Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : https://ijpsdronline.com/index.php/journal

Research Article

Quercetin Ameliorated Diabetic Stress through Upregulation of Antioxidant Enzymes and the Nrf-2/HO-1 Axis in the Spleen of Mice

Subhrata Sarma, Anubhuti Kashyap, Arjita Chakrabarti, Shiv Shankar Singh*

Molecular Endocrinology Laboratory, Department of Zoology, Tripura University, Suryamaninagar, Tripura, India.

ARTICLE INFO

Article history:

Received: 23 September, 2024

Revised: 03 November, 2024

Accepted: 09 November, 2024

Published: 30 November, 2024

Keywords:

Diabetes mellitus, Spleen, Peritoneal macrophage, Quercetin, Oxidative stress, Nrf-2/HO-1 Axis.

DOI:

10.25004/IJPSDR.2024.160611

ABSTRACT

Diabetes is an enduring endocrine complication, increasing with tremendous challenges all over the world. Quercetin, being the predominant flavonoid, has pentahydroxy polyphenolic structure that bears antioxidant activity. Our study assessed the efficacy of quercetin in mice against diabetes-caused oxidative stress by modifying the radical quencher, the body's antioxidants and their regulator-Nrf-2 and HO-1. Hyperglycemia attributes (p <0.01) raised levels of lipid peroxidation, lowered levels of catalase, GSH, SOD, higher intracellular ROS generation and suppressed Nrf-2/HO-1 reactivity in the studied organ (spleen). Quercetin minimizes the sugar level significantly (p <0.01) in blood, causes restoration of altered spleen and body weight, also brings down the lipid peroxide level significantly (p <0.01) and further enhances SOD, catalase, and GSH antioxidant levels. Both splenic & peritoneal macrophages revealed lower intracellular ROS generation. Quercetin uplifted the reactivity of Nrf-2 & HO-1. The study's outcome showed that quercetin supplementation attenuated diabetes-caused oxidative stress through upregulation of antioxidant enzyme activity and Nrf-2 and its regulated gene-HO-1 in immune organ-spleen. Thus, quercetin might be fruitful in reducing ROS-mediated damage in diabetic individuals.

Introduction

One of the major metabolic disorders faced by people worldwide is diabetes mellitus, with an increasing case in developing and newly industrialized countries.[1] The continuous presence of high blood glucose levels is a characteristic feature of this multi-factorial chronic health disease, i.e., diabetes mellitus or diabetes. It could result in a deficiency in insulin biosynthesis or resistance to insulin.[2] Diabetes mellitus (Type 1 & 2) are the two major kinds of diabetes mellitus; gestational diabetes is another type noted in pregnant women. In type 1, β cells of the pancreas are affected because of autoimmune destructions of these cells and type 2 arises from resistance of body cells against insulin produced by the β cells. Consequently, lack of insulin occurs in type-1 and an impairment in glucose uptake from blood occurs in type-2 diabetes mellitus.[3]

Streptozotocin is isolated from *Streptomyces achromogenes* which is a soil bacterium, possessing anti-tumor, oncogenic, and diabetogenic properties.[4] A wide implementation has been documented to generate diabetes mellitus (type-1) by streptozotocin in laboratory animals. Streptozotocin imposes its diabetogenic property by causing β cell death of the pancreas, leading to deprivation of secretion of insulin and this deficiency further causes hyperglycemia, polydipsia, and polyurea; all these characteristics mimic the traits of type 1 diabetes mellitus.[5]

The emergence and progression of diabetic complications list oxidative stress as a major factor.[6] Escalation of superoxides and peroxides is categorized as reactive oxygen species (ROS) when it overpasses the antioxidant system that neutralizes ROS, resulting in oxidative stress. The ROS are oxygen containing reactive molecules

***Corresponding Author:** Dr. Shiv Shankar Singh

Address: Molecular Endocrinology Laboratory, Department of Zoology, Tripura University, Suryamaninagar, Tripura, India.

Email ✉**:** shivssingh@tripurauniv.ac.in

Tel.: +91-9402169695

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

that react with other molecules of the cells to stabilize themselves. Persistent hyperglycemic conditions and mitochondrial dysfunction due to diabetes cause excess production of ROS, thus resulting in oxidative stress.[7] Regarding ROS, the most explored research area has turned out to be lipid peroxidation, an essential indicator of oxidative stress.[8] The most important first-line antioxidant defense comprises superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), and glutathione (GSH).[9]

Hyperglycemia causes dysfunction of the immune system and thus fails in the prevention of invading pathogen's propagation in hyperglycemic individuals.[10] The immune system shields individuals from harmful, invading pathogens. Impairment in the immune system due to diabetes includes increased production of proinflammatory cytokines, dysfunction of immune cells, and fault in phagocytosis.[11] The spleen, a lymphatic organ of the body performs a number of immunological tasks as it contains numerous lymphocytes and macrophages along with its role in red blood cell formation and clearance.[12] Histoarchitecturally, red pulp and white pulp are the main regions of the spleen. Among the two marginal zones is present in rodents, distinguishing it from the perifollicular zone present in humans.[13] When the spleen is injured, the body's immunity goes down automatically,[14] and ROS generation in the spleen is one of the utmost factors of splenic injury.[15] The pathophysiology of tissue macrophages is studied using peritoneal macrophages.[16] Collecting peritoneal fluid is a well-established method for isolating macrophages from rodents in the laboratory.[17] Over the past several years, flavonoids have gained much attention in treating various diseases because of their non-toxic, free radical quenching and inflammation reduction role.[18] Dietary polyphenols manage diabetes in several ways, diminishing the absorption of carbohydrates by the intestine and enhancing insulin secretion and sensitivity.[19] Quercetin (QE) is a naturally occurring polyphenol belonging to the class of flavonoids. Leaves, seeds, grains, flowers, and barks of numerous plants as well as many fruits and vegetables, are rich sources of quercetin, among which onions contain an enormous amount of quercetin.[20] Reported previously, quercetin prevents hyperglycemia in diabetic rats by lowering blood sugar levels and improvement of glucose tolerance.[21] Quercetin scavenges free radicals by inhibiting lipid peroxidation, increases immune functions, and prevents proneness to illness.[22] The report suggested that quercetin supplementation minimized the oxidative stress in hepatic tissue in diabetic mice.[23] but there are limited reports that suggest the preventive effects of quercetin against the detrimental effects caused by diabetes on immune function in laboratory mice. Thereby, in this study, we have tried to examine the role played by quercetin in minimizing the stress generated on account of diabetes in the spleen and peritoneal macrophage of mice. By examining the antioxidant potential of quercetin, the current study will contribute to the therapeutic efficacy of natural antioxidants in promoting health benefits in human wellness/treating complications caused by metabolic disorders, i.e., diabetes mellitus. Further, the outcome of the study will add more dimension to the investigation of the pharmacological properties of quercetin for its potential clinical implications.

