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DIETARY POLYPHENOL COMBINATION POTENTIATES ANTIHYPERGLYCEMIC EFFECT OF GLIMEPIRIDE IN EXPERIMENTALLY INDUCED DIABETES IN RATS

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Abstract

Diabetes mellitus (DM) is one of the leading causes of morbidity and mortality all over the world. The application of a traditional system of medicine in the treatment of DM is having a boom in recent years. The use of phytochemicals like quercetin, resveratrol, gallocatechin, rutin, gallic acid, and genestin has a beneficial effect in the management of the severity of DM and related complications and the combined effect of these phytochemicals is need to be evaluated. In present research antidiabetic activity of quercetin (Q) and resveratrol (R) alone and in combination were tested in the presence and absence of modern antidiabetic agents on the alloxan-induced DM and associated complications. Alloxan monohydrated (120 mg/kg, i. p.) being used as an inducer for DM in experimental animals and treatment of quercetin (50 mg/kg, p.o.) and resveratrol (50 mg/kg, p.o.) alone and combination (1:1) was given for 21 days; glimepiride (0.09 mg/kg, p.o.) was used as standard antidiabetic agents for the present research. Biochemical parameters such as plasma glucose, serum lipid profile, creatinine, total protein, and albumin; liver glycogen content, and morphological parameters such as body weight were evaluated. Plasma glucose was evaluated on days 0, 7, 14, and day 21, and other biochemical and morphological parameters were measured at the start and the end of treatment respectively. Treatment with quercetin (Q) and resveratrol (R) alone and in combination showed a significant reduction in the levels elevated levels of plasma glucose, dyslipidemia, altered kidney parameters with improvement in the body weight and glycogen content in the liver. Whereas; resveratrol-quercetin (RQ) in combination with glimepiride depicted a significant ($p<0.001$) decline in plasma glucose, dyslipidemia, altered kidney function, and improvement in body weight and liver glycogen content when compared with untreated diabetic animals.Phytoconstituents such as quercetin and resveratrol possess prominent antidiabetic activity owing to their antidiabetic mechanism of glucose utilization and insulin secretagogue-like activity. The combination of quercetin and resveratrol was highly beneficial as a supportive treatment for diabetes and associated complication.

Keywords: Diabetes, Plasma glucose, Quercetin, Resveratrol

1. Introduction:

Diabetes mellitus (DM) is one of the epidemics of public fitness, troubles for the duration of an arena [1]. The incidence charge of diabetes is growing exponentially, and the World Health

Organization predicts that by the year 2030, diabetes is predicted to be the seventh leading cause of death globally [2,3]. DM is a metabolic disorder characterized by the elevation of blood glucose either may be due to decreased insulin secretion or impaired action or both [4].

Since ancient times natural medicines having an essential function in human health care. Plants were traditionally been used to combat sicknesses inside the clinical traditions of different cultures. Therefore, no longer surprisingly many modern medicines constitute plant-derived substances for treating Type 2 diabetes (T2D), inclusive of acarbose, andrographolide, and galegine, which contributed to the invention of biguanides. With so many successful facts, the advantages of natural sources possess a discovery spotlight on biodiversity of plant resources with structural and chemical variety, drug-likeness and biological friendliness, biocompatibility, and biological validation for chemical changes to optimize potency. The biodiversity of sources of herbal products from wealthy ecosystems includes vegetation, fungi, micro-organism, algae, animals, minerals, and their metabolites, which give a unique and renewable resource for the discovery of abilities of new polyphenols with novel bioactivities [5].

Polyphenol utilization is generally connected with the use in certain metabolic and obesity-related issues. Quercetin is a polyphenolic flavonoid found in leafy foods, alongside tomatoes, apples, onions, broccoli, and berries. It has an enormous collection of organic exercises and wellness advancing outcomes, along with hostile to cancer-causing [6], antiviral [7], cell reinforcement [8], antidiabetic [9], calming [10], against developing old [11] and angioprotective properties. Quercetin supplementation has known for its beneficial effect owing through reduction in plasma glucose, increasing glycogen content and stabilizing dyslipidemic conditions.Further; quercetin helps to reduce the oxidative stress induced damage towards the pancreatic beta cell and restored the pancreas to secrete adequate insulin in diabetic conditions [12].

Resveratrol also possess glucose utilizing ability in skeletal muscle and also stimulate pancreatic insulin activity, along with these it has ability to reduced free radical induced harm to the pancreatic beta cells [13]. Many researchers explored the role of quercetin and resveratrol in combination in the treatment of various diseases and disorder. Resveratrol and quercetin in combination significantly suppresses cell multiplication and apoptosis which further helpful in the cell line studies related to malignancy [12]. Combination of such polyphenols had reported for its antitumor activity in different kinds of cancer like oral cancer, colon cancer etc. [14]. Co-treatment with resveratrol and quercetin also possesses beneficial effect in few diseases where control over angiogenesis is the prime concern [15].

Based on the literature review we propose the use of such flavonoids in combination for the treatment of diabetes and related complications in animals. The present investigation was to assess the impact of co-treatment with resveratrol-quercetin on plasma glucose and other related biochemical parameters associated for diabetic complications in alloxan-induced diabetes in experimental animals.

2. Materail and Methods

2.1 Animals

Albino Wistar rats of either sex (140-210 g) were received from S. N. Institute of Pharmacy, Pusad and housed at 22 ± 3 ^{0}C with 30% relative humidity and 12 h light and dark cycle. The animals had free access to standard pellet diet and water *ad-libitum.* The experimental protocol was approved by the institutional animal ethical committee (SNIOP/CPCSEA/IAEC/CP-PL/20/2022).

2.2 Acute oral toxicity study

The acute oral toxicity of resveratrol and quercetin was performed to establish the safety of these bioactive compounds according to the OECD guidelines (Organization for Economic Cooperation and Development) No. 423 [16,17]. Three female Wistar rats were used for each step. A test at each dose level was carried out with three animals (n=3 / step). For assessing the acute toxicity of resveratrol, quercetin, and resveratrol-quercetin combination (1:1) fixed-dose level was used likewise 5, 50, 300, and 2000 mg/kg body weight and observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 h, with special attention was given during the first 4 h, (lacrimation, salivation, tremor, lethargy, and diarrhea) or mortality and daily thereafter for a total of 14 days.

2.3. Collection of the plant phytoconstituents

Quercetin and resveratrol were purchased from Yucca Enterprises, Mumbai and sorted in the standard condition for further use.

2.4. Induction of diabetes in rats

Pre-standardized dose (150 mg/kg i.p.) of alloxan monohydrate was given to the animals and kept for the next 24 h on a 5% glucose solution to prevent possible hypoglycemia [18]. Rats with plasma glucose >200 mg/dl were rendered diabetic and used for further study after 48h of alloxan injection.

2.5. Antidiabetic study of co-treatment of resveratrol-quercetin in alloxan-induced diabetic rats

Elevation in the plasma glucose of alloxanized animals was considered for induction of diabetes and a treatment schedule was prepared accordingly. Diabetic animals were then divided into different groups (n=6) while, plasma glucose was measured before starting the treatment, considered day 1 and at the same day treatment was started [19–21].

Assessment of blood glucose level was done on the 1st, 7th, 14th, and 21stdays of the study period. The treatment design using forty-two rats of either sex (6 normal rats and 36diabetic surviving rats) was separated into six groups (n=6). Group I: Normal control; treated with 1% gum acacia suspension (1 ml/kg, p.o.). Group II: Diabetic control; diabetic rats treated with a suspension of 1% gum acacia (1 ml/kg, p.o.). Group III: Glim; diabetic rats treated with glimepiride (0.09 mg/kg, p.o.). Group IV: Q 50; diabetic rats treated with a suspension of quercetin in 1% gum acacia (50 mg/kg, p.o.). Group V: R 50; diabetic rats treated with a suspension of resveratrol in 1% gum acacia (100 mg/kg, p.o.). Group VI: RQ 100; diabetic rats treated with suspension of resveratrol-quercetin combination (1:1) in 1% gum acacia (100 mg/kg, p.o.). Group VII: RQ 200; diabetic rats treated with a suspension of resveratrol-quercetin combination (1:1) in 1% gum acacia (200 mg/kg, p.o.). Group VIII: RQ 100 + Glim; diabetic rats treated withsuspension of resveratrol-quercetin combination (1:1) in 1% gum acacia (100 mg/kg, p.o.) + glimepiride (0.09 mg/kg, p.o.).

2.6. Evaluation of biochemical parameters

Assessment of the antidiabetic effect of co-treatment of resveratrol-quercetin was carried out by plasma glucose monitoring at 1th, 7th, 14th and 21st days of the treatment. Estimation of lipid profile, and serum protein, albumin and creatinine were performed at the end of treatment for assessment of possible changes therein using standard diagnostic kits from Crest Diagnostic, India [22].

2.7. Evaluation of liver glycogen content

To access the effect of co-treatment of resveratrol-quercetin on glucose utilizing ability in liver was assessed using evaluation of glycogen content in liver at the end of the treatment as described by Carroll [23].

2.8.Body weight changes

Assessment of change in animal body weight was carried out on the 1st, 7th, 14th, and 21st day of co-treatment of resveratrol and quercetin [24].

2.9.Histological study

Effect of nephroprotective action of co-treatment of resveratrol and quercetin was performed on the 21stday of treatment. Animals were sacrificed to isolated kidneys and stored in 10 % formalin solution for fixation and embedding in paraffin. A rotary microtome was used to cut the kidney tissues into fine sections of 5 µm thickness. Further; these sections were treated with xylene and ethanol for deparaffinization and then stained with hematoxylin and eosin and further examined microscopically.

2.10. Statistical analysis

All the results were presented as Mean ± S.E.M. Statistical significance was done using analysis of variance (ANOVA) followed by Dunnett's test with $p<0.05$ considered as statistically significant using GraphPad Prism version 9.0.0.

3. Results

3.1. Acute oral toxicity

The rats that received individual resveratrol and quercetin at 5, 50, and 300 mg/kg showed no signs of toxicity during the first 30 minutes, periodically during the first 24 h, and daily thereafter, for a total of 14 days. Furthermore; resveratrol and quercetin at a dose of 2000 mg/kg showed the toxic event in the treated animals. Two out of three dosed animals were observed a sign of toxicity referred to as moribund or mortality in animals at each step. Based on the acute oral toxicity resveratrol and quercetin individually fall in the GSH category 4 where the

LD_{50} was fall in the range of 300 to 2000 mg/kg body weight. While considering acute toxicity for resveratrol-quercetin combination (1:1) the LD_{50} was found to be 1000 mg/kg body weight. In the present study, acute toxicity profiling gives an idea about the selection of doses; 10% of LD_{50} was considered as the therapeutic dose for resveratrol-quercetin combination i.e. 100 mg/kg in equal proportion and two-dose level were selected for further screening methods (100 and 200 mg/kg, p.o.).

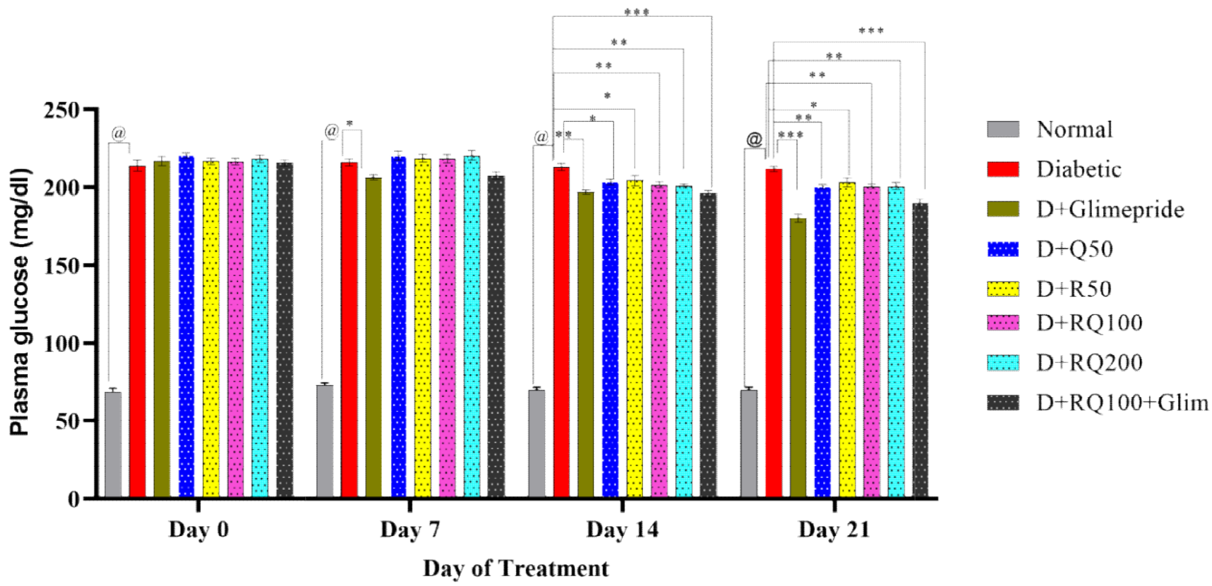
3.2. Effect of co-treatment of resveratrol-quercetin on plasma glucose

Diabetic animals treated with RQ-100 + glimepiride showed a significant fall in the plasma glucose (p<0.001) which was much more prominent in comparison to that of individual glimepiride treated animals. An equal proportion of resveratrol-quercetin in RQ-200 depicted a significant (p<0.01) reduction in the elevated plasma glucose level when compared with diabetic animals whereas RQ-100 showed marginal alteration in the plasma glucose (Table 1).

Table 1: Effect of co-treatment of resveratrol-quercetin on plasma glucose in alloxan induced diabetic animals

Day	Normal	Diabetic	D+Glimepiride	D+Q50	D+R50	D+RQ100	D+RQ200	D+RQ100 +Glim
Day 0	68.66±2.34	213.80±1.94$^{@}$	216.91±2.80	220.52±1.64	216.81±1.94	216.46±2.03	218.13±2.36	215.47±2.08
Day 7	72.82±1.44	216.01±2.52$^{@}$	206.31±1.69*	220.14±2.90	218.72±2.52	218.45±2.69	220.34±3.04	207.35±2.29
Day 14	70.02±1.58	212.90±3.18$^{@}$	196.80±1.32**	203.53±1.65*	204.43±3.18*	201.47±1.93**	200.48±1.54**	195.19±1.61***
Day 21	70.06±1.59	211.70±2.76$^{@}$	180.30±2.47***	200.55±1.48**	203.33±2.76*	200.71±2.43**	199.7±1.60**	189.26±3.00***

Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05, **p<0.01, ***p<0.001 when compared with Diabetic animals. Diabetic animals showed (p<0.001) significant increment in the plasma glucose when compared with normal control animals. Treatment with resveratrol and quercetin alone and in combination depicted a significant reduction in the plasma glucose level from day 14 onwards. Furthermore; resveratrol-quercetin co-treatment with glimepiride showed (p<0.001) a significant reduction in elevated plasma glucose when compared to that of diabetic animals.



Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05,**p<0.01,***p<0.001 when compared with Diabetic animals.

Graph 1: Effect of Co-treatment of resveratrol-quercetin on plasma glucose in alloxan induced diabetic animals

3.3.Effect of co-treatment of resveratrol-quercetin on serum lipid profile

Lipid profile was considered as a marker for diabetic cardiovascular complication; in the present study the RQ-200 showed a significant reduction in serum total cholesterol, serum triglyceride, and LDL cholesterol levels; whereas; serum HDL level was considerably increased by RQ-100 and 200 mg/kg dose level. The present finding depicted that the effect of RQ-200 over the lipid profile parameters was comparable to that of glimepiride-treated animals. Whereas; diabetic animals treated with RQ-100 + glimepiride represent promising action of RQ in association with glimepiride while considering the individual effect of either (Table 2).

Table 2: Effect of co-treatment of resveratrol-quercetin on serum lipid profile

Parameter	**Normal**	**Diabetic**	**D + Glimepiride**	**D+Q50**	**D+R50**	**D+RQ100**	**D+RQ200**	**D+RQ100+ Glim**
Total Cholest	75.76±2.404	175.9±1.558$^{@}$	102.5±2.304***	168.1±3.496	167.5±3.351	158.6±1.611***	143.3±3.208***	99.90±1.216***

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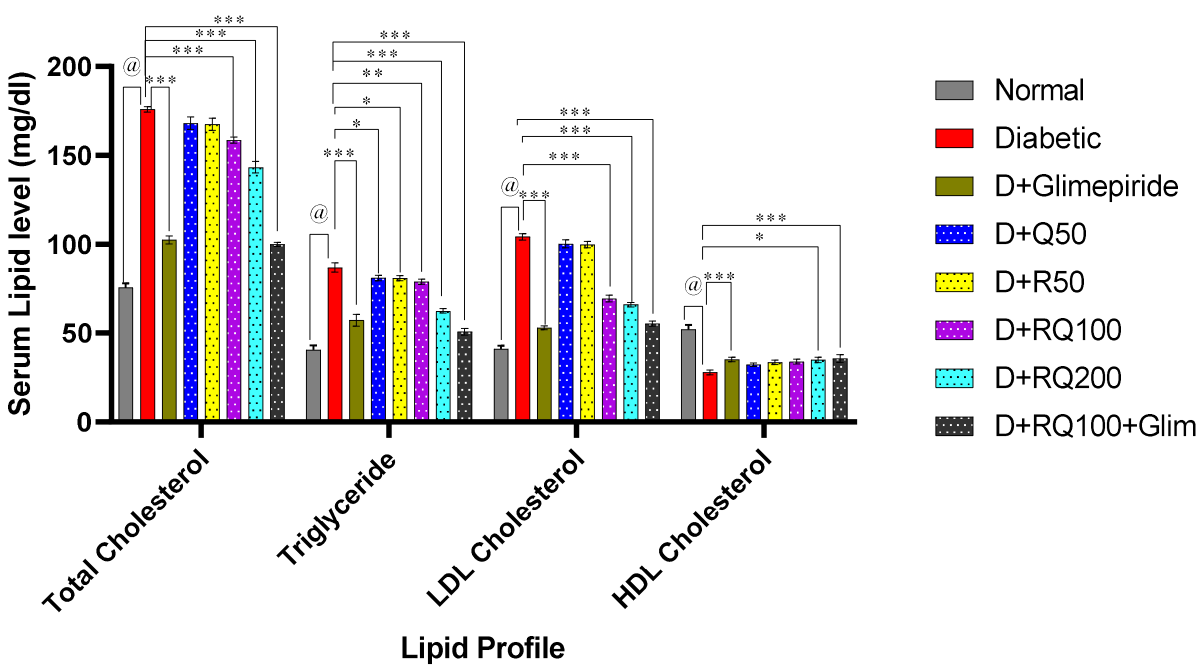
Triglyceride	40.62±1.00	87.66±2.05@	57.32±1.36***	80.50±1.57*	80.03±0.943*	70.54±0.835**	62.49±1.27***	50.82±1.84***
LDLc	41.04±1.848	104.2±1.701@	52.94±1.072***	100.3±2.268	99.81±1.795	69.48±1.921***	66.01±1.209***	55.35±1.390***
HDLc	52.07±2.430	27.93±1.345@	35.09±1.245***	32.27±1.055	33.51±1.202	33.95±1.401	34.92±1.500*	35.77±2.202***

Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @ p<0.001 when compared with Control; *p<0.05, **p<0.01, ***p<0.001 when compared with Diabetic animals. Alloxan-induced diabetic animals showed (p<0.001) a significant rise in the level of serum total cholesterol, triglyceride, LDLc, and a fall in the level of serum HDLc when compared to that normal control animals. Diabetic animals treated with resveratrol and quercetin alone and in combination showed a dose-dependent reduction in the level of serum total cholesterol, triglyceride, LDLc, and increased serum HDLc levels. Whereas; resveratrol-quercetin co-treatment with glimepiride ameliorate lipid-related complication in diabetic animals when compared to that of diabetic untreated animals.



Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05,**p<0.01,***p<0.001 when compared with Diabetic animals.

Graph 2: Effect of co-treatment of resveratrol-quercetin on serum lipid profile

3.4.Effect of co-treatment of resveratrol-quercetin on serum total protein, albumin and creatinine level

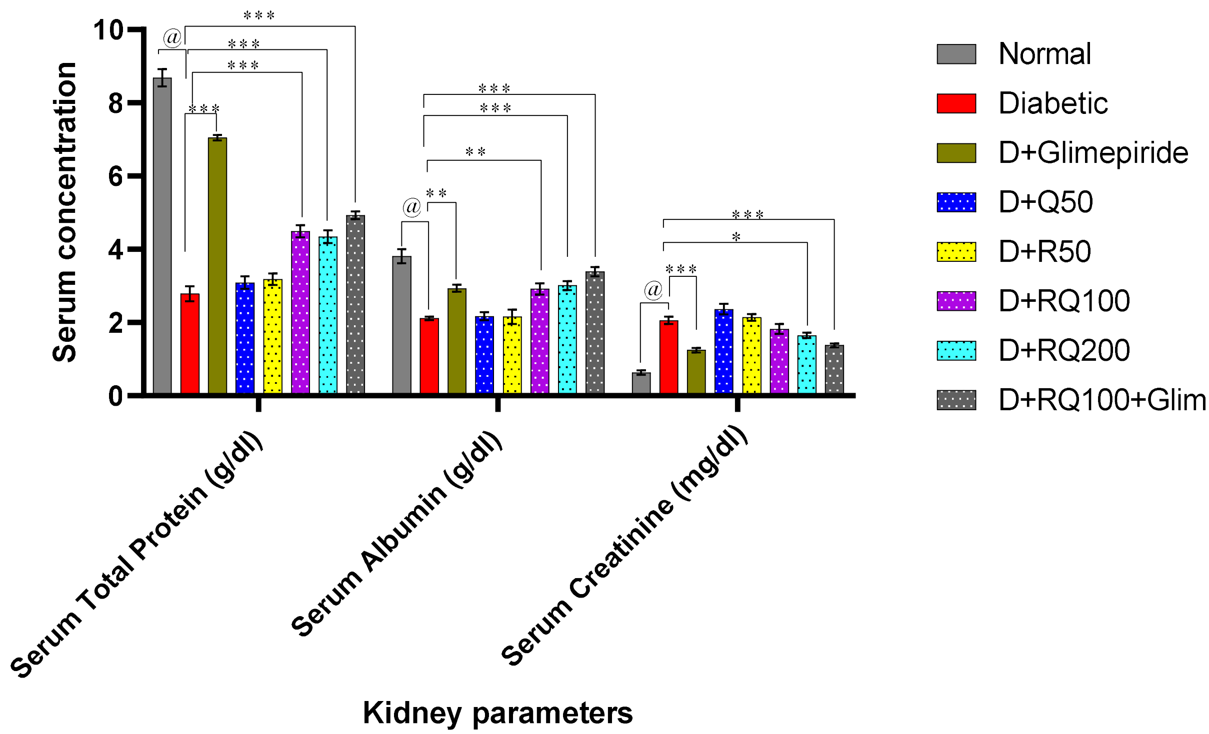
In diabetic animals, there was a significant fall in the level of serum total protein and albumin whereas rising in the level of serum creatinine when compared to that of normal control animals.

Diabetic animals treated with RQ-100 and 200 showed significant alterations in these levels when compared to that diabetic untreated animal. RQ-100 + glimepiride postulated a significant increase in the serum total protein and albumin level whereas; a significant reduction in the levels of serum creatinine when compared to that of diabetic untreated animals (Table 3).

Table 3: Effect of co-treatment of resveratrol-quercetin on serum total protein, albumin and creatinine level

Day	Normal	Diabetic	D+ Glimepiride	D+Q50	D+R50	D+RQ100	D+RQ200	D+RQ100 +Glim
Total Protein	8.683±0.235	2.785±0.201$^{@}$	7.045±0.0716***	3.089±0.169	3.177±0.158	4.490±0.166***	4.347±0.176***	4.927±0.102***
Albumin	3.810±0.190	2.115±0.050$^{@}$	2.932±0.097**	2.172±0.106	2.157±0.200	2.918±0.152**	3.010±0.123***	3.387±0.128***
Creatinine	0.6267±0.058	2.057±0.101$^{@}$	1.243±0.062***	2.362±0.141	2.138±0.091	1.823±0.132	1.647±0.0749*	1.377±0.053***

Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05, **p<0.01, ***p<0.001 when compared with Diabetic animals. In nephropathy-related complications in diabetic animals decrease in total protein and albumin were observed whereas an increase in serum creatinine levels. Resveratrol-quercetin combination helps to alter the nephropathic complication by significant increment (p<0.001) in the level of total protein and albumin, further reduction in the level of (p<0.05) serum creatinine. Co-treatment of Resveratrol-quercetin with glimepiride reverses the diabetic alteration in kidney function test when compared to that of diabetic animals.



Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05,**p<0.01,***p<0.001 when compared with Diabetic animals

Graph 3: Effect of co-treatment of resveratrol-quercetin on serum total protein, albumin and creatinine level

3.5.Effect of co-treatment of resveratrol-quercetin on change in body weight

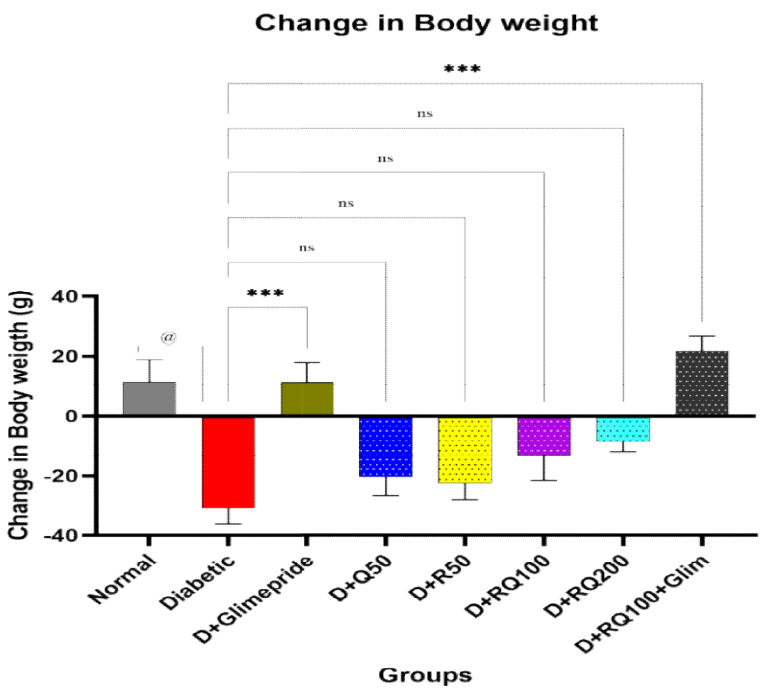
Untreated diabetic animals showed considerable fall (p<0.001) in the body weight due to disturbed glycemic control, whereas; diabetic animals treated with RQ-100+glimepiride depicted positive alteration (p<0.001) in the bodyweight when compared to that diabetic animal. RQ 100 and 200 also protected diabetic animals from a possible reduction in body weight which was comparable to that of standard antidiabetic drugs i. e. glimepiride treated animals (Table 4).

Table 4: Effect of co-treatment of resveratrol-quercetin on change in body weight

Groups	Change in body weight (g)
Normal	11.50±7.438
Diabetic	-30.83±5.275$^{@}$
D+ Glimepiride	11.33±6.561***
D+Q50	-20.33±6.381

D+R50	-22.50±5.548
D+RQ100	-13.17±8.340
D+RQ200	-8.500±3.314
D+RQ100+Glim	21.67±5.213***

Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05, **p<0.01, ***p<0.001 when compared with Diabetic animals. Due to disturbed glycemic control diabetic animals depicted significant reduction in body weight where as Co-treatment of Resveratrol-quercetin with glimepiride showed significant improvement (p<0.001) in the body weight of diabetic animals. Resveratrol-quercetin also showed improvement in the change in body weight owing through the glycemic control.



Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05,**p<0.01,***p<0.001 when compared with Diabetic animals.

Graph 4: Effect of co-treatment of resveratrol-quercetin on change in body weight

3.6.Effect of co-treatment of resveratrol-quercetin on liver glycogen content

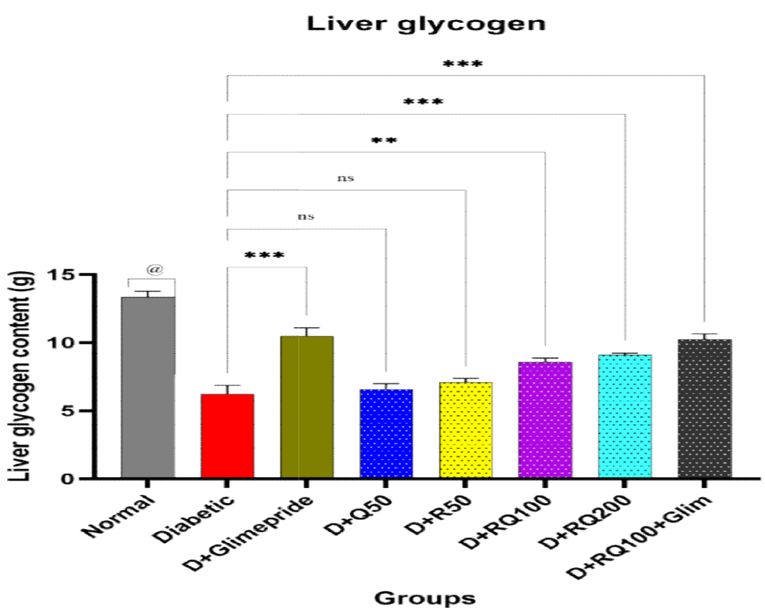
In diabetic animals' disturbance in the carbohydrate, protein and fat metabolism responsible for considerable reduction in liver glycogen content where as Co-treatment of RQ-100 with glimepiride showed significant improvement (p<0.001) in the liver glycogen in diabetic animals. Resveratrol-quercetin also showed improvement in the liver glycogen content owing through the glycemic control (Table 5).

Table5*:*Effect of co-treatment of resveratrol-quercetin on liver glycogen content

Groups	Liver glycogen (mg/100g)

Normal	13.35±0.4298
Diabetic	6.236±0.6320@
D+Glimepiride	10.46±0.6309***
D+Q50	6.576±0.4062
D+R50	7.088±0.2723
D+RQ100	8.592±0.2786**
D+RQ200	9.105±0.1510***
D+RQ100+Glim	10.24±0.3918***

Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05, **p<0.01, ***p<0.001 when compared with Diabetic animals. Due to disturbed glycemic control diabetic animals depicted significant reduction in liver glycogen content where as Co-treatment of Resveratrol-quercetin with glimepiride showed significant improvement (p<0.001) in the liver glycogen in diabetic animals. Resveratrol-quercetin also showed improvement in the liver glycogen content owing through the glycemic control.

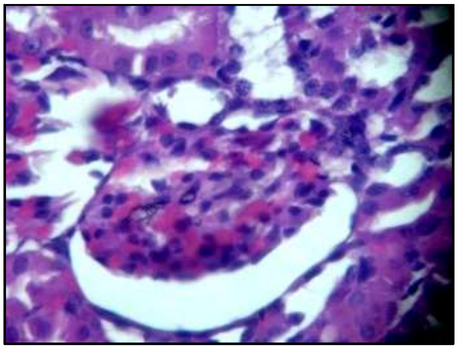


Values were presented as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. @p<0.001 when compared with Control; *p<0.05,**p<0.01,***p<0.001 when compared with Diabetic animals.

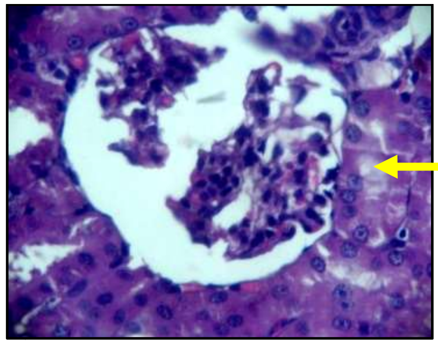
Graph 5: Effect of co-treatment of resveratrol-quercetin on liver glycogen content

Table 6: Effect of co-treatment of resveratrol-quercetin on kidney histology

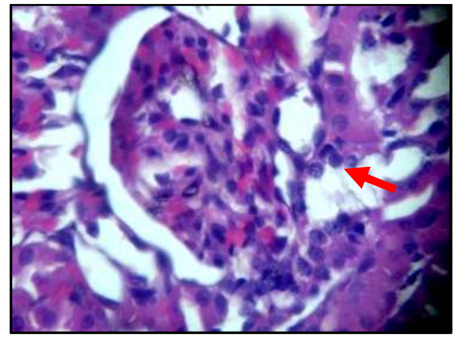
Control **Diabetic** **D + Glimepiride**



Photomicrograph ofControl group kidney showingthe normal structure of glomerulus (H & E 100X).

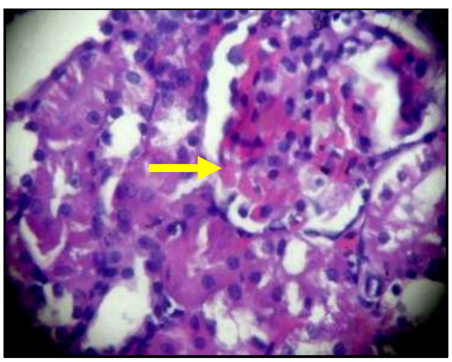


Diabetic control group kidneyshowing significant mark ofglomerulosclerosis (nephritis) and Hyalinization.Glomerular capillaries and tubular epithelium was affected (arrowhead) (H & E 100X)



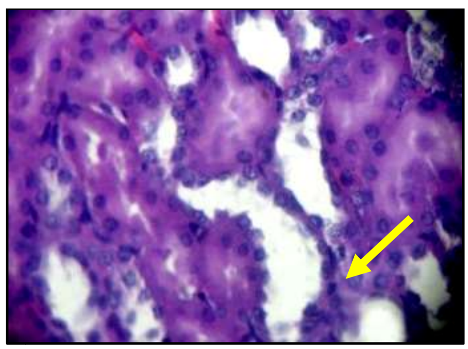
Photomicrograph ofD + Glimepiride: The histological features are relatively improved compared to the non-treated diabetic group. The glomerular capillaries showed normal size (arrow). (H & E 100X)

D+Q50



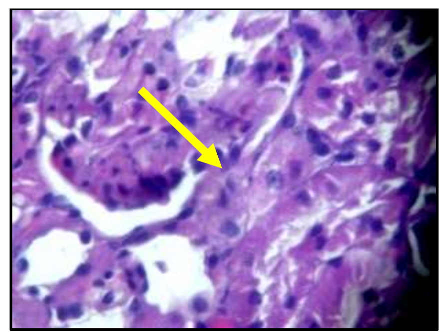
Photomicrograph of D + Q50 group kidneyshowinga little mark of glomerulosclerosis (nephritis)and Hyalinization (H & E 100X) (arrow).

D+R50



Photomicrograph ofD + R50 group kidney showingasignificant mark of glomerulosclerosis(nephritis) and mild hyalinization(H & E 100X)(arrow).

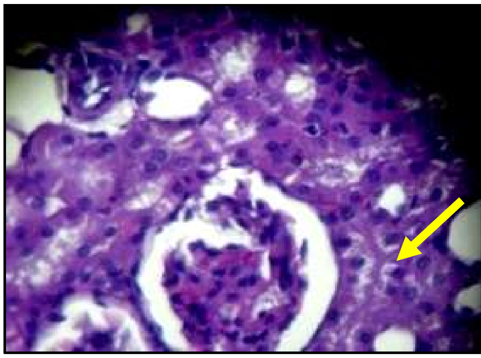
D+RQ100



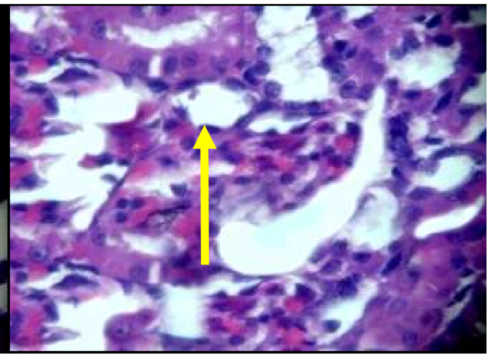
Photomicrograph of D + RQ100 group kidney showing significant decline in glomerulosclerosis (nephritis) but no sign of hyalinization. (H & E 100X) (arrow).

D+RQ200

D+RQ100 + Glim



Photomicrograph ofD + RQ200 group kidney showingvery mild glomerulosclerosis(nephritis) but no sign of hyalinization. (H & E 100X)(arrow).



Photomicrograph of D+ RQ100+ Glim group kidney showing structure of glomerulus close towards normalization. (H & E 100X) (arrow).

4. Discussion

Diabetes is the most common well-being concern worldwide and its frequency is expanding at a high rate, bringing about huge social expenses. DM is a metabolic issue described by the annihilation of pancreatic cells or decreased insulin emission. Obesity causes the improvement of metabolic issues like DM, hypertension, cardiovascular sicknesses, and inflammation-based pathologies [25]. Secondary metabolite in the plant constituents possesses a promising role in the treatment of various diseases and disorders; one of the constituents is flavonoids which is a 15-carbon skeleton structure containing two phenyl rings and a heterocyclic ring. Flavonoids have additionally been considered as wellbeing advancing specialists with demonstrated in vitro and in vivo effects, which incorporate nephroprotective, antibacterial, free radical scavenging action, anticancer, pain-relieving and anti-inflammatory potential.

More than 5000 unique flavonoids have been separated and distinguished from plant sources; these compounds are widely dispersed in the plant kingdom, especially in photosynthesizing plant cells [26]. Flavonoids are an assorted gathering of polyphenolic compounds fundamentally known as pigments creating the many types of shading in plants, flowers, and fruits. These polyphenolic compounds were notable for their therapeutic properties in well-being in humans for treating various diseases and disorders.

In several metabolic disorders, such as cardiovascular disease, cancer, obesity, and diabetes flavonoids depicted several affirmative health benefits [26]. In the management of free radical-induced oxidative stress flavonoids possesses an important role by neutralizing nitrogen and oxygen-free radical species, thus preventing the disease progression [27]. Flavonoids targets various pathways that regulate beta cell proliferation, improved insulin secretion, and signaling, suppress cell apoptosis, and improve glycemic control through the regulation of carbohydrate, protein, and fat metabolism [28,29].

Alloxan induced diabetic animals treated with individual resveratrol and quercetin have shown to have ameliorative effects on type 2 diabetes through significant improvement in glycemic control through a reduction in plasma glucose, serum total cholesterol, triglyceride, LDLc and improvement in serum HDL cholesterol; further; kidney-related parameters also improved by treatment. While considering diabetic complications related to change in body weight and liver glycogen content were significantly improved by resveratrol and quercetin treatment. In type 2 diabetes treatments with resveratrol were reported for its anti-diabetic activity owing to its radical scavenging assay [30]. Whereas, quercetin treatment in alloxan-induced diabetic animals is known to reduce elevated plasma glucose and other diabetic-related biochemical parameters, such as serum lipid- parameters and liver gluconeogenesis [31].

A combination of resveratrol-quercetin depicted a significant reduction in plasma glucose and lipid-related biochemical parameters along with significant improvement in kidney function tests [32]. The co-treatment of resveratrol-quercetin reported for significant alteration of glucose/lipid metabolism owing to modification in the other diabetic complications, which were altered by alloxan-induced diabetes [33].

In the present research, alloxan is a diabetogenic agent responsible for the release of insulin from pancreatic islets for a short time and decreasing beta-cell responsiveness towards blood glucose. Alloxan is a known diabetogenic agent cause pancreatic beta-cell damage by the production of reactive oxygen species also damages DNA structure furthermore; alloxan is responsible for the rise in the levels of intracellular calcium to increase the depletion of store insulin from islets [34,35] this could be the possible mechanism for induction of diabetes in an experimental animal by elevating plasma glucose (Graph 1).

Diabetic animals treated with a resveratrol-quercetin combination where resveratrol is already known for its antioxidant activity by reducing ROS production via chelation at the binding site and increasing endogenous antioxidant enzyme level [36,37]. Quercetin was also reported for its activity against free radical-induced oxidative stress and maintains a normal level of antioxidant enzymes which protect against cellular injury [38,39].

Furthermore, resveratrol-quercetin is a polyphenolic compound that possesses redox properties based on the structure as it contains phenolic hydroxyl groups and the ability to donate the electron to stabilize the oxidative stress condition [40]. Therefore, the resveratrol-quercetin combination helps restore the concentration of endogenous antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, catalase, which protect pancreatic beta cells from oxidative damage by ROS, this could be the possible mechanism of resveratrol-quercetin combination to prevent alloxan-induced hyperglycemia in rats [41,42].

Besides the antioxidant property of resveratrol-quercetin they also help to maintain glycemic control in diabetic animals [43–45]. Resveratrol-quercetin in combination showed an increment in insulin secretion and was sensitively to participate in the reduction of plasma glucose in diabetic animals [46,47]. Furthermore, resveratrol-quercetin co-treatment with glimepiride also depicted promising results by reducing plasma glucose as glimepiride is known for its anti-diabetic effect through insulin secretion and modulating insulin sensitivity.

Uncontrolled diabetes may responsible for alteration in the lipid metabolism and possible changes in the serum lipid biomarkers. In the present investigation, alloxan-induced diabetes causes an increment in the serum total cholesterol, triglyceride, LDL, and a fall in the level of serum HDL cholesterol. Whereas; treatment with resveratrol; quercetin alone and in combination significantly ameliorate the diabetic condition by reducing the levels of serum lipid biomarkers and improvement in serum HDLc levels. Resveratrol in normal rats reduces lipid production from glucose in adipocytes in the presence or absence of insulin was already reported [48]. Several studies justify the role of quercetin in lipid management among them some create controversial data. Various studies deny the effective role of quercetin in lipid management [33,49]. In contrast, supplementation of a quercetin-rich diet alters total cholesterol level and serum concentrations of HDLc [50,51], which justifies the effectiveness of quercetin in the present findings (Graph 2).

Diabetic animals depicted alteration in the kidney-related parameters as aincreased level of serum creatinine and a decrease in the levels of serum total protein and albumin this could be the possible nephrotoxic effect of alloxan. Treatment with resveratrol and quercetin showed significant modification in the diabetic condition individually and in combination owing to an increment in serum total protein and albumin whereas; serum creatinine was significantly reduced (Graph 3). Quercetin was reported for its nephroprotective action in the kidney by altering the oxidative stress condition and prevention of the release of renal biomarkers in the blood [52]. Histological studies confirmed the changes in kidney morphology as it gets altered by administration of alloxan, and reversal in the altered kidney architecture was seen in the animals co-treated with resveratrol-quercetin (Table 6).

Resveratrol was reported for its nephroprotective action by reducing intracellular ROS levels by the NADP oxidase in high glucose-treated human tubular epithelial cells (HK2) [53]; along with that resveratrol ameliorate diabetic nephropathy like condition be reducing IL-1beta and cell apoptosis in acute kidney injury[54] which justifies the present finding that resveratrol-quercetin combination regulates oxidative response and showed protective action against alloxan-induced kidney damage. Furthermore; the RQ-glim combination depicted a beneficial effect over alloxan-induced diabetic nephropathic conditions.

The most common finding in uncontrolled diabetes is changes in body weight that may be due to decreased control over blood glucose further causing altered metabolism of carbohydrates, proteins, and fats that could be responsible for a fall in body weight in diabetic animals [55]. In the present study treatment of resveratrol-quercetin combination significantly improves the glycemic control and reduction in protein catabolism which was disturbed by alloxan administration; further RQ combination helps to regain the metabolic loss owing to improvement in the bodyweight of diabetic animals (Graph 4) [56].

In diabetic animals altered glucose homeostasis leads to altered metabolism of carbohydrates, protein, and fats which was confirmed by a reduction in the liver's glycogen content in alloxanized rats. Treatment with resveratrol-quercetin combination significantly improves the glycemic control that helps to increase the liver glycogen content, further RQ along with glimepiride is also responsible for the improvement in liver glycogen content as glimepiride was known insulin secretagogues that increase glucose uptake in liver and skeletal muscle (Graph 5)[57,58].

5. Conclusions

In the present study resveratrol, quercetin alone, and in combination were assessed for their antidiabetic potential in alloxan-induced diabetes in experimental animals. Also, the resveratrol-quercetin-glimepiride co-treatment was evaluated for its beneficial effect over known antidiabetic agents. Resveratrol-quercetin combination depicted significant improvement in glycemic control owing to a reduction in plasma glucose in diabetic animals. Considering diabetic cardiovascular-related parameters; the resveratrol-quercetin combination significantly reduced serum total cholesterol; triglyceride and LDLc levels while improvement in HDLc levels. Serum total protein, albumin, and creatinine are the beneficial biomarker that identifies the health of the Kidney; in the present study treatment with a resveratrol-quercetin combination significantly alters the kidney function-related parameters towards normalization. Liver glycogen and bodyweