ISSN 2475-5451

Research Article

International Journal of Diabetes & Metabolic Disorders

Protective Action of Aegeline and Quercetin Against Streptozotocin-Induced Diabetic Nephropathy

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Submitted: 2025, Mar 10 ; **Accepted:** 2025, May 20; **Published:** 2025, Jun 05

Citation: Parmar, M. Y., Niranjan, S. D., Shah, K., Khare, S., Baghban, I. et al. (2025). Protective Action of Aegeline and Quercetin against Streptozotocin-Induced Diabetic Nephropathy. *Int J Diabetes Metab Disord, 10*(2), 01-16.

Abstract

Background: *Diabetic nephropathy (DN) is a major cause of end-stage renal disease characterized by persistent albuminuria, declining glomerular filtration rate, and increased cardiovascular mortality. This study investigates the protective effects of Aegeline and Quercetin against streptozotocin (STZ)-induced diabetic nephropathy.*

Methods: *STZ was used to induce diabetes in Wistar rats. Treated groups received Aegeline, Quercetin, or their combination. Physical parameters (body weight, food, and water intake), biochemical markers (blood glucose, serum insulin, %HbA1c), renal parameters (albumin, creatinine, uric acid, cholesterol, total protein, triglycerides), oxidative stress markers (SOD, GSH, CAT, MDA), and histopathological evaluations were assessed.*

Results: *Treatment with Aegeline and Quercetin significantly ameliorated hyperglycemia, improved insulin sensitivity, restored renal parameters, reduced oxidative stress, and mitigated histopathological alterations in kidneys compared to diabetic control.*

Conclusion: *Aegeline and Quercetin exhibit substantial nephroprotective effects against diabetic nephropathy, indicating potential therapeutic roles.*

Keywords: Diabetic nephropathy, Streptozotocin, Aegeline, Quercetin, Antioxidants, Kidney protection.

1. Introduction

Diabetic nephropathy (DN) is a significant complication of both type 1 and type 2 diabetes mellitus, affecting approximately 20-40% of diabetic individuals and contributing to about 40% of new ESRD cases globally [1]. Characterized by persistent albuminuria, glomerulosclerosis, and decreased glomerular filtration rate, DN results from a complex interplay of metabolic and hemodynamic disturbances, oxidative stress, and inflammation [2,3].

Hyperglycaemia activates multiple damaging pathways including advanced glycation end products (AGEs), protein kinase C (PKC), polyol and hexosamine pathways, leading to increased reactive oxygen species (ROS) and inflammatory cytokines such as TGF-β1 and IL-6 [4,5,5a]. These mechanisms culminate in glomerular hypertrophy, basement membrane thickening, and interstitial fibrosis [6].

Natural plant-based compounds have gained attention for their pleiotropic pharmacological activities. Aegeline, an alkaloid from Aegle marmelos, has shown hypoglycemic, antioxidant, and anti-inflammatory properties [7,7a]. Quercetin, a flavonoid present in fruits and vegetables, exhibits strong antioxidant, anti-fibrotic, and anti-diabetic activities [8,9]. This study investigates the nephroprotective effects of Aegeline and Quercetin, alone and in combination, against STZ-induced diabetic nephropathy in rats.

2. Materials and Method

2.1 Chemicals and Reagents

Streptozotocin (STZ), Aegeline, Quercetin, and biochemical kits were procured from certified suppliers. Diagnostic kits for blood glucose, lipid profile, creatinine, uric acid, albumin, total protein, and oxidative stress markers were from Erba Mannheim.

2.2 Experimental Animals

Male Wistar rats (150-200g) were acclimatized under standard

conditions and provided with a standard diet and water ad libitum. Experiments were approved by the Institutional Animal Ethics Committee (IAEC).

2.3 Experimental Design

The rats were randomly divided into five groups (n=6 each):

- Group I: Normal control
- Group II: Diabetic control (STZ 55 mg/kg, i.p.)
- Group III: Diabetic + Aegeline (10 mg/kg, orally)
- Group IV: Diabetic + Quercetin (50 mg/kg, orally)
- Group V: Diabetic + Aegeline + Quercetin

2.4 Induction of Diabetes

Diabetes was induced via a single intraperitoneal injection of STZ (55 mg/kg) in citrate buffer (pH 4.5). Fasting blood glucose >250 mg/dL after 72 hours was considered diabetic.

2.5 Biochemical Parameters

Fasting blood glucose (oxidase-peroxidase method), serum insulin (ELISA), HbA1c (%), serum creatinine, uric acid, albumin, cholesterol, triglycerides, and total proteins were evaluated.

2.6 Antioxidant Assays

Renal tissues were homogenized and tested for:

- Superoxide dismutase (SOD)
- Catalase (CAT)
- Glutathione (GSH)
- Malondialdehyde (MDA)

2.7 Inflammatory Marker

C-reactive protein (CRP) was measured using a latex-enhanced immunoassay.

2.8 Histopathology

Kidneys, pancreas, and liver were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic evaluation.

3.Results

3.1 Physical Parameters

- **Body Weight:** Diabetic rats (Group II) showed a significant reduction in body weight compared to normal controls (Group I). Aegeline and Quercetin treatments improved body weight, with combination therapy showing maximum improvement (Table 41, Figure 45).

Day's	Normal Control						Diabetic Control					
0	250	252	260	255	258	255	255	265	250	250	252	260
2	270	260	269	265	270	265	198	190	192	195	196	192
7	272	265	262	267	265	267	200	195	197	199	200	196
14	270	268	271	268	266	268	202	198	202	201	202	200
21	270	265	270	268	266	268	205	200	206	202	204	205
28	272	270	270	270	264	270	208	202	210	205	207	208
Day's	**STZ + Standard Treatment Metformin**						**STZ + Standard Treatment Aegelin + Quercetin**					
0	195	200	198	195	190	192	192	195	191	190	195	190
2	200	210	205	205	200	203	201	200	202	203	205	206
7	215	130	220	215	220	222	230	226	230	230	225	232
14	225	240	235	220	228	230	245	247	245	251	228	249
21	250	260	255	245	245	243	250	255	246	240	244	
28	260	268	265	255	258	253	271	275	275	272	275	

Table 1: Body Weight

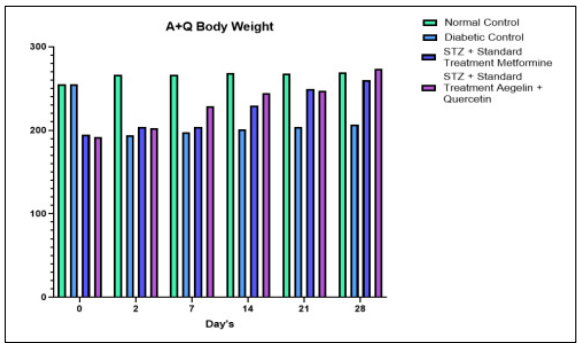


Figure 1: Graphical Representation of Body Weight

Water Intake: Diabetic rats (Group II) exhibited a significant increase in water intake due to polydipsia, reaching 75 ml by day 28 compared to stable levels around 30 ml in normal controls (Group I). Treatment with Metformine and Aegeline + Quercetin

notably reduced water intake to 32 ml and 30 ml respectively, nearing normal levels. Combination therapy showed the greatest improvement, highlighting its potential to alleviate diabetes symptoms effectively.

Day's	Normal Control						Diabetic Control					
0	25	28	25	25	28	25	28	28	25	30	29	30
2	30	28	30	29	28	30	35	38	32	38	37	37
7	28	30	25	26	30	28	45	48	42	49	48	47
14	30	26	28	28	25	26	53	59	52	61	60	58
21	27	28	30	30	24	28	65	70	63	77	68	68
28	30	30	28	28	29	30	75	77	72	79	70	75
Day's	**STZ + Standard Treatment Metformin**						**STZ + Standard Treatment Aegelin + Quercetin**					
0	69	70	70	77	75	74	65	67	60	61	70	69
2	65	70	68	77	75	72	62	64	57	67	67	66
7	62	67	66	74	72	69	56	58	50	61	61	60
14	48	50	46	49	48	59	48	50	43	53	53	52
21	38	42	36	39	40	49	38	40	33	43	43	
28	26	32	28	30	32	30	28	30	23	33	33	

Table 2: Water Intake

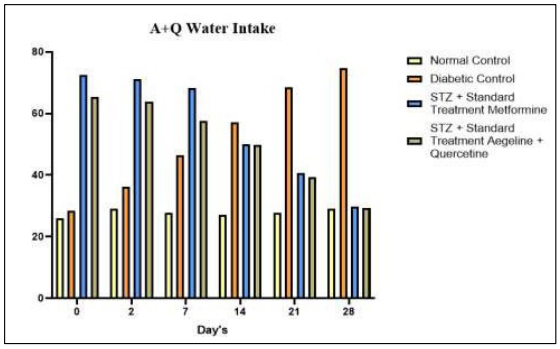


Figure 2: Graphical Representation of Water Intake

- **Food Intake:** Diabetic rats (Group II) demonstrated a significant reduction in food intake compared to normal controls (Group I), with consumption dropping to 20-22 g/day by day 28. Treatment with Metformine and the combination of Aegeline + Quercetine significantly improved food intake, reaching 47 g/day and 51 g/day respectively by day 28 ($p < 0.05$). The combination therapy was particularly effective, restoring food intake to near-normal levels and indicating its potential benefits in improving metabolic health and mitigating diabetes-related disturbances.

Day's	Normal Control						Diabetic Control					
0	52	44	51	46	50	44	30	35	32	35	30	38
2	50	49	45	50	48	47	29	32	31	32	29	37
7	45	50	48	48	49	49	24	27	27	27	24	34
14	48	52	50	48	51	50	20	22	22	22	20	30
21	48	45	52	50	50	52	25	24	24	24	25	26
28	50	48	52	51	52	52	21	20	20	20	21	22
Day's	**STZ + Standard Treatment Metformin**						**STZ + Standard Treatment Aegelin + Quercetin**					
0	30	35	32	35	30	38	26	25	20	23	26	24
2	29	32	31	32	29	37	31	25	25	28	27	28
7	24	27	27	27	24	34	35	29	29	32	32	33
14	20	22	22	22	20	30	40	34	34	37	36	28
21	25	24	24	24	25	26	48	39	42	45	41	
28	21	20	20	20	21	22	51	46	47	48	49	

Table 3: Food Intake

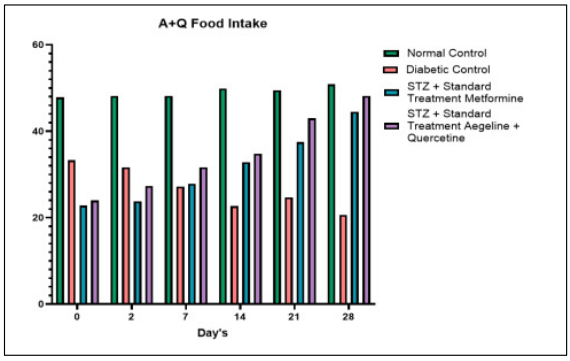


Figure 3: Graphical representation of Food Intake

- **Urine Output:** Diabetic rats (Group II) exhibited a significant decrease in food intake, dropping to 20-22 g/day by day 28 compared to normal controls (Group I), which maintained stable levels of around 50-52 g/day. Treatments with Metformine and Aegeline + Quercetine significantly improved food consumption, reaching 47 g/day and 51 g/day respectively by day 28 ($p < 0.05$). Combination therapy showed maximum improvement, restoring food intake to near-normal levels and demonstrating its potential to enhance metabolic health and alleviate diabetes-related disturbances.

Day's	**Normal Control**						**Diabetic Control**					
0	12	13	15	14	16	15	14	15	12	12	17	16
2	17	16	16	16	17	15	23	20	21	22	22	21
7	17	16	15	15	16	17	26	22	23	24	26	22
14	17	15	17	15	15	15	21	23	28	26	26	26
21	16	15	16	16	15	16	23	26	24	27	26	25
28	16	17	16	16	15	17	30	28	35	25	24	25
Day's	**STZ + Standard Treatment Metformin**						**STZ + Standard Treatment Aegelin + Quercetin**					
0	30	28	26	24	22	20	28	20	22	22	25	20
2	29	27	25	24	22	20	23	20	21	19	22	18
7	26	23	21	20	18	17	22	17	20	18	20	16
14	22	20	18	17	15	14	21	14	18	15	17	15
21	18	17	15	14	12	11	17	11	15	12	16	13
28	16	14	12	11	9	8	15	8	12	9	11	12

Table 4: Urine Output

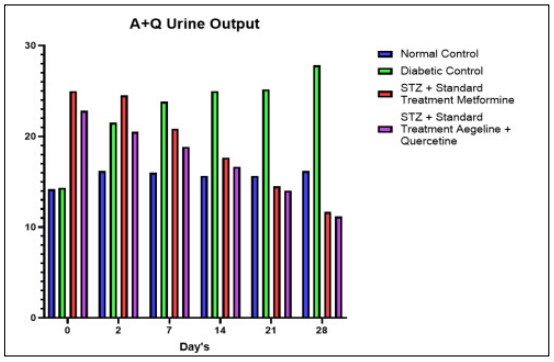


Figure 4: Graphical Representation of Urine Out

3.2 Biochemical Parameters

- **Blood Glucose:** This study compares blood glucose levels across four groups over 28 days: Normal Control, Diabetic Control, STZ + Metformin, and STZ + Aegelin + Quercetin. The Normal Control group maintains stable glucose levels, while the Diabetic Control group experiences severe hyperglycemia. The STZ + Metformin group shows improved glucose management over time. However, the STZ + Aegelin + Quercetin group demonstrates the most significant improvement, achieving near-normal glucose levels by day 14 and maintaining them through day 28, indicating a potentially highly effective diabetes treatment. Further research is required to evaluate its long-term efficacy and safety.

Day's	Normal Control						Diabetic Control					
0	108	110	112	105	109	106	200	202	210	216	220	222
2	134	135	109	111	109	111	203	198	201	233	246	241
7	111	115	110	112	110	108	208	200	209	234	248	247
14	109	108	112	111	110	108	213	204	208	234	248	247
21	110	110	107	105	101	105	243	239	240	277	276	270
28	103	107	106	105	101	103	259	263	267	290	299	287
Day's	**STZ + Standard Treatment Metformin**						**STZ + Standard Treatment Aegelin + Quercetin**					
0	240	245	248	242	241	243	370	391	408	351	394	536
2	240	244	247	242	240	243	362	290	322	323	283	520
7	220	224	227	222	220	223	322	260	284	315	245	496
14	190	194	197	192	190	193	252	160	237	238	200	416
21	150	154	157	152	150	159	172	140	177	177	147	
28	95	99	102	97	95		103	111	121	121	111	

Table 5: Blood Glucose

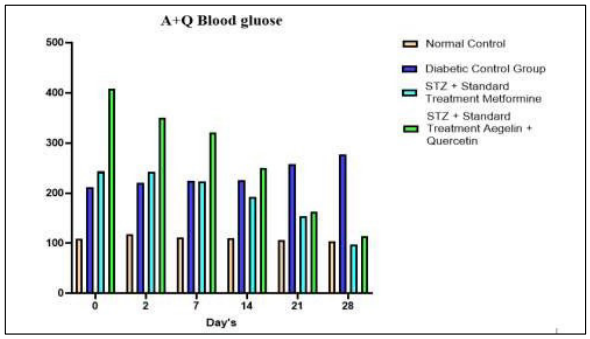


Figure 5: Graphical Representation of Blood Glucose

- **% HbA1c:** This study examines HbA1c levels in five groups over 28 days: Normal Control, Diabetic Control, STZ+Metformin, STZ+Pre-treatment, and STZ+Aegeline+Quercetin. The Normal Control group maintains stable HbA1c levels (4-6%), while the Diabetic Control group experiences a significant increase (9% to over 12%), indicating poor glucose control. The STZ+Metformin group shows gradual improvement, reaching about 6% by day 28. The STZ+Pre-treatment group follows a trend similar to the Diabetic Control. The Aegeline+Quercetin group exhibits the most remarkable improvement, reducing elevated HbA1c levels to near-normal by day 14 and maintaining this through day 28. These findings suggest that Aegeline+Quercetin may offer superior long-term glycemic control, potentially exceeding Metformin's effectiveness. Further research is needed to confirm its safety and mechanisms.

Day's	Normal Control						Diabetic Control					
0	4.1	3.4	3.6	3.7	3.6	3.8						
2	4.7	4.1	4	4.4	4.2	4.7	9.1	10	9.7	9.4	8.3	8.9
7	5.1	4.3	4.2	4.5	4.7	4.9	9.9	10.7	10.2	9.9	9.7	9.8
14	5.9	4.7	4.6	5.1	5.3	5.8	10.7	11.4	11.2	10.8	10.1	10.8
21	6.2	5.5	5.4	5.7	5.9	6	11.6	12.9	12	12.1	11.4	12.4
28	103	107	106	105	101	103	259	263	267	290	299	287
Day's	**STZ + Met**						**STZ + AQ**					
0												
2	7.2	6.8	7.8	7.6	8.1	7.9	9.2	8.1	12.3	7.9	8.4	8.2
7	6.6	5.8	6.4	6.1	7.2	6.3	7.9	7	X	6.4	7.1	6.7
14	5	5.1	5.3	5.7	6.3	5.2	6.6	6	X	5.7	6.2	5.9
21	3.9	4.1	4.3	4.5	5.2	4	5.9	5.6	X	5	5.7	5.2

28	95	99	102	97	95		103	111	121	121	111	

Table 6: %HbA1c

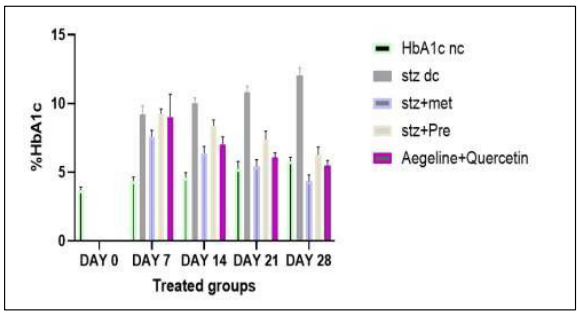


Figure 6: Graphical representation of %HbA1c

- **Serum Insulin:** This study examines serum insulin levels across four groups over 28 days. While the Normal Control group maintains stable levels (3-6 ng/ml) and the Diabetic Control shows a steep decline (below 1 ng/ml), the STZ + Metformin group has minimal impact on insulin production. The STZ + Aegeline + Quercetin (AQ) group, however, demonstrates significant improvement, with insulin levels increasing to around 8 ng/ml by day 28, surpassing the Normal Control. This suggests that AQ treatment may effectively stimulate insulin production or preserve beta cell function, showing potential for managing diabetes. Further research is needed to confirm its safety and mechanisms.

Day's	**Normal Control**						**Diabetic Control**					
0	3.1	3.4	3.6	3.7	3.4	3.8						
2	4.7	4.1	4	4.4	4.2	4.7	2.5	2.7	2.3	2.2	2.8	2.6
7	5.1	4.3	4.2	4.5	4.7	4.9	1.9	1.6	1.8	1.5	1.7	1.3
14	5.7	4.7	4.6	5.1	5.3	5.4	0.98	0.88	0.78	0.76	0.94	0.67
21	6.2	5.5	5.4	5.6	5.9	6	0.45	0.37	0.27	0.2	0.41	0.15
28	103	107	106	105	101	103	259	263	267	290	299	287
Day's	**STZ + Standard Treatment Metformin**						**STZ + Standard Treatment Aegelin + Quercetin**					
0												
2	4.7	4.6	4.4	5.4	5.6	4.6	4.4	5.1	5.7	5.3	5.7	5.9
7	5.7	5.8	5.2	6.6	6.3	5.8	5.4	5.8	6.5	6.3	6.7	6.9
14	6.6	6.7	6.4	7.2	7.4	6.9	6.6	6.5	7.4	7.3	7.4	7.5
21	7.8	7.9	7.8	8	8.3	8	7.6	7.9	8	8	8.3	8.4
28	95	99	102	97	95		103	111	121	121	111	

Table 7: Serum Insulin

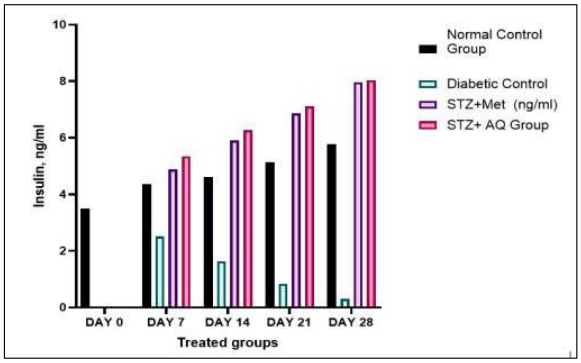


Figure 7: Graphical Representation of Serum Insulin

3.3 Kidney Function Markers

- **Albumin:** This study evaluates albumin levels across four groups: Normal Control, Diabetic Control, DC + Metformin, and DC + Aegeline + Quercetin (AQ). The Diabetic Control group displays the highest albumin levels (4.94 ± 0.32 g/dl), significantly elevated compared to the Normal Control group

(3.59 ± 0.06 g/dl). Treatment groups, DC + Metformin (3.73 ± 0.08 g/dl) and DC + AQ (3.55 ± 0.10 g/dl), show reductions in albumin levels, approaching those of the Normal Control group. Statistical analysis confirms that diabetes increases albumin levels ($p < 0.05$) while AQ treatment significantly reduces them ($p < 0.05$), highlighting its potential in mitigating albumin elevation caused by diabetes.

Normal Control Group	Diabetic Control Group	DC+ Metformin Group	DC+ AQ Group
3.59	4.44	3.86	3.8
3.7	4.6	3.59	3.87
3.59	5.07	3.7	3.22
3.57	5.19	3.73	3.28
3.73	5.04	3.97	3.55
3.65	5.12	3.6	

Table 8: Albumin

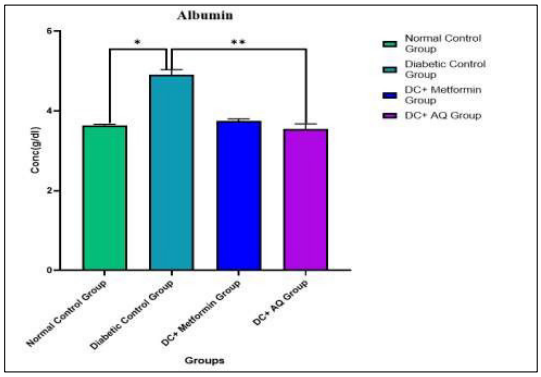


Figure 8: Graphical Representation for Albumin

- **Uric Acid:** This study evaluates uric acid levels across four groups: Normal Control, Diabetic Control, DC + Metformin, and DC + Aegeline + Quercetin (AQ). The Diabetic Control group exhibits the highest uric acid levels (8.55 ± 0.42 mg/dl), significantly elevated compared to the Normal Control group (5.92 ± 0.06 mg/dl). Both treatment groups, DC + Metformin (5.96 ± 0.24 mg/dl) and DC + AQ (5.95 ± 0.05 mg/dl), show reductions in uric acid levels, approaching those of the Normal Control group. Statistical analysis indicates that diabetes raises uric acid levels ($p < 0.05$), and AQ treatment significantly lowers them, potentially mitigating diabetic nephropathy.

Normal Control Group	Diabetic Control Group	DC+ Metformin Group	DC+ AQ Group
5.58	7.77	5.61	5.74
6.2	8.13	6.18	5.69
5.82	8.07	5.55	6.00
6.04	8.15	6.00	5.96
6.17	10.64	6.42	5.98
5.99	9.01	5.89	

Table 9: Uric Acid

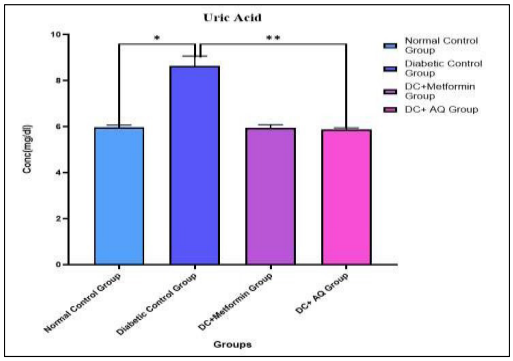


Figure 9: Graphical Representation for Uric Acid

- **Creatinine:** This study examines creatinine levels across four groups: Normal Control, Diabetic Control, DC + Metformin, and DC + Aegeline + Quercetin (AQ). The Diabetic Control group shows significantly elevated creatinine levels (3.84 ± 0.58 mg/dl) compared to the Normal Control group (0.96 ± 0.16 mg/dl), indicating diabetic nephropathy. Treatment groups, DC + Metformin (1.51 ± 0.14 mg/dl) and DC + AQ (1.43 ± 0.19 mg/dl), exhibit reduced creatinine levels closer to the Normal Control, suggesting that Metformin and AQ may help mitigate nephropathy effects. Statistical analysis supports these findings, showing diabetes-induced creatinine increases and a potential therapeutic role for AQ.

Normal Control Group	Diabetic Control Group	DC+ Metformin Group	DC+ AQ Group
0.8	2.46	1.5	1.59
0.45	3.6	1.4	1.84
0.75	3.3	0.98	1.17
1.3	4.89	1.22	2
1.2	3.7	1.35	1.82
1.1	4.96	2	

Table 10: Creatinine

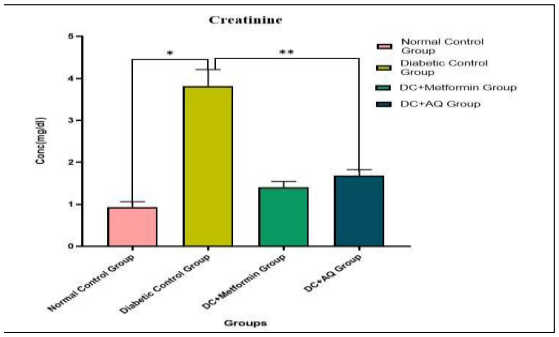


Figure 10: Graphical Representation for Creatinine

- **Cholestrol:** This study examines cholesterol levels across four groups: Normal Control, Diabetic Control, DC + Metformin, and DC + Aegeline + Quercetin (AQ). The Diabetic Control group shows significantly elevated cholesterol levels (445.26 ± 72.59 mg/dl) compared to the Normal Control group (240.39 ± 13.46 mg/dl). Treatment groups, DC + Metformin (248.34 ± 16.57 mg/dl) and DC + AQ (187.82 ± 9.98 mg/dl), exhibit reduced cholesterol levels, nearing those of the Normal Control group. Statistical analysis confirms diabetes-induced cholesterol increases ($p < 0.05$) and highlights AQ's significant reduction of cholesterol, showcasing its potential therapeutic benefits.

Normal Control Group	Diabetic Control Group	DC+ Metformin Group	DC+ AQ Group
200.07	425.6	208.6	150.9
245	629.1	213.9	159.4
255.09	666.4	277.5	207.9
207.7	244	263.8	200.2
210.04	227.3	200.6	178.4
256.9	455.3	278.6	

Table 11: Cholesterol

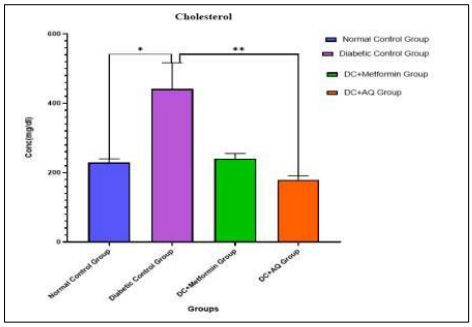


Figure 11: Graphical Representation for Cholesterol

- **Total Protein:** This study evaluates total protein levels in four groups: Normal Control, Diabetic Control, DC + Metformin, and DC + Aegeline + Quercetin (AQ). The Diabetic Control group displays significantly elevated total protein levels (15.58 ± 0.62 g/dl) compared to the Normal Control group (6.37 ± 0.41 g/dl), indicative of diabetic impact. Treatment groups, DC + Metformin (13.47 ± 0.17 g/dl) and DC + AQ (6.97 ± 0.42 g/dl), show reduced protein levels, with AQ-treated groups closely approaching normal levels. Statistical analysis highlights diabetes-induced total protein increases ($p < 0.05$) and AQ's significant ability to normalize levels, showcasing its potential therapeutic benefits.