Materials And Methods

The study was performed following the institutional guideline on animal experiments with Ethical Clearance No. TU/IAEC/2022/I/2-7.This study was done in Swiss albino mice. Healthy mice were maintained with proper hygiene in laboratory conditions with 12 hours light and 12 hours dark with temperature (25 ± 2°C) and humidity (55 ± 5%). For the experiment, healthy mice of 25 g body weight were chosen and they were provided timely with proper feed and water.

Diabetes Induction

To induce experimental diabetes, streptozotocin (STZ; SRL, India) (50 mg/kg body wt.) was dissolved in a citrate buffer of 0.1M maintaining a pH of 4.5. STZ was given for 5 days continuously and during the treatment period, mice were provided with sucrose water (10%). After STZ treatment, blood glucose was determined on each alternate day through the tail vein, and mice whose blood glucose levels exceeded 250 mg/dL were regarded as diabetic.[11]

Experimental Set up

Mice were assigned in 4 groups. Each group contains 5 mice.

1. CON: Control mice
2. DB: Diabetic mice
3. DQ: Diabetic + Quercetin mice
4. Q: Quercetin mice

Group 1, control mice received ethanolic saline (0.01% ethanol) throughout the experimental period. Group 2 contained diabetic mice. Group 3 diabetic mice received quercetin (Sigma-Aldrich, USA) (30 mg/kg body weight/day, orally).[24] Group 4 control mice received quercetin only. Quercetin supplementation was made for 15 days. Mice were euthanized after the last day of quercetin treatment. Blood glucose level and body weight were noted. Peritoneal macrophages were collected for intracellular ROS analysis. The spleen was removed and weighed, and a piece of it was kept in fixative for immunohistochemical study. A part of the spleen was processed for NBT assay, and a part was kept to evaluate the oxidative stress markers, whereas for reverse transcriptase PCR, part of the spleen was minced in PureZol following the further steps of the protocol.

Parameters Studied

Blood glucose determination

The sugar level in blood was measured through tail pricking of mice by Accu-check active blood glucose monitoring system.

Oxidative Stress Analysis

Splenic lipid peroxidation

Malondialdehyde (MDA) assay was conducted using the methodology of Ohkawa *et al*. [25] In this assay, the measurement of the reaction between MDA and thiobarbituric acid (TBA) was done. Splenic homogenate (10%) was prepared in phosphate buffer for estimation of total protein. 3.3 mL of TBA reagent was added with 10% splenic homogenate and kept on boiling for 30 minutes, centrifuged, and collection of supernatant was done. Optical density measurement has been taken at 532 nm. MDA level was presented as TBARS (nmol) produced per milligram of proteins.

SOD enzyme activity

The assay was done in accordance with the methodology of Das *et al*. [26]. Tissue homogenate (100 µL) was added with SOD reaction mixture (1.4 mL). The mixture exposed to 20 W fluorescence after the addition of 50 mM riboflavin. Further, in the mixture, Griess reagent (1-mL) was mixed, followed by the measurement of optical density at 532 nm. The SOD amount, which inhibits 50% of nitrites formed during the process, has been considered as a unit of SOD activity and was presented as a unit per milligram protein.

Catalase enzyme activity

Splenic tissue was analyzed for catalase activity by protocol of Sinha[27], and Hadwan.[28] A mixture containing hydrogen peroxide and potassium dichromate was mixed with 100 µL of splenic homogenate, followed by boiling and centrifugating the mixture. At the wavelength of 570 nm, the supernatant's optical density has been taken. Catalase enzyme activity measurement was determined as degradation of H_2O_2 per minute. Catalase activity was presented as kU/mg protein.

GSH level

The method of Ellman,[29] modified by Gupta *et al*.[30] was used. An equal proportion of 10% spleen homogenate was combined with 20% of trichloroacetic acid, followed by centrifugation. Ellman reagent, which is the key reagent of the procedure, was further mixed with tissue supernatant (200 µL), and reduced GSH concentration was further calculated as mg/g tissue.

Isolation of Splenic macrophage for NBT assay

The spleen was dissected out and kept in chilled PBS, and a single-cell suspension was prepared. The viability of the cell was assessed, and cell number was adjusted ($1x10^7$ cells/mL) in culture media RPMI-1640 contains 500 µg/mL of streptomycin, 5000 U/mL of penicillin, 2 mM/mL of L-glutamine, 10% of FCS and 2-mercaptoethanol (0.1%).

