin (RF). Generally known as vitamin B2 is a water-soluble vitamin and is one such vitamin that is necessary for healthy cellular growth and development. RF is found in a wide range of dietary products, usually as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Humans are susceptible to developing RF deficiency during food restriction or physiological and pathological stress. These include growth retardation, anaemia, skin lesions, renal damage, and degenerative alterations in the neurological system, among other clinical problems. RF deficiency has been noted in the elderly and those with eating disorders, diabetes, chronic heart disease, inflammatory bowel disease, and HIV [19]. Recent research has demonstrated that RF can shield tissues from oxidative damage in an alloxan-induced diabetic rat model [20]. RF decreases myocardial lipid peroxidation, leukocyte infiltration, cytokine production, and cardiac allograft vasculopathy in a mouse heart transplantation model [21]. Furthermore, it has been discovered that riboflavin increases the survival rate of mice with endotoxin-induced sepsis and gram-positive and gram-negative bacterial infection [22]. Therefore, it was hypothesized that combining p-CA with vitamin D and with riboflavin could prevent diabetes related complication due to the cumulative damage produced by ROS over time, in a better way than p-CA alone, expecting the effect being synergistic.

2. Materials and method

Materials

Vitamin D (Code-97389), Nitro Blue Tetrazolium (Code-48898), Riboflavin (34392), α-amylase (28588), 2,7 Dichlorofluorescein Diacetate (85048), p-nitro phenol-a-D-glucopyranoside (96226), 2,2-dipheny-1-picrylhydrazyl (29128) and Histopaque--1077 were purchased from Sisco Research Laboratory India. α-glucosidase (CAS number 9001-42-7) was purchased from Sigma Aldrich, India. Para coumaric acid (p-CA) was obtained from Koch-Light Laboratory Ltd England. All other chemicals used were of analytical grade.

Methods

2.1. Antioxidant activity assays

2.1.1. NBT test

Generation of the superoxide anion was measured by the Nitro Blue Tetrazolium (NBT) assay with slight modifications [23]. 50mM Phosphate Buffer Saline, 1mM NBT, 1mM EDTA, and 0.06% triton X-100 were added to the experimental setup consisting of 50 μM of p-CA, vitamin D and riboflavin each alone and in combination with p-CA in the ratios 1:1. The absorbance of all samples was read at 560nm after 2 hours of incubation at room temperature.

2.1.2. DPPH radical scavenging activity

DPPH was also used to evaluate the antioxidant potential of the two compounds [24]. The radical scavenging activity was determined using 1mM of DPPH solution. 300μL of DPPH solution was added to the experimental setup consisting of 50μM p-CA, 50μM vitamin D, 50μM riboflavin, and synergistic mixtures of p-CA with vitamin D/riboflavin in the ratios 1:1 was incubated at room temperature for 30 minutes and read at 517nm.

To calculate the scavenging activity of p-CA, following formula was used:

$$\text{DPPH radical scavenging activity}(\%) = \frac{A\text{control} - A\text{sample}}{A\text{control}} \text{ x } 100 \quad (1)$$

2.1.3. FRAP assay

The antioxidant potential of p-CA-vitamin D and p-CA-riboflavin was also assessed using the Ferric Reducing Antioxidant Power assay (FRAP assay). The method of Muller was used with modifications [25]. The 2.5mL of TPTZ solution (10mM), 25mL acetate buffer (0.1M, pH 3.6), and 2.5mL FeCl3 were prepared and incubated at 370C for 10 minutes. Then 2mL of freshly prepared FRAP solution was added to the experimental set consisting of 50 µM p-CA, 50 µM vitamin D, 50 µM riboflavin. In case of combination study, p-CA and vitamin D and riboflavin ratio (1:1) was incubated at room temperature for 10 minutes and then reading was taken at 593nm.