Normal Control Group	Diabetic Control Group	DC+ Metformin Group	DC+ AQ Group
6.86	15.59	15.59	6.43
6.74	15.88	15.88	6.45
5.68	18.58	18.58	7.05
5.48	18.51	18.51	7.08
6.01	13.16	13.16	6.35
6.12	12.45	12.45	

Table 12: Total Protein

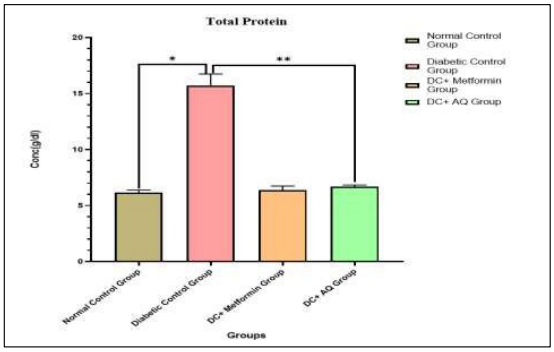


Figure 12: Graphical Representation for Total Protein

- **Triglycerides:** This study examines triglyceride levels across four groups: Normal Control, Diabetic Control, DC + Metformin, and DC + Aegeline + Quercetin (AQ). The Diabetic Control group exhibits the highest triglyceride levels (393.63 ± 23.95 mg/dl), significantly elevated compared to the Normal Control group (207.14 ± 13.84 mg/dl). Treatment groups, DC + Metformin (212.32 ± 12.41 mg/dl) and DC + AQ (215.28 ± 12.89 mg/dl), show substantial reductions in triglyceride levels, bringing them closer to normal values. Statistical analysis confirms diabetes-induced triglyceride elevation ($p < 0.05$) and highlights both Metformin and AQ's efficacy in managing triglyceride levels effectively.

Normal Control Group	Diabetic Control Group	DC+ Metformin Group	DC+ AQ Group
206.32	419.42	248.41	223.39
200.29	450.33	217.95	228.55
193.52	406.76	195.58	191.61
202.45	349.52	191.75	201.61
204.34	304.34	198.55	207.29
219.422	410.12	230.16	

Table 13: Triglycerides

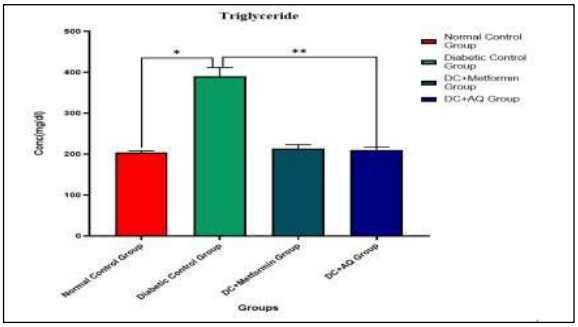


Figure 13: Graphical Representation for Triglycerides

3.4 Inflammatory and Oxidative Stress Markers

- **CRP Levels:** This study evaluates C-Reactive Protein (CRP) levels across four groups: Normal Control, Diabetic Control, DC + Metformin, and DC + Aegeline + Quercetin (AQ). The Diabetic Control group exhibits the highest CRP levels (3.18 ± 0.39 mg/L), significantly elevated compared to the Normal Control group (1.00 ± 0.18 mg/L). Treatment groups, DC + Metformin (1.56 ± 0.27 mg/L) and DC + AQ (1.42 ± 0.17 mg/L), show reduced CRP levels, with AQ treatment being slightly more effective in reducing inflammation to near-normal levels. Statistical analysis confirms diabetes-induced inflammation ($p < 0.05$) and highlights both treatments' potential in mitigating this effect.

Normal Control Group	Diabetic Control Group	STZ + Standard Treatment Metformin	STZ + AQ Group
1.42	1.52	0.5	1.05
0.98	2.62	0.96	2.2
0.56	4.78	1.30	1.78
1.32	3.00	2.50	1.73
1.20	2.63	1.56	0.91
0.79	3.98	2.03	

Table 14: CRP

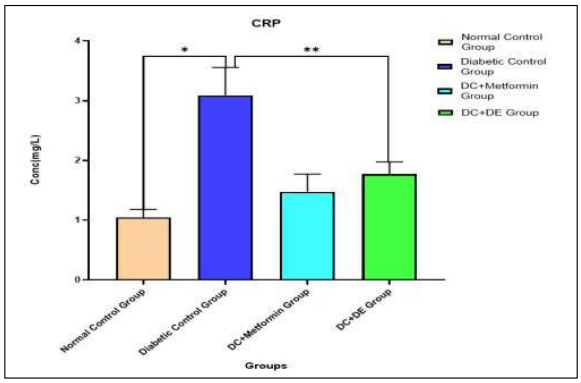


Figure 14: Graphical representation of CRP

- **Superoxide Dismutase (SOD):** This study evaluates superoxide dismutase (SOD) levels across four groups: Normal Control, Diabetic Control, STZ + Metformin, and STZ + Aegeline + Quercetin (AQ). The Normal Control group exhibits the highest SOD levels (around 45 U/mg protein), while the Diabetic Control group shows the lowest levels (around 25 U/mg protein). Both treatment groups demonstrate improved SOD levels compared to the Diabetic Control, with STZ + Metformin showing slightly higher SOD levels (47 U/mg protein) than AQ (45 U/mg protein), close to normal values. Statistical significance confirms the effectiveness of both treatments, with Metformin showing a slightly stronger impact.

Normal Control Group	Diabetic Control Group	STZ + Standard Treatment Metformin	STZ + AQ Group
45.8	25.6	48.9	47.5
42.5	23.6	45.6	45.6
43.5	25.9	43.5	48.5
44.5	24.5	44.5	43.8
41.5	27.2	47.5	42
48.5	24.3	49.6	43.1

Table 15: SOD

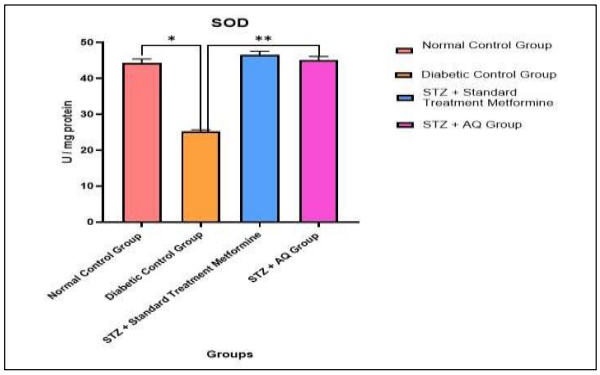


Figure 15: Graphical representation of SOD

- **Catalase (CAT):** This study evaluates catalase (CAT) activity across four groups: Normal Control, Diabetic Control, STZ + Metformin, and STZ + Aegeline + Quercetin (AQ). The Diabetic Control group exhibits significantly reduced CAT levels (~3.3 U/mg protein) compared to the Normal Control group (~9.5 U/mg protein). Both treatment groups show notable improvement, with Metformin (~8.6 U/mg protein) demonstrating slightly higher CAT levels than AQ (~7.7 U/mg protein). Statistical analysis confirms diabetes-induced reduction in CAT activity and the efficacy of both treatments in restoring this antioxidant enzyme, though not fully to normal levels.