Isolation of Macrophages from Peritoneal Cavity

Following the euthanization, the belly region of the mice was disinfected with 70% alcohol. 5 mL of PBS (sterilized) was injected into the peritoneal cavity. The belly region was gently rubbed with a finger for a few minutes and a sterilized 15 mL centrifuge tube was used to collect approximately 5 mL of peritoneal fluid. PBS was used to wash the pellet of peritoneal macrophage and centrifuged twice. 10% fetal calf serum (FCS) having culture medium was used to suspend the pellet

Spectrophotometric ROS analysis

NBT Assay was performed as elucidated by Majewski *et al*.[31] 96-well culture plates were used for plating of peritoneal leukocytes and splenic cell suspension after incubating at 5% CO2 incubator for an hour. NBT (Nitroblue tetrazolium) was prepared in PBS, and 100 µL solution was added and kept for one hour in the incubator. 70% methanol was used to fix the cells after the medium was discarded. KOH and DMSO were added to dissolve formazan crystals, and optical density was taken at 630 nm.

Microscopic analysis of cellular ROS

H_2DCFDA (2,7-dichlorodihydrofluorescein diacetate) is a fluorescent probe that is used to analyze the microscopic generation of ROS. Clean cover glasses were placed on sterilized petri dishes and the isolated peritoneal leukocytes (106 cells/mL) were flooded on them and placed for one hour a 5% CO_2 incubator. 40 mM solution of H_2DCFDA was used to stain for 30 minutes. Cover glasses were placed on new slides and a fluorescence microscope (Leica 2500) was used to take fluorescent images at 488 nm. Quantification of fluorescence intensity was done by ImageJ software.

Immunohistochemistry (Nrf-2, HO-1)

The procedure of Singh *et al*. [32] was followed for Nrf-2 and HO-1 immunohistochemistry in the spleen. Slides were coated with gelatine, and 5 µm thick sections of spleen tissue were mounted. Antibodies against Nrf-2, ab31162, and HO-1, ab31163, [1:100] were used for the detection of the above-mentioned proteins. HRP enzyme-catalyzed DAB+H2O2 reaction was employed for visualization of antigen-antibody interaction. Leica Microscope DM2500 was used to capture the images at 40X objectives.

RNA Isolation, cDNA Synthesis and PCR

Spleen samples were homogenized with PureZol (Biorad, USA) for RNA extraction and iScript (Biorad, USA) kit for cDNA synthesis. Specific primers of SOD (F 5'-GAGAGGCATGTTGGAGACCT-3' and R

5'-CCACCTTTGCCCAAGTCATC-3' give 158 bp amplicon), catalase (F 5'-CGCAATCCTACACCATGTCG-3' and R 5'-TCCGCTCTCTGTCAAAGTGT-3' give 219 bp amplicon) and GAPDH (F 5'-AACTTTGGCATTGTGGAAGG-3'and R 5'-ACACATTGGGGGTAGGAACA-3' give 132 bp amplicon). Amplicons were resolved with the help of agarose gel, and amplicon band intensity was analyzed using ImageJ software. The expressions were shown in terms of O.D relative to GAPDH.

Statistical Analysis

One-way ANOVA was conducted by the SPSS 17.0 (SPSS Corp., USA) program and for comparing the data, Tukey's multiple range test was performed. Mean ± SEM was done to present the data. Differences are viewed as significant at p <0.05. For graph preparation, Microsoft Excel has been used.

RESULTS

Blood Glucose Level

Sugar level in blood was expressed in %change of final glucose levels to initial glucose levels in all groups. The positive value denotes a percentage rise in the sugar level of blood, whereas the negative value denotes a percentage fall in blood sugar level. Diabetic mice showed highly increased blood glucose levels relative to control (p <0.01), and quercetin supplementation reduced the levels of blood glucose (Fig. 1A).

Effect of Quercetin on Body Weight

The body weight was expressed in % change of final body weight to initial body weight in respective experimental groups. The negative value reflects a percentage reduction, and the positive value reflects a percentage rise in the weight of mice in the respective groups. Reduced body weight was noted in the mice having high blood sugar relative to the control mice group (p <0.01). Further supplementation with quercetin caused an increase in body weight significantly(p <0.01) relative to the DB group (Fig. 1B).

Effect of Quercetin on Relative Spleen Weight

(Organ weight/body weight) x100 was the formula for calculating the relative organ weight. A significant (p <0.01) decline in the relative organ weight has been seen in the DB group, whereas quercetin administration significantly altered the depletion that occurred in the relative organ weight of the spleen (Fig. 1C).

Lipid Peroxidation

Compared to the control, levels of MDA content were markedly higher in the spleen of the DB group (p < 0.01) (Fig 2A). A remarkable reduction of MDA level has been observed in the quercetin group (DQ).

SOD Enzyme Activity

Superoxide radicals are converted into H_2O_2 and O_2 by the enzyme SOD. Highly reduced activity of the SOD enzyme was seen in the splenic tissue of the DB group relative to the CON group at a significance of p <0.01. (Fig. 2B). SOD activity was remarkedly increased (p <0.01) in the DQ group, relative to the DB group.

Catalase Enzyme Activity

Hydrogen peroxide is broken down by catalase enzyme into water and oxygen. Catalase enzyme activity (kU/mg protein) was substantially lowered in the splenic tissue of the DB group relative to the CON group (Fig. 2C). Quercetin supplementation caused remarked (p < 0.01) increase in catalase activity relative to the DB group.

Effect of Quercetin on GSH Level

Diabetes caused remarked depletion in GSH levels in the spleen of the DB group (p <0.01) relative to the CON group (Fig. 2D). Quercetin elevates the GSH level significantly (p <0.01) in the DQ group relative to the DB group.