2.2. Lymphocyte-based study

2.2.1. Isolation of Human lymphocytes:

In order to isolate lymphocytes, human blood was mixed with normal saline and Histopaque 1077 in a 1:1:1 ratio at room temperature. The mixture was centrifuged at 2400 rpm for 20 minutes, and a white buffy layer of lymphocytes, just above Histopaque 1077, was obtained. The cells were pelleted by centrifugation at 10000 rpm for 10 minutes and washed thrice with normal saline. Then, the lymphocytes were suspended in normal saline to make 5% (v/v) cell suspension. A small quantity was used to perform fluorescence microscopy, and the rest was processed further.

2.2.2. Treatment of Isolated Lymphocytes with H2O2 to Induce Oxidative Stress

The healthy control lymphocytes were incubated with PBS. Stressed control lymphocytes were incubated with H2O2. Other samples were prepared by adding H2O2 along with 50 µM p-CA, 50 µM vitamin D, 50 µM riboflavin alone, and in combination of p-CA with vitamin D as well as with riboflavin in ratio 1:1 and incubated for 1 hour at 37o C. The lymphocytes were centrifuged at 12000 rpm for 10 minutes after the H2O2 treatment, and the pellets were collected and resuspended in lysis buffer (0.2% Triton X-100, 100mM NaCl, 1mM EDTA, and 20mM Tris HCl, pH 7.4) and incubated at 400C for 20 minutes. The samples were centrifuged again at 12000 rpm for 10 minutes. The supernatants were collected and finally quickly frozen for further study.

2.2.3. SOD activity analysis

The method based on the auto-oxidation of pyrogallol was used [26]. Tris succinate buffer (0.05M, pH 8.2) was added to 50µL of lysate samples and was incubated at 250C for 20 minutes. Then 0.1 mL of 8mM pyrogallol was added to the sample mixture, and absorbance was taken at 412 nm every 30 seconds for 3 minutes using a spectrophotometer.

2.2.4. Catalase activity assay

It is an indirect method for estimating ROS generation, which is inversely proportional to the enzyme catalase activity [27]. The 7 samples were added to 0.05M (pH 7.0) potassium phosphate buffer and 30 mM H2O2. The absorbance was recorded at 240 nm every 30 seconds for 2 minutes.

2.2.5. Lipid peroxidation

It was performed using the method of Beuge and Aust to determine the extent of lipid peroxidation [28]. H2O2 causes the oxi-

dation of lipids, forming aldehydic compounds like malondialdehyde (MDA), which reacts with TBA reagent, forming thiobarbituric acid reactive substances (TBARS). The amount of TBARS present was determined using the molar extinction coefficient, 1.56 × 10-5 M-1 cm-1 for the MDA–thiobarbituric acid-colored complex. TBA (0.6%) and TCA (0.3%) were added to 100µL of lysate samples. The samples were heated in the water bath at 1000C for 20 minutes and then centrifuged at 10000 rpm for 10 minutes. The absorbance was recorded at 535nm.

2.2.6. Dichlorofluorescein Assay (DCFH-DA)

DCFH-DA staining and microscopy were performed to measure the intracellular ROS in isolated lymphocytes [29]. It is based on forming 2, 7- dichlorofluorescein (DCF), a fluorescent compound from non-fluorescent DCFH-DA in the presence of ROS. 10µM of DCFH-DA was added to the samples containing 100µl of treated lymphocyte suspension. The reaction mixture was incubated for one hour at 370C. Pictures were captured using a fluorescence microscope-attached camera.

2.2.7. Scanning electron microscopy (SEM)

Suspensions of unwashed, untreated, or treated cells in 0.82% NaCl were incubated for 20 minutes at 220C. The cells were fixed in 2.5% glutaraldehyde in PBS for 1 hour at 40C. The cells were centrifuged and washed with PBS at room temperature. Thereafter, the cells were dehydrated using a graded series of ethyl alcohol and layered on glass slides. Then, the metal stubs bearing the dried specimens were coated with gold. A scanning electron microscope was used to observe the cell surface.