Normal Control Group	Diabetic Control Group	DC+ Metformin Group	DC+ AQ Group
8.6	2.9	8.9	7.5
9.2	3.3	6.2	8.3
9.7	2.9	7.2	6.9
8.4	3.5	10.2	8.7
10.2	4.1	8.6	7.6
9.5	3.3	9	9.11

Table 16: Catalase

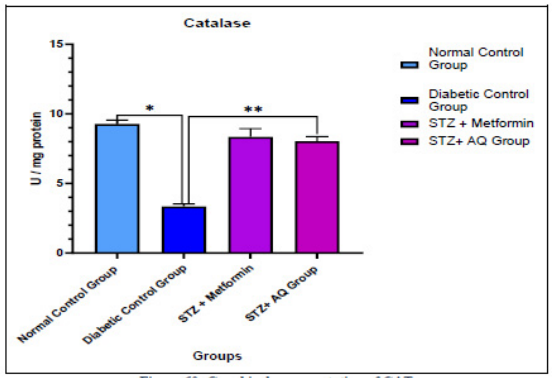


Figure 16: Graphical Representation of CAT

- **Glutathione perioxidase (GSH):** This study demonstrates that diabetes significantly depletes glutathione (GSH) levels, a crucial antioxidant, as observed in the Diabetic Control group (~4.7 U/mg protein) compared to the Normal Control group (~9.7 U/mg protein). Both STZ + Metformin and STZ + Aegeline + Quercetin (AQ) treatments effectively restore and even slightly exceed normal GSH levels (10.2 and 10.1 U/mg protein, respectively). These results highlight the potent antioxidant effects of both treatments, with comparable efficacy, in managing oxidative stress induced by diabetes.

Normal Control Group	Diabetic Control Group	STZ + Standard Treatment Metformin	STZ + AQ Group
9.7	4.8	11.6	10.12
9.5	5.9	12.5	10.5
8.7	6	8.9	9.5
9.5	3.9	9.5	8.9

10.1	4.3	10.12	11.2
10.8	3.9	8.8	10

Table 17: GSH

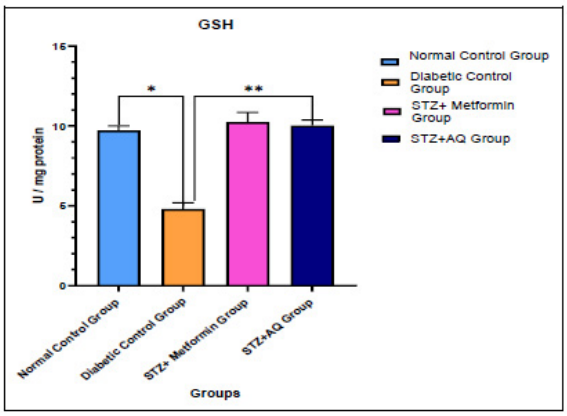


Figure 17: Graphical representation of GSH

- **Malondialdehyde (MDA):** This study evaluates malondialdehyde (MDA) levels, a marker of lipid peroxidation and oxidative stress, across four groups: Normal Control, Diabetic Control, STZ + Metformin, and STZ + Aegeline + Quercetin (AQ). The Diabetic Control group exhibits significantly elevated MDA levels (~49.5 U/mg protein) compared to the Normal Control (~28.3 U/mg protein), reflecting heightened oxidative stress due to diabetes. Both treatment groups, STZ + Metformin (~30.1 U/mg protein) and STZ + AQ (~30.6 U/mg protein), significantly reduce MDA levels, bringing them close to normal. These findings highlight diabetes-induced oxidative stress and the efficacy of both treatments, with comparable antioxidant effects, in mitigating this condition.

Normal Control Group	Diabetic Control Group	DC+ Metformin Group	DC+ AQ Group
27.5	49.8	29.3	30.2
28.2	51.1	31.2	29.6
24.2	47.8	27.8	27.4
24.9	52.9	32.5	29.4
30.5	50.8	28.9	32.1
30	46.9	30.5	30.4

Table 18: MDA

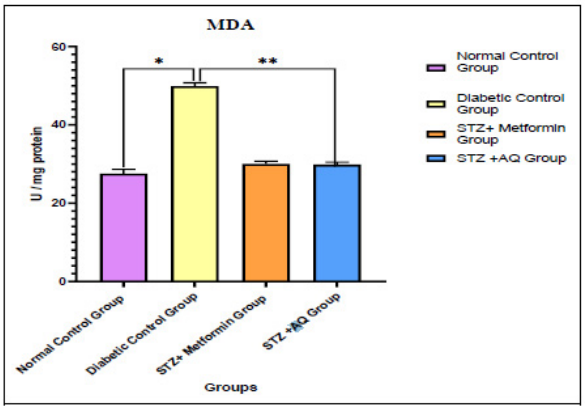


Figure 18: Graphical Representation of MDA

3.5 Histopathology

- **Kidney:**

A. Section of kidney from normal control group showing normal architecture of kidney. (H & E x 100). G-Glomeruli, T-Tubules. While the second figure shows the magnification (H & E x 400).

B. Section of kidney from Diabetic group showing increase in glomerulus diameter and Bowman's space (Yellow arrow) as well as increased number of proximal tubules with necrosis nuclei as compared to control group. (H & E x 100) While the second figure shows the magnification (H & E x 400).

C. Section of kidney from Diabetic + Std Control group showing increase in glomerulus diameter and Bowman's space (Yellow arrow) as well as increased number of proximal tubules with necrosis nuclei as compared to control group. (H & E x 100) While the second figure shows the magnification (H & E x 400).

D. Microscopic section of Kidney of Aegeline & Quercetin group showing congestion and increase the bowman's space in the glomeruli compared to Diabetic group. (H & E Stain X 100) While the second figure shows the magnification (H & E x 400).