Effect of Quercetin on Reverse Transcriptase-PCR Analysis of SOD and Catalase

Reverse transcription PCR analysis was done to see the consequences of diabetes on gene expression. SOD and Catalase mRNA levels were notably decreased in diabetic mice (p <0.01) relative to CON mice (Fig. 3). Quercetin supplementation caused a notable increase in mRNA (p <0.01) levels of both SOD and catalase relative to DB mice

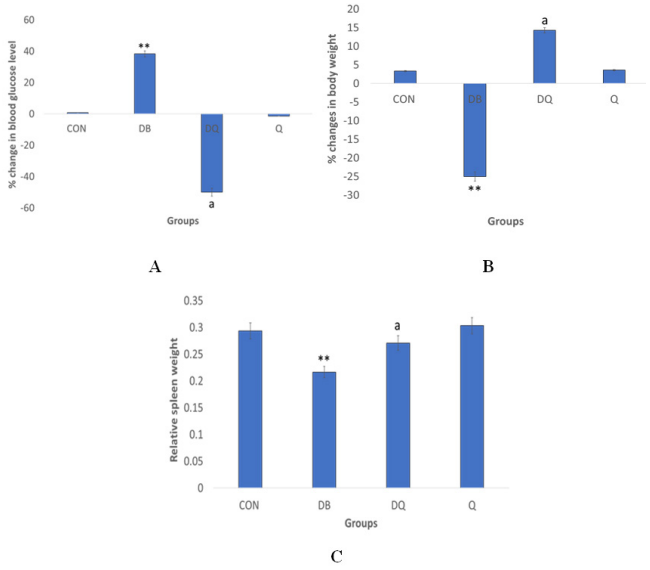

Fig. 1: Histogram shows (A) blood glucose level (percentage change), (B) body weight (percentage change), and (C) relative spleen weight. Change in blood glucose and body weight calculated from the initial value to the final value and represented as the ratio with respect to the initial value as a percent change. Data are shown as Mean ± SEM (n = 5). Comparisons were made **(p <0.01) with control and [a](p <0.01) with diabetes

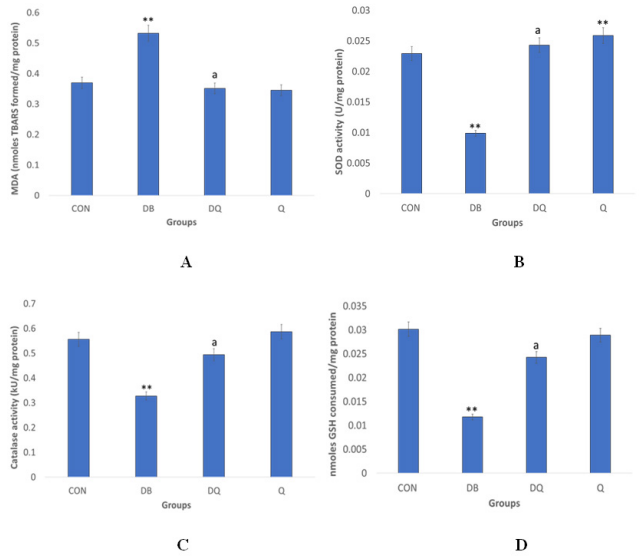

Fig. 2: Histogram shows the effect of quercetin on (A) MDA level, (B) SOD enzyme activity, (C) catalase enzyme activity and (D) GSH level in splenic tissue of mice. Values are represented as Mean ± SEM (n = 5). Comparisons were made **(p <0.01) with control and [a](p <0.01) with diabetes

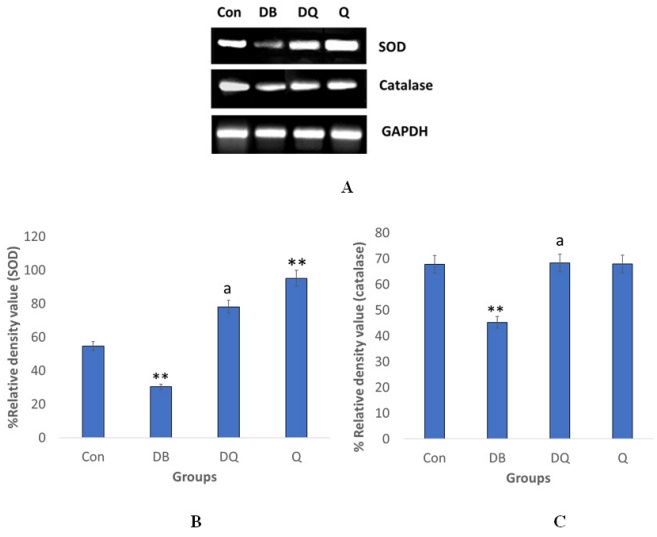

Fig. 3: Reverse transcription PCR revealed the action of quercetin on SOD and catalase mRNA expression in spleen tissue mice. GAPDH was considered as the control expression (A). Histogram shows SOD (B) and catalase (C) % relative density value. Values are represented as Mean ± SEM, (n = 5). Comparisons were made **(p <0.01) with control and [a](p <0.01) with diabetes

Effect of Quercetin on Intracellular ROS Generation

Intracellular ROS in peritoneal macrophage and Splenic macrophage was measured through the proportion of NBT converted by superoxide radicals to formazan crystal, which is water insoluble. ROS level in both peritoneal and splenic macrophages of DB mice group was much higher at the significance of p <0.01 relative to the CON group (Fig. 4). Quercetin supplementation markedly reduced the

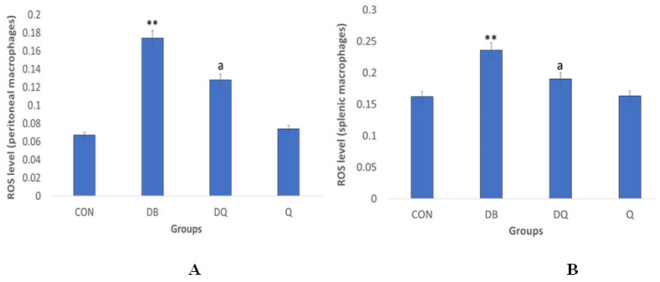

Fig. 4: Histogram shows the effect of quercetin on ROS level in (A) peritoneal macrophages and (B) splenic macrophages in experimental diabetic mice. Values are represented as Mean ± SEM (n = 5). Comparisons were made **(p <0.01) with control and [a](p <0.01) with diabetes

ROS level in peritoneal and splenic macrophages compared to diabetic mice.