2.3. Antidiabetic properties

2.3.1. α -Glycosidase inhibitory activity

With a few modest changes, the substrate p-nitro phenol-α -D-glucopyranoside (pNPG) was used to measure the inhibitory activity of the -glycosidase enzyme [30]. Briefly, samples and acarbose (a positive control) were mixed with α-glycosidase (1.25 U/mL) in sodium phosphate buffer (20 mM, pH 6.9) and pre-incubated for 10 min at 37 °C. The reaction was initiated by the addition of the substrate pNPG, and the mixture was then incubated at the same temperature for 10 min. When 0.1M Na2CO3 was added to terminate the reaction, the amount of produced p-nitrophenol (a yellow product) was measured spectrophotometrically at 405 nm. Using equation (2), the percent inhibition of enzyme activity was calculated.

$$\text{Inhibition (\%)} = [(OD_{control} - OD_{sample})/(OD_{sample})] \times 100 \quad (2)$$

2.3.2. Alpha-amylase activity inhibition

The procedure previously described was used to measure the activity of α-amylase [30]. Acarbose (used as a positive control) or the enzyme (5 U/mL) were mixed with 360 µL of sodium phosphate buffer (0.02 M, pH 6.9 with 6mM NaCl) and 200 µL of the sample using various concentrations before being incubated at 37 °C for 20 minutes. 300 µl of starch solution (1% in sodium phosphate buffer, pH 6.9) was added to the reaction mixture and incubated for 20 min. 200 µl of 3,5-dinitro salicylic acid was added to the mixture to stop the process, and it was then heated for five minutes and cooled to room temperature. After being diluted with 10 ml of pure water, it was finally read at 540 nm. The calculation was done using Equation 2.

3. Statical analysis

All experiments were carried out in triplicates. Statistical analysis was done with the help of Graph Pad Prism 8.0.2. One-way ANOVA was followed by the Bonferroni multiple comparison test. * Presenting the significant different from P50 at $p \leq 0.05$.

4. Results

This experiment measured the generation of reactive oxygen species (ROS) using an NBT assay. The results presented in (Figure 1) show that the highest level of ROS was detected in the sample treated with riboflavin alone. Minimum production of superoxide radicles was observed in p-CA and vitamin D equimolar combination compared to the combination with riboflavin.

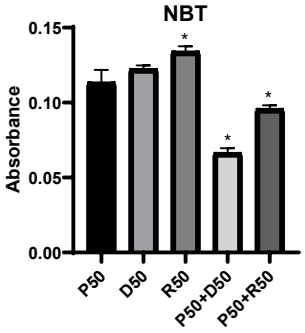

Figure 1: Shows the superoxide radicle generation in samples with p-CA, vitamin D, riboflavin alone, and combinations of p--CA with vitamin D and riboflavin (ratio 1:1). * indicating significantly different from P50 at p≤ 0.05.

4.1. DPPH

The DPPH assay is a commonly used method to measure the radical scavenging activity of a compound by assessing its ability to reduce a stable free radical, DPPH, accompanied by a loss in its violet color. The results are expressed as the percentage of radical scavenging activity. (Figure 2) presents the radical scavenging activity of the samples p-CA, vitamin D, riboflavin, and the combinations in the ratios of 1:1. The results indicate that the sample with a ratio of P50+D50 (1:1) showed the highest radical scavenging activity, which was significantly higher than P50+R50 and alone concentrations.

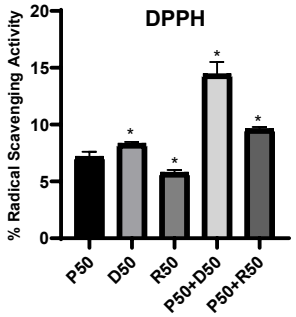

Figure 2: Is showing the percent radical scavenging activity of alone as well as combinations. * indicating significantly different from P50 at p≤ 0.05.

4.2. FRAP

The FRAP assay is commonly used to determine the antioxidant capacity of a compound by measuring its ability to reduce a complex of Ferric iron and TPTZ to its ferrous form. This reduction reaction results in a blue color that can be detected by measuring the increase in absorbance at 593 nm. The results indicate that the sample with a P50+D50 (1:1) ratio had the highest absorbance at 593 nm, indicating the maximum antioxidant potential among the tested samples.