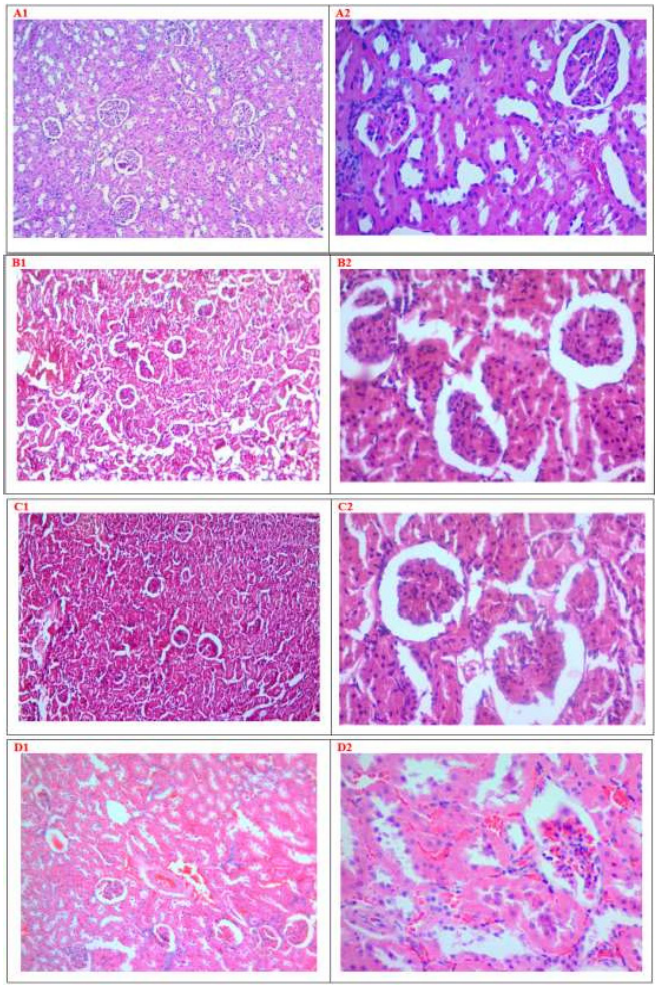


Figure 19: Histopathology of Kidney

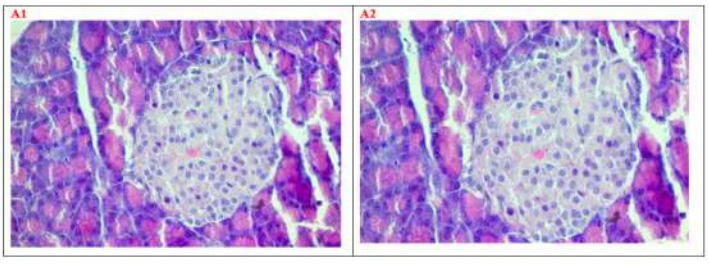
- **Pancreas:**

A.Microscopic section of Pancreas of Control group showing normal architecture. Note: Normal Population of Exocrine Cell- α cell and Endocrine part - β cell. (H & E Stain X 400).

B.Microscopic section of Pancreas of Diabetic group showing severe decrease the Population and degeneration and necrosis of - β cell compared to control group. (H & E Stain X 40) While the second figure shows the magnification (H & E x 400).

C.Microscopic section of Pancreas of Diabetic group + Metformin showing mild increase the Population and degeneration and necrosis of of - β cell compared to control group. (H & E Stain X 400)

D.Microscopic section of Pancreas of Aegeline & Quercetin group showing mild decrease the Population of - β cell compared to Diabetic group. (H & E Stain X 100) While the second figure shows the magnification (H & E x 400).



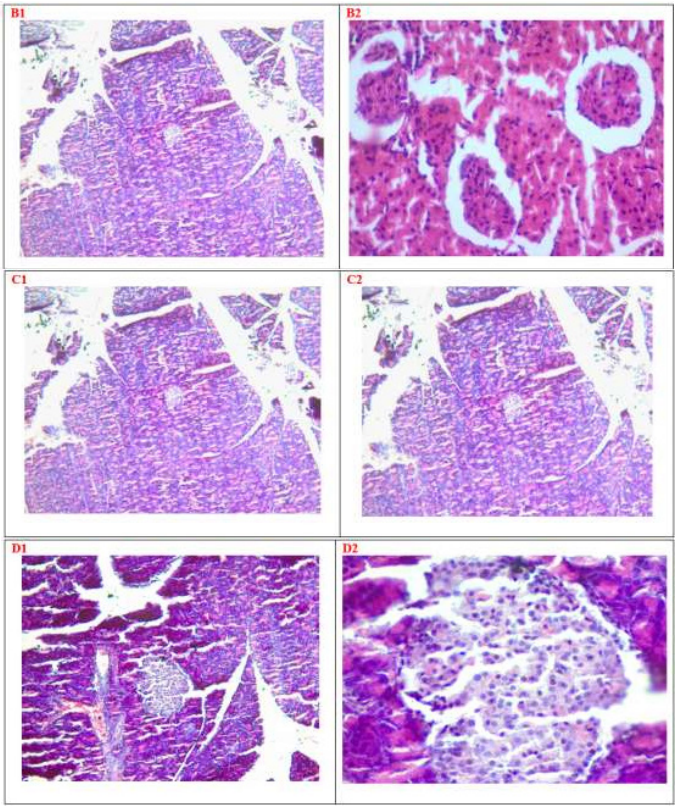


Figure 20: Histopathology of Pancreas

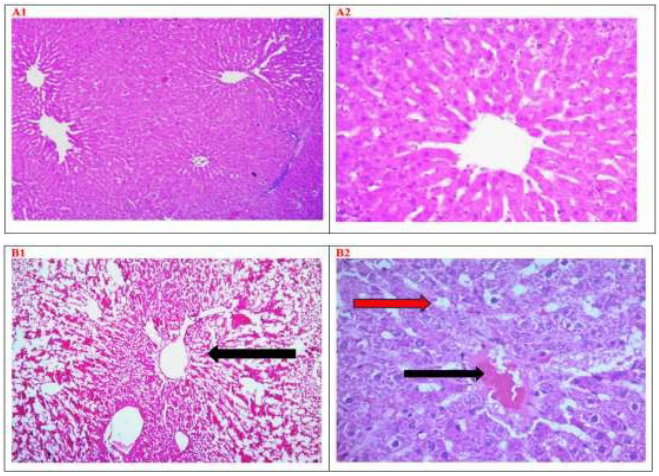
- **Liver**

A .Section of liver from Normal control group showing almost normal architecture of hepatic lobules (H & E x 100) while the second figure is section of liver from Normal control group showing almost normal architecture of hepatic lobules (H & E x 400).

B. Section of liver from Diabetic group showing vacuolar degeneration and necrosis in hepatocytes (black arrow). The hepatic cords are also severely distorted. (H & E x 40) while section of liver from Diabetic group showing congestion in central vein (Black arrow) and vacuolar degeneration and necrosis in hepatocytes (red arrow). The hepatic cords are also severely distorted. (H & E x 400).

C. Section of liver from Diabetic + Std Control group showing moderately vacuolar degeneration and necrosis in hepatocytes (black arrow) as compared to diabetic group. The hepatic cords are also less distorted as compared to diabetic group. The hepatic cords are also less distorted as compared to diabetic group as well as congestion in central vein is also noted (red arrow). (H & E x 100).

D.Section of liver from Aegeline + Quercetine group showing mild congestion of central vein (black arrow) and mild vacuolar degeneration of hepatocytes (Yellow arrow) (H & E, x40) while second image Same figure at higher magnification ((H & E, x 400).



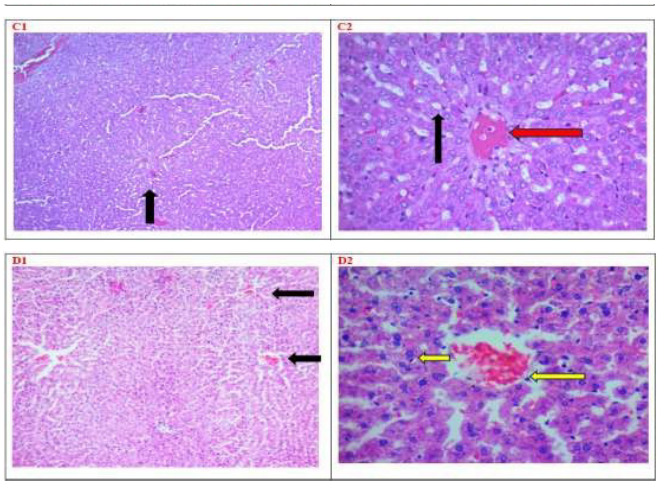


Figure 21: Histopathology of Liver

4. Discussion

The present study provides comprehensive evidence that both Aegeline and Quercetin exert protective effects against STZ-induced diabetic nephropathy through multiple mechanisms. Diabetic rats exhibited hallmark symptoms including hyperglycemia, weight loss, polyuria, elevated oxidative stress, and renal dysfunction, aligning with previous findings on DN pathology [10]. Treatment with Aegeline demonstrated significant antihyperglycemic and antioxidant effects, which may be attributed to its action on GLUT4 translocation and anti-inflammatory cytokine modulation, as suggested by Gautam et al. [11]. Quercetin, known for its multifaceted pharmacological actions, significantly reduced blood glucose, improved insulin secretion, and corrected dyslipidemia, consistent with findings by Peng-Bin Lai et al. and Rahmani et al. [12,13]. Combination therapy yielded superior results compared to monotherapies. This synergistic effect is likely due to the complementary mechanisms of action: Aegeline enhances insulin sensitivity while Quercetin reduces oxidative stress and fibrosis. The significant improvement in renal histoarchitecture, reduction in inflammatory biomarkers (CRP), and normalization of antioxidant enzymes (SOD, CAT, GSH) further support their nephroprotective roles.

The observed reduction in serum creatinine, uric acid, and albumin levels corroborates the kidney-protective action of both phytocompounds. These findings align with previous studies where herbal extracts ameliorated STZ-induced renal injury through suppression of ROS and pro-inflammatory cytokines [14, 14a–17]. Histological examination revealed that Aegeline and Quercetin could restore glomerular and tubular structures, minimizing necrosis and fibrosis. This complements findings by Ansari et al. and Bhatti et al., who observed structural kidney recovery with these phytocompounds [18,19]. In summary, the combination of Aegeline and Quercetin offers a promising adjunctive therapy in the management of diabetic nephropathy due to their collective antioxidant, anti-inflammatory, and hypoglycemic properties.

5.Conclusion

This study concludes that Aegeline and Quercetin, both individually and synergistically, exhibit potent nephroprotective effects in STZ-induced diabetic rats. Their combined administration significantly restored renal function markers, improved glycemic control, enhanced antioxidant enzyme levels, and reduced inflammation. Histological evaluations further confirmed preservation of kidney, pancreas, and liver structures. These findings support the potential of Aegeline and Quercetin as effective natural therapeutics for managing diabetic nephropathy. Further clinical investigations are warranted to confirm their translational applicability in human subjects.

Acknowledgements

The authors are thankful to Management of Krishna School of Pharmacy & Research, Dr. Kiran and Pallavi Patel Global University (KPGU), Vadodara, Gujarat, India for providing all the supports to carry out the research work. We also acknowledge the student start up innovation policy 2.0 (SSIP), Gujarat for providing the research grant.

Disclosures No conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions

MP, DS, PP, B, KS had done review of literature and experimental work. MP, SK drafted manuscript, DS, PP, BP, SK, KS, ZD, PL, IB, KP evaluated and analyse the data. MP, DS, PP, BP, IB edited and revised manuscript; MP approved final version of manuscript.

References

1. Kimmelstiel, P., & Wilson, C. (1936). Intercapillary lesions in the glomeruli of the kidney. *The American journal of pathology, 12*(1), 83.
2. Sagoo, M. K., & Gnudi, L. (2019). Diabetic nephropathy: an overview. Diabetic nephropathy: methods and protocols, 3-7.
3. Samsu, N. (2021). Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. *BioMed research international, 2021*(1), 1497449.
4. Agarwal, R. (2021). Pathogenesis of diabetic nephropathy.
5. Freedman, B. I., Bostrom, M., Daeihagh, P., & Bowden, D.

W. (2007). Genetic factors in diabetic nephropathy. *Clinical Journal of the American Society of Nephrology, 2*(6), 1306-1316.
6. Suliska, N., Praviska, M., Kurniati, N. F., & Sukandar, E. Y. (2021). Nephroprotective effect of ethanol extract of Sonchus arvensis L. leaves in gentamicin-piroxicam induced rat renal failure. *Journal of Research in Pharmacy, 25*(4), 441-449.
7. Bhatti, R., Sharma, S., Singh, J., Singh, A., & Ishar, M. P. S. (2013). Effect of Aegle marmelos (L.) Correa on alloxan induced early stage diabetic nephropathy in rats.
8. Sharma, G. N., Dubey, S. K., Sharma, P., & Sati, N. (2011). Medicinal values of bael (Aegle marmelos)(L.) Corr.: A review. *Int J Curr Pharm Rev Res, 2*(1), 12-22.
9. 7a. Transaminase, P. Nephroprotective Potential of Ethanolic Extract of Barks of Tricholepis Glaberrima Against Gentamicin Induced Nephrotoxicity.
10. David, A. V. A., Arulmoli, R., & Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacognosy reviews, 10*(20), 84.
11. Parasuraman S. Pharmacological Actions of Quercetin. *Pharmacogn Rev. 2016;10*(20):84–89.
12. Lakshmi, V. J. (2021). Syamala. R, Santhosh Kumar Ranajit. A Review On Pharmacological Effects Of Bilwa: Aegle Marmelos L.(Rutaceae). *Am. J. PharmTech Res, 11*(2).
13. Gautam, S., Ishrat, N., Singh, R., Narender, T., & Srivastava, A. K. (2015). Aegeline from Aegle marmelos stimulates glucose transport via Akt and Rac1 signaling, and contributes to a cytoskeletal rearrangement through PI3K/Rac1. *European journal of pharmacology, 762, 4*19-429.
14. Lai, P. B., Zhang, L., & Yang, L. Y. (2012). Quercetin ameliorates diabetic nephropathy by reducing the expressions of transforming growth factor-β1 and connective tissue growth factor in streptozotocin-induced diabetic rats. *Renal failure, 34*(1), 83-87.
15. Rahmani, A. H., Alsahli, M. A., Khan, A. A., & Almatroodi, S. A. (2023). Quercetin, a plant flavonol attenuates diabetic complications, renal tissue damage, renal oxidative stress and inflammation in streptozotocin-induced diabetic rats. *Metabolites, 13*(1), 130.
16. Ansari, P., Choudhury, S. T., Seidel, V., Rahman, A. B., Aziz, M. A., Richi, A. E., ... & Abdel-Wahab, Y. H. (2022). Therapeutic potential of quercetin in the management of type-2 diabetes mellitus. *Life, 12*(8), 1146.
17. 14a. Abuohashish, H. M., Al-Rejaie, S. S., Al-Hosaini, K. A., Parmar, M. Y., & Ahmed, M. M. (2013). Alleviating effects of morin against experimentally-induced diabetic osteopenia. *Diabetology & metabolic syndrome, 5,* 1-8.
18. Yang, D., Wang, T., Long, M., & Li, P. (2020). Quercetin: its main pharmacological activity and potential application in clinical medicine. *Oxidative Medicine and Cellular Longevity, 2020*(1), 8825387.
19. Baliga, M. S., Bhat, H. P., Joseph, N., & Fazal, F. (2011). Phytochemistry and medicinal uses of the bael fruit (Aegle marmelos Correa): A concise review. *Food Research International, 44*(7), 1768-1775.
20. Arulmoli R et al. Protective action of flavonoids in DN. J Mol Cell Biochem. 2022;476:345–359.
21. Sati N et al. Aegle marmelos: A functional nutraceutical. J Funct Foods. 2021;82:104464.
22. Singh, A., Srinivasan, A. K., Chakrapani, L. N., & Kalaiselvi, P. (2019). LOX-1, the Common Therapeutic Target in Hypercholesterolemia: A New Perspective of Antiatherosclerotic Action of Aegeline. *Oxidative medicine and cellular longevity, 2019*(1), 8285730.