A fluorescence probe, H_2DCFDA, determined ROS generated in peritoneal leukocytes. In the DB group, intense fluorescence activity showed a high level of reactive oxygen species relative to the control group (Fig. 5). Quercetin curbed the cellular ROS and thus decreased fluorescent activity.

Effect of Quercetin on Nrf-2/HO-1 Reactivity

Nuclear factor (Nrf-2) regulates antioxidant protein expressions, which protects against reactive species. Mild immunostaining of Nrf-2 antisera was examined in DB group, likened to spleen of a control group. The increased reactivity of Nrf-2 was found in the quercetin-supplemented group (DQ) (Fig. 6).

HO-1 is a Nrf-2 regulated gene. HO-1 reactivity was noticed throughout the splenic tissue section in the control mice group. Weak immunoreactivity of HO-1 antisera was observed in DB group relative to control group. Quercetin-supplemented mice showed higher reactivity of HO-1 antisera in splenic tissue relative to DB group (Fig. 7).

Discussion

In diabetes mellitus (Type1), patients require lifelong insulin administration in patients. Insulin therapy and the other hypoglycemic drugs available in the market have several side effects, including allergic reactions, prone to various infections, and other complications of diabetes. Insulin therapy has a myriad of side effects that include hypoglycemia along with other complications like palpitation, dizziness, paralysis, and, in some cases, even coma. The lower-income group is deprived of insulin therapy due to its high cost. Several herbal remedies have been used for ages in treating diabetes and minimizing complications due to fewer side effects and being cost-effective. Plant flavonoids have drawn more attention in scientific society due to their high abundance, structural diversity, low toxicity, and multiple pharmacological properties. Quercetin is the most abundantly found plant

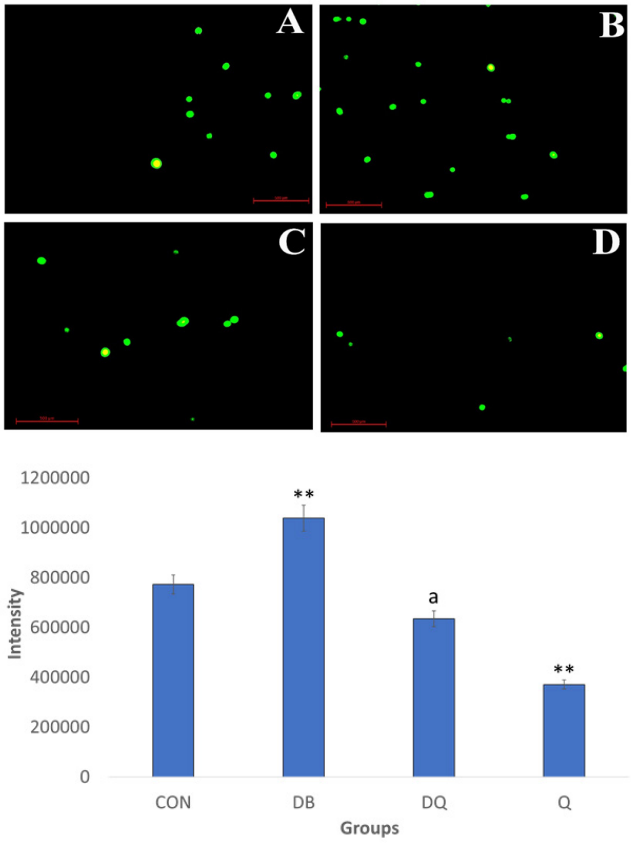

Fig. 5: Micrographs showing the effect of quercetin treatment on H_2DCFDA fluorescence in peritoneal macrophages in experimental diabetic mice. The histogram represents fluorescence intensity. Mean ± SEM, (n = 5). (A) Control, (B) Diabetes, (C) Diabetes + Quercetin, and (D) Quercetin treated. Comparisons were made **(p <0.01) with control and [a](p <0.01) with diabetes

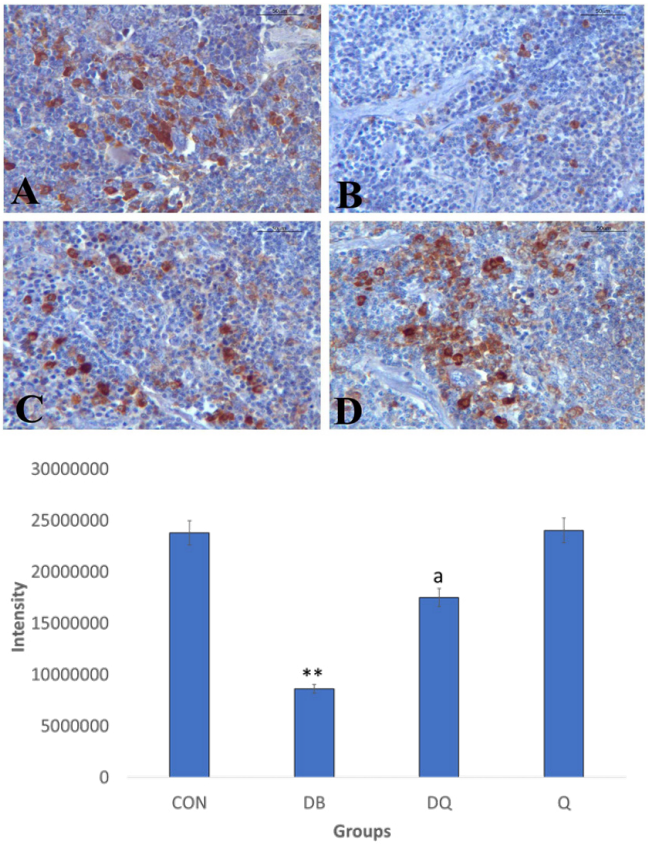

Fig. 6: Micrographs displaying the Nrf-2 antisera reactivity in the spleen of experimental mice. Histogram represents the intensity of antisera reactions fluorescence in peritoneal macrophages in experimental diabetic mice. The histogram represents fluorescence intensity. Mean ± SEM, (n = 5). (A) Control, (B) Diabetes, (C) Diabetes + Quercetin, and (D) Quercetin treated. Comparisons were made **(p <0.01) with control and [a](p <0.01) with diabetes

flavonoid. In the present study, quercetin supplementation caused significant suppression in blood sugar levels. Quercetin minimizes the effects of diabetes and normalizes the change in tissue and body weight of hyperglycemic mice. Rahmani *et al*.[33] reported that quercetin treatment increases the body weight in diabetic mice. Organ weight analysis is considered the end point of any toxicological study, where the effect of a particular compound in the specific organ is studied.[34] Sutradhar *et al*.[35] reported the restoration of spleen weight by supplementation of melatonin in diabetic Swiss albino mice.