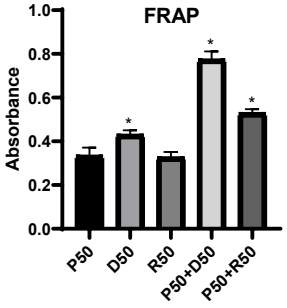

Figure 3: shows the FRAP of the samples p-CA, vitamin D, and riboflavin alone and their combinations in ratio (1:1). * indicating significantly different from P50 at p≤ 0.05.

4.3. SOD assay

Superoxide dismutase (SOD) is an enzyme that is critical in protecting cells from oxidative damage by breaking down reactive oxygen species. SOD activity can be measured by inhibiting the auto-oxidation of pyrogallol in the presence of molecular oxygen in an aqueous or alkaline medium. The results indicate that the supernatant of P50+D50 (1:1) treated lymphocytes possess the highest SOD activity compared to other samples.

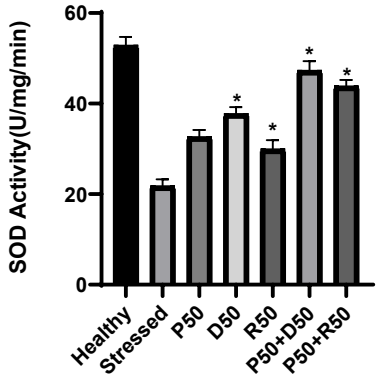

Figure 4: Presenting the SOD activity in samples categorized as healthy, stressed, and treated with p-CA, vitamin D, riboflavin and their combinations in ratio of (1:1). * indicating significantly different from P50 at p≤ 0.05.

4.4. Catalase Assay

The activity of catalase was measured by monitoring the decrease in H2O2 concentration. The samples treated with p50, D50, and R50 have shown less activity, while P50+D50 sample (1:1) showed higher catalase activity (Figure 5). Combination of p-CA with riboflavin also restored the catalase activity.

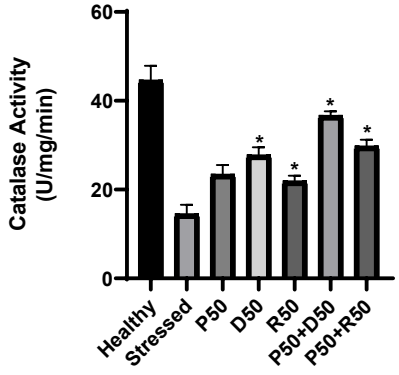

Figure 5: Depicts the catalase activity in healthy, stressed samples and sample treated with p-CA, vitamin D riboflavin alone, and their combinations in ratio of (1:1). * indicating significantly different from P50 at p≤ 0.05.

4.5. MDA Level

The MDA assay was conducted to assess the level of lipid peroxidation in lymphocyte lysate induced by oxidative stress The results indicated that the treated samples had lower MDA production than the stressed sample. Moreover, the sample treated with P50+D50 in a 1:1 ratio demonstrated the most least MDA production among the treated samples (Figure 6).

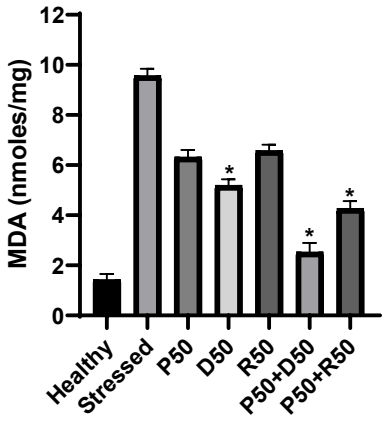

Figure 6: Is presenting the MDA concentration in healthy, stressed, and treated samples. * indicating significantly different from P50 at p≤ 0.05.

4.6. DCFH-DA

The DCFH-DA assay was used to measure intracellular ROS levels. In (Figure 7A), the stressed lymphocytes showed the highest fluorescence, indicating high levels of ROS production. Native (Figure 7B) is showing significantly less fluorescence. Treatment with p-CA and vitamin D equimolar combination resulted in a significant decrease in ROS production and negligible fluorescence, indicating effective inhibition of intracellular ROS production (Figure 7C). P50+R50 reduced the intensity but less than P50+D50 (Figure 7D).

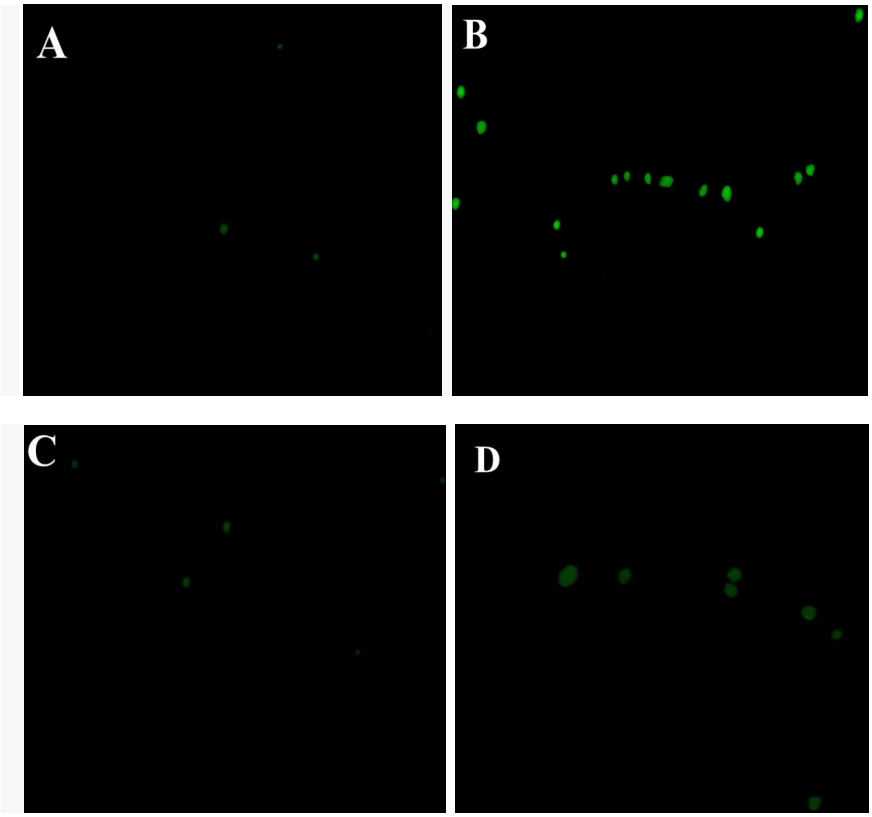

Figure 7: Is showing (A) Healthy Lymphocytes (B) Stressed lymphocytes (C) treated with P50+D50 (D) treated with P50+R50 (1:1).

4.7. SEM

Scanning electron microscopy was used to observe the morphology of lymphocytes at x10000 magnification. This allowed for a detailed analysis of the surface features and structures of the lymphocytes. The disrupted morphology (Figure 8B) of the lymphocyte observed in the stressed lymphocytes was significantly restored in the treated lymphocytes (8C and 8D). This is probably the result of a protective effect provided by p-CA and vitamin D when used in the ratio (1:1).

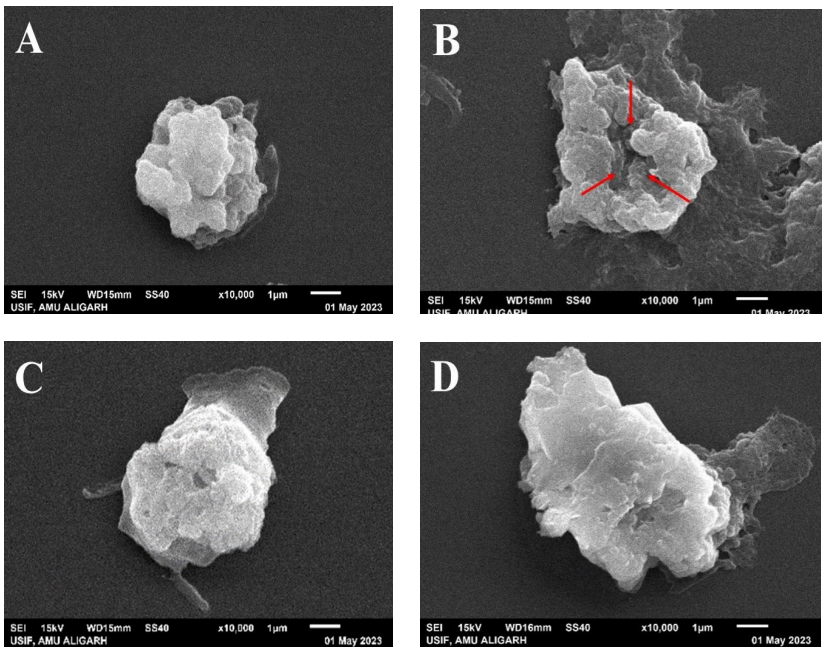

Figure 8: Is showing (A) Control (untreated) lymphocytes. (B) Lymphocytes in stressed conditions. (C) Lymphocytes were incubated with p-CA and vitamin D (1:1) (D) treated with p-CA and Rf (1:1) ratio for 2 hours at 37 °C. Red arrows indicate the disrupted morphology of lymphocytes due to membrane damage.

4.8. α-glucosidase inhibition

α-glucosidase inhibition assay was carried out to test the antidiabetic potential of the compound alone and in combinations. A-glucosidase acts on α-1, 4 glycosidic linkages and releases glucose, thereby increasing glucose concentration in blood. A promising antidiabetic compound should be able to inhibit this enzyme to prevent the increased glucose concentration. It was observed that the sample (1:1), i.e., P50+D50, showed maximum inhibition of the enzyme compared to other treated samples (Figure 9).

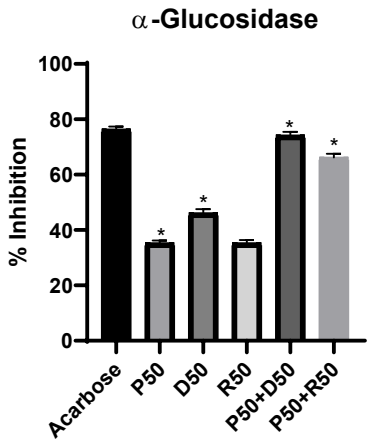

Figure 9: illustrates the α-glucosidase inhibition (%) in acarbose (control) and samples carrying p-CA, vitamin D, and riboflavin alone and their combination in the ratios (1:1). * indicating significantly different from P50 at p≤ 0.05.

4.9. Alpha-amylase activity inhibition

Alpha-amylase is a significant carbohydrate catabolizing enzyme that plays a significant role in metabolism homeostasis. Inhibition of this enzyme activity is directly linked to blood sugar level control. Although P50, D50, R50, and the equimolar combination of p-CA and riboflavin have amylase activation inhibition potential, P50+D50 exhibited the highest inhibition, as depicted in (Figure 10)

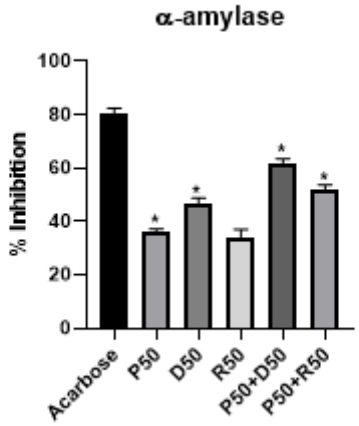

Figure 10: Is depicting the α-amylase inhibition (%) in acarbose (control), and samples carrying p-CA, vitamin D, riboflavin alone and their combination in the ratios (1:1). * indicating significantly different from P50 at p≤ 0.05.