The complications in diabetes are induced by hyperglycemia due to an imbalance in ROS production, which causes cellular death resulting from oxidative stress. Thus, the complications of diabetes may be managed by the downregulation of these reactive species.[36] Lipids are the primary target of ROS, causing lipid peroxidation.[37] Our study revealed that significantly enhanced MDA levels were present in the studied tissue of hyperglycemic mice, whereas the treatment of quercetin significantly suppressed levels of MDA. Previously, it was reported that lipid peroxidation was lowered by quercetin in various tissues, including the liver, kidney and pancreas of diabetic laboratory mice.[38] Antioxidant enzymes are responsible for neutralizing ROS in various components of cells and overcoming stressful conditions. In the current study, the activity of both the antioxidant enzyme (SOD and catalase) was seen to be lowered in the studied tissue during a hyperglycemic state. On the other way, quercetin treatment minimized diabetes-caused suppression and enhanced the SOD and catalase enzyme activities in experimental mice. Reverse-transcriptase PCR analysis of SOD and Catalase enzymes showed similar changes with a significant reduction in mRNA levels in diabetes mice, and quercetin-treated diabetes mice showed markedly increased SOD and catalase mRNA levels. Aside from enzymatic antioxidants that are present in the body, there is also an important non-enzymatic antioxidant called Reduced glutathione (GSH). We have noted reduced GSH levels in the spleen of studied hyperglycemic mice. Escalation of GSH level was noted in the quercetin-treated mice. Quercetin supplementation minimized the oxidative stress in hepatic tissue in diabetic mice.[23]

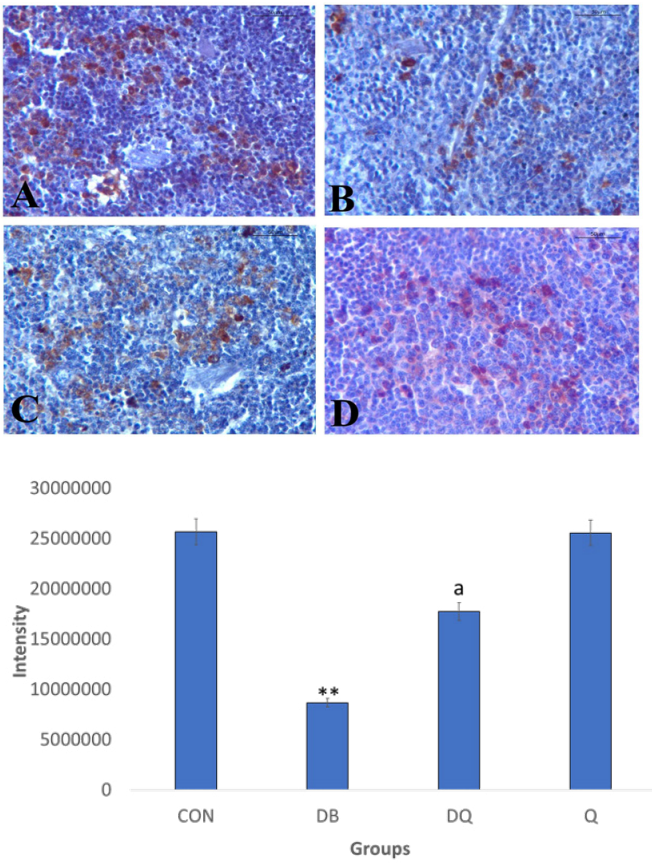

Fig. 7: Micrographs showing HO-1 antisera reactivity in spleen of experimental mice. The histogram represents the intensity of antisera reactions. Mean ± SEM, (n = 5). (A) Control, (B) Diabetes, (C) Diabetes + Quercetin, and (D) Quercetin treated. Comparisons were made **(p <0.01) with control and [a](p <0.01) with diabetes

A transcription factor (Nrf-2) mediated by antioxidant response element (ARE) transcribed in response to oxidative stress, activating detoxifying and antioxidant genes like HO-1, involved in maintaining cellular redox homeostasis. Diabetic induction caused reduced expression of Nrf-2 and its dependent HO-1 in splenic tissue. Quercetin supplementation upregulates Nrf-2 and HO-1 axis in studied tissue. Downregulation of Nrf-2 signaling inhibits antioxidant enzyme activations; hence the chance of occurrence of oxidative stress is higher.[39] ROS generation is common in diabetic mice in the splenic and peritoneal macrophages. In diabetic conditions, a glycolytic metabolic shift occurs in monocytes and macrophages, which results in increased ROS formation.[40] The intracellular ROS generation was measured through the proportion of NBT converted by superoxide radicals to formazan crystal, which is water-insoluble.[41] The present study showed that intracellular ROS generation was significantly higher in splenic and peritoneal macrophages. 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) reacts to ROS, resulting in a fluorescent compound (2',7'-DCF), which produces a bright green fluorescence. Emitted fluorescence can be directly related to the amount of presence ROS, as both are directly proportional. The reaction of fluorescent dye, H_2DCFDA, revealed higher ROS generation in diabetic groups. Quercetin supplementation lowered the ROS generation in both splenic and peritoneal macrophages. Polyphenols can modulate cellular mechanisms and thus protect glucose homeostasis.[42]