5. Discussion

Free radicals are produced by immune cells and by metabolic reactions. They play an important role in maintaining biological homeostasis. However, when the production of free radicals exceeds the antioxidant capacity of the system, it leads to oxidative stress. Free radicals include reactive oxygen species (ROS), reactive nitrogen species (RNS), and iron and copper ions. These highly reactive elements have unpaired electrons that can interact with other biomolecules and cause damage, such as oxidizing proteins, lipids, and nucleic acids, leading to toxic byproducts and tissue dysfunction. The body has an intrinsic defense mechanism against oxidative stress that involves various enzymes, such as superoxide dismutase (SOD), catalase (CAT), GPR, GR and GSH as the reducing buffer. They work together to neutralize free radicals and maintain a healthy balance in the body. Vitamin D also plays a crucial role in glucose metabolism via regulating insulin function. It has been linked to diabetes for many years. The discovery of vitamin D receptors and related proteins in pancreatic tissue and immune cells further supports the idea of vitamin D as a possible therapeutic agent for both type 1 and type 2 diabetes.

The antioxidant potential of p-CA and vitamin D, riboflavin individually and in combinations (1:1), was evaluated using NBT, FRAP, and DPPH assays. The results indicated that the combination of the two compounds produced an enhanced antioxidant effect, as evidenced by increased radical scavenging values in the sample with a 1:1 ratio (P50+D50) and (P50+R50) in the DPPH assay, as well as increased absorbance of the same sample in the FRAP assay. The lowest ROS generation was observed in the sample containing both compounds in a 1:1 ratio (P50+D50) during the NBT assay. The protective effect of p-CA and vitamin D in combination on human lymphocytes against the free radicals generated by H2O2 was assessed by performing DCFH-DA assay, Malondialdehyde (MDA) estimation assay, Catalase (CAT) and Superoxide dismutase (SOD) assay, etc. We observed that the levels of the generated ROS were significantly reduced in the group containing both p-CA and vitamin D.

ROS generation was the highest in R50 alone. This was evident by the reduced levels of DCF and MDA and increased catalase and superoxide dismutase enzyme activity.

The a-glucosidase inhibition assay was utilized to assess the antidiabetic potential of p-CA alone and in combinations of 1:1, 1:2, and 1:3 with vitamin D. The results indicated that the sample P50+D50 exhibited the most effective antidiabetic potential, as evidenced by its highest a-glucosidase inhibition value (%). Scanning electron microscopy was employed to investigate the morphology of lymphocytes at a magnification of x10000, providing a comprehensive examination of the surface features and structures of the lymphocytes in healthy, stressed, and treated conditions.

6. Conclusion

Although alone concentrations were found to alleviate the damage by improving oxidative stress, overall results of this study suggested that the combination of p-CA and vitamin D may have therapeutic potential in the treatment of diabetes and other age-related disorders as they both intensify the therapeutic potential of each other through reduction of oxidative stress.

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CRediT authorship contribution statement

Rizwan Khan: Conceptualization, Methodology, Validation, Formal analysis, Investigation. Aqsa and Aiman- Performed experiments and did writing of original draft.

Imrana Naseem: Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

There is no conflict of interest related to this study.

Data availability

Data will be made available when will be asked.

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References

1. Bokkon I (2012) Recognition of functional roles of free radicals. Current Neuropharmacology 10: 287.

2. Halliwell B, Gutteridge JM (2015) Free Radicals in Biology and Medicine. Oxford University Press, USA.

3. Maritim AC, Sanders A, Watkins JB III (2003) Diabetes, oxidative stress, and antioxidants: A review. Journal of Biochemical and Molecular Toxicology 17: 24-38.

4. Yaribeygi H, Farrokhi FR, Rezaee R, Sahebkar A (2018) Oxidative stress induces renal failure: A review of possible molecular pathways. Journal of Cellular Biochemistry 119: 2990-2998.

5. Hurrle S, Hsu WH (2017) The etiology of oxidative stress in insulin resistance. Biomedical Journal 40: 257-262.

6. Yaribeygi H, Farrokhi FR, Butler AE, Sahebkar A (2019) Insulin resistance: Review of the underlying molecular mechanisms. Journal of Cellular Physiology 234: 8152-8161.

7. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A (2020) Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxidative Medicine and Cellular Longevity 2020.

8. Hunt JV, Smith CC, Wolff SP (1990) Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 39: 1420-1424.

9. Ceriello A (2003) New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 26: 1589-1596.

10. Kuyvenhoven JP, Meinders AE (1999) Oxidative stress and diabetes mellitus: Pathogenesis of long-term complications. European Journal of Internal Medicine 10: 9-19.

11. Aldaba Muruato LR, Ventura Juárez J, Perez Hernandez AM, Hernández Morales A, Muñoz Ortega MH, Martinez Hernández SL, et al. (2021) Therapeutic perspectives of p-coumaric acid: Anti-necrotic, anti-cholestatic and anti-amoebic activities. World Academy of Sciences Journal 3: 1-8.

12. Sheikh-Ali M, Chehade JM, Mooradian AD (2011) The antioxidant paradox in diabetes mellitus. American Journal of Therapeutics 18: 266-278.

13. Malik N, Dhiman P (2022) New approaches and advancements in drug development from phenolic p-coumaric acid. Current Topics in Medicinal Chemistry 22: 1515-1529.

14. Al-Shoumer KA, Al-Essa TM (2015) Is there a relationship between vitamin D with insulin resistance and diabetes mellitus? World Journal of Diabetes 6: 1057.

15. Epstein S (2012) Vitamin D. Rheumatic Disease Clinics 38: 1.

16. Danescu LG, Levy S, Levy J (2009) Vitamin D and diabetes mellitus. Endocrine 35: 11-17.

17. Beach RS, Mantero-Atienza E, Shor-Posner G, Javier JJ, Szapocznik J, Morgan R, et al. (1992) Specific nutrient abnormalities in asymptomatic HIV-1 infection. AIDS 6: 701-708.

18. Alam MM, Iqbal S, Naseem I (2015) Ameliorative effect of riboflavin on hyperglycemia, oxidative stress and DNA damage in type-2 diabetic mice: Mechanistic and therapeutic strategies. Archives of Biochemistry and Biophysics 584: 10-19.

19. Iwanaga K, Hasegawa T, Hultquist DE, Harada H, Yoshikawa Y, Yanamadala S, et al. (2007) Riboflavin-mediated reduction of oxidant injury, rejection, and vasculopathy after cardiac allotransplantation. Transplantation 83: 747-753.

20. Betz AL, Ren XD, Ennis SR, Hultquist DE (1994) Riboflavin reduces edema in focal cerebral ischemia. Brain Edema IX: 314-317.

21. Husain MA, Sarwar T, Rehman SU, Ishqi HM, Tabish M (2015) Ibuprofen causes photocleavage through ROS generation and intercalates with DNA: A combined biophysical and molecular docking approach. Physical Chemistry Chemical Physics 17: 13837-13850.

22. Kim WR, Kim EO, Kang K, Oidovsambuu S, Jung SH, Kim BS, et al. (2014) Antioxidant activity of phenolics in leaves of three red pepper (Capsicum annuum) cultivars. Journal of Agricultural and Food Chemistry 62: 850-859.

23. Müller L, Fröhlich K, Böhm V (2011) Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. Food Chemistry 129: 139-148.

24. Marklund S, Marklund G (1974) The involvement of the superoxide radical in the auto-oxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47: 469-474.

25. Aebi H (1984) Catalase in vitro. Methods in Enzymology 105: 121-126.

26. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods in Enzymology 52: 302-310.

27. Negrei C, Hudita A, Ginghina O, Galateanu B, Voicu SN, Stan M, et al. (2016) Colon cancer cells gene expression signature as response to 5-fluorouracil, oxaliplatin, and folinic acid treatment. Frontiers in Pharmacology 7: 172.

28. Khalid A, Naseem I (2022) Antidiabetic and antiglycating potential of chrysin is enhanced after nano formulation: An in vitro approach. Journal of Molecular Structure 1261: 132906.

29. Khan R, Naseem I (2023) Antiglycation, antifibrillation and antioxidative effects of para coumaric acid and vitamin D: An in-vitro and in-silico comparative-cum-synergistic approach. Biochimica et Biophysica Acta - General Subjects 130455.

30. Khan R, Naseem I (2023) Antiglycation and antioxidant potential of coumaric acid isomers: A comparative in-vitro study. SSRN 4354174.