Conclusion

Diabetes is the most prevalent chronic disease, causing death worldwide, and cases have been steadily increasing over the past few decades. Supplementation of natural compounds has always been an age-old technique for treating diabetes. In the present study, diabetic hyperglycemia caused increased MDA content and suppression of SOD, GSH, and catalase and altered macrophage activity in the studied mice. Quercetin minimized lipid peroxidation, restored the altered antioxidant enzymes, upregulated the Nrf-2 and HO-1 axis in the spleen, and reduced intracellular ROS generation in spleen and peritoneal macrophage, thus subsequently reducing the generated oxidative stress in hyperglycemic mice. The study hence concludes that quercetin prevents splenic damage and macrophage activity against diabetes toxicity by upregulating antioxidants enzymes, the Nrf-2/HO-1 axis in studied laboratory mice.

Acknowledgment

CSIR, New Delhi, DST-FIST, Tripura University's state Biotech Hub is highly acknowledged by all the authors.

References

1. Mohamed SF, Mwangi M, Mutua MK, Kibachio J, Hussein A, Ndegwa Z, Kyobutungi C. Prevalence and factors associated with pre-diabetes and diabetes mellitus in Kenya: results from a national survey. BMC Public Health. 2018;18(3):1-11. Available from: doi.org/10.1186/s12889-018-6053-x
2. Shi GJ, Shi GR, Zhou JY, Zhang WJ, Gao CY, Jiang YP, Zi ZG, Zhao HH, Yang Y, Yu JQ. Involvement of growth factors in diabetes mellitus and its complications: A general review. Biomedicine and Pharmacotherapy. 2018;101:510-527. Available from: doi.org/10.1016/j.biopha.2018.02.105
3. Li M, Lu Y, Wang AR. View insulin resistance from an interaction between pancreatic islets and peripheral tissues. Clinical Medicine and Research. 2018;7(5):124-130. Available from: doi.org/10.11648/j.cmr.20180705.14
4. Like AA, Rossini AA. Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science .1976;193(4251):415-417. Available from: doi.org/10.1126/science.180605
5. Kolb H. Mouse models of insulin dependent diabetes: Low-dose streptozocin-induced diabetes and nonobese diabetic (NOD) mice. Diabetes/Metabolism Reviews. 1987;3(3):751-778. Available from: doi.org/10.1002/dmr.5610030308
6. Keane KN, Cruzat VF, Carlessi R, De Bittencourt PIH, Newsholme P. Molecular events linking oxidative stress and inflammation to insulin resistance and β-cell dysfunction. Oxidative Medicine and Cellular Longevity. 2015;181643. Available from: doi.org/10.1155/2015/181643
7. Caturano A, D'Angelo M, Mormone A, Russo V, Mollica MP, Salvatore T, Galiero R, Rinaldi L, Vetrano E, Marfella R, Monda M, Giordano A, Sasso FC. Oxidative stress in type 2 diabetes: impacts from

pathogenesis to lifestyle modifications. Current Issues in Molecular Biology. 2023;45(8):6651-6666. Available from: doi.org/10.3390/cimb45080420
8. Valgimigli L. Lipid peroxidation and antioxidant protection. Biomolecules. 2023;13(9):1291. Available from: doi.org/10.3390/biom13091291
9. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine. 2018;54(4):287–293. Available from: doi.org/10.1016/j.ajme.2017.09.001
10. Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R. Type 2 diabetes and its impact on the immune system. Current Diabetes Reviews. 2020;16(5):442-449. Available from: doi.org/10.2174/1573399815666191024085838
11. Sutradhar S, Deb A, Singh SS. Protective efficacy of melatonin and insulin against LPS caused toxicity in diabetic mice. Immunopharmacology and Immunotoxicology. 2022;44(6):902-914. Available from: doi.org/10.1080/08923973.2022.2093739
12. Lewis SM, Williams A, Eisenbarth SC. Structure and function of the immune system in the spleen. Science Immunology. 2019;4(33):1-25. Available from: doi.org/10.1126/sciimmunol.aau6085
13. Bronte V, Pittet MJ. The spleen in local and systemic regulation of immunity. Immunity. 2013;39(5):806-818. Available from: doi.org/10.1016/j.immuni.2013.10.010
14. Perrotta G, Guerrieri E, Guerrieri M. Splenic trauma: Definition, classifications, clinical profiles and best treatments. Open Journal of Trauma. 2021;5(1):019-036. Available from: doi.org/10.17352/ojt.000038
15. Sun W, Liu S, Yan Y, Wang Q, Fan Y, Okyere SK. Pseudorabies virus causes splenic injury via inducing oxidative stress and apoptosis related factors in mice. Scientific Reports. 2023;13(1):23011. Available from: doi.org/10.1038/s41598-023-50431-7
16. Pavlou S, Wang I, Xu H, Chen M. Higher phagocytic activity of thioglycolate-elicited peritoneal macrophages is related to metabolic status of the cells. Journal of Inflammation. 2017;14(4):1-6. Available from: doi.org/10.1186/s12950-017-0151-x
17. Zhang X, Goncalves R, Mosser DM. The isolation and characterization of murine macrophages. Current Protocols in Immunology. 2008;83:14. Available from: doi.org/10.1002/0471142735.im1401s83
18. Yan L, Vaghari-Tabari M, Malakoti F, Moein S, Qujeq D, Yousefi B, Asemi Z. Quercetin: An effective polyphenol in alleviating diabetes and diabetic complications. Critical Reviews in Food Science and Nutrition. 2023;63(28):9163-9186. Available from: doi.org/10.1080/10408398.2022.2067825
19. Aryaeian N, Sedehi SK, Arablou T. Polyphenols and their effects on diabetes management: A review. Medical Journal of the Islamic Republic of Iran. 2017;31:134. Available from: doi.org/10.14196/mjiri.31.134
20. Chen C, Zhou J, Ji C. Quercetin: a potential drug to reverse multidrug resistance. Life Sciences. 2010;87(11-12):333-338. Available from: doi.org/10.1016/j.lfs.2010.07.004
21. Yang DK, Kang HS. Anti-diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats. Biomolecules and Therapeutics. 2018;26(2):130. Available from: doi.org/10.4062/biomolther.2017.254
22. Rastogi S, Haldar C. Comparative effect of melatonin and quercetin in counteracting LPS induced oxidative stress in bone marrow mononuclear cells and spleen of Funambulus pennanti. Food and Chemical Toxicology. 2018;120:243-252. Available from: doi.org/10.1016/j.fct.2018.06.062
23. Jeong SM, Kang MJ, Choi HN, Kim JH, Kim JI. Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. Nutrition Research and Practice. 2012;6(3):201-207. Available from: doi.org/10.4162/nrp.2012.6.3.201
24. Ravikumar N, Kavitha CN. Immunomodulatory effect of Quercetin on dysregulated Th1/Th2 cytokine balance in mice with both type 1 diabetes and allergic asthma. Journal of Applied Pharmaceutical Science. 2020;10(3):080-087. Available from: doi.org/10.7324/JAPS.2020.103010
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979;95(2):351-358. Available from: doi.org/10.1016/0003-2697(79)90738-3
26. Das K, Samanta L, Chainy GBN. A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Indian Journal of Biochemistry and Biophysics. 2000;37:201-204.
27. Sinha AK. Colorimetric assay of catalase. Analytical Biochemistry. 1972;47(2):389-394. Available from: doi.org/10.1016/0003-2697(72)90132-7
28. Hadwan MH. New method for assessment of serum catalase activity. Indian Journal of Science and Technology. 2016;9:80499. Available from: doi.org/10.17485/ijst/2016/v9i4/80499
29. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics.1959;82(1):70-77.
30. Gupta YK, Kumar MV, Srivastava AK. Effect of Centella asiatica on pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. Pharmacology Biochemistry and Behavior. 2003;74(3):579-585. Available from: doi.org/10.1016/S0091-3057(02)01044-4
31. Majewski P, Dziwinski T, Pawlak J, Waloch M, Skwarlo-Sonta K.Anti-inflammatory and opioid-mediated effects of melatonin on experimental peritonitis in chickens. Life Sciences. 2005;76(17):1907-1920. Available from: doi.org/10.1016/j.lfs.2004.04.062
32. Singh SS, Sarma S, Kashyap A, Chakrabarti A. Melatonin protects against oxidative damage in spleen and deterioration of immune function in forced swim-stressed laboratory mice. Journal of Applied Biological Sciences. 2024;18(1):78-93. Available from: doi.org/10.5281/zenodo.10614148
33. Rahmani AH, Alsahli MA, Khan AA, Almatroodi SA. Quercetin, a plant flavonol attenuates diabetic complications, renal tissue damage, renal oxidative stress and inflammation in streptozotocin-induced diabetic rats. Metabolites. 2023;13(1):130. Available from: doi.org/10.3390/metabo13010130
34. Bailey SA, Zidell RH, Perry RW. Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? Toxicologic Pathology. 2004;32(4):448–466. Available from: doi.org/10.1080/01926230490465874
35. Sutradhar S, Deb A, Singh SS. Melatonin attenuates diabetes-induced oxidative stress in spleen and suppression of splenocyte proliferation in laboratory mice. Archives of Physiology and Biochemistry. 2020;128(5):1401-12. Available from: doi.org/10.1080/13813455.2020.1773506
36. Volpe CMO, Villar-Delfino PH, Dos Anjos, PM, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death and Disease. 2018;9(2):119. Available from: doi.org/10.1038/s41419-017-0135-z
37. Feng J, Wang J, Wang Y, Huang X, Shao T, Deng X, Zhao C. Oxidative stress and lipid peroxidation: prospective associations between ferroptosis and delayed wound healing in diabetic ulcers. Frontiers in Cell and Developmental Biology. 2022;10:898657. Available from: doi.org/10.3389/fcell.2022.898657
38. Alam MM, Meerza D, Naseem I. Protective effect of quercetin on hyperglycemia, oxidative stress and DNA damage in alloxan induced type 2 diabetic mice. Life Sciences. 2014;109(1):8-14. Available from: doi.org/10.1016/j.lfs.2014.06.005
39. Gambhir L, Tyagi G, Bhardwaj R, Kapoor N, Sharma G. Perturbation of cellular redox status: Role of Nrf2, a master regulator of cellular redox. In: Reactive Oxygen Species. Vol. 28, IntechOpen, London, United Kingdom, 2022; pp. 181-196. Available from: doi.org/10.5772/intechopen.94870
40. Rendra E, Riabov V, Mossel DM, Sevastyanova T, Harmsen MC, Kzhyshkowska J. Reactive oxygen species (ROS) in macrophage activation and function in diabetes. Immunobiology.

2019;224(2):242-253. Available from: doi.org/10.1016/j.imbio.2018.11.010
41. Choi HS, Kim JW, Cha YN, Kim CA. Quantitative nitroblue tetrazolium assay for determining intracellular superoxide anion production in phagocytic cells. Journal of Immunoassay and Immunochemistry. 2006;27(1):31-44. Available from: doi.org/10.1080/15321810500403722
42. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutrition & Metabolism. 2015;12:1-20. Available from: doi.org/10.1186/s12986-015-0057-7

HOW TO CITE THIS ARTICLE: Sarma S, Kashyap A, Chakrabarti A, Singh SS. Quercetin Ameliorated Diabetic Stress through Upregulation of Antioxidant Enzymes and the Nrf-2/HO-1 Axis in the Spleen of Mice. Int. J. Pharm. Sci. Drug Res. 2024;16(6):1004-1012. **DOI:** 10.25004/IJPSDR.2024.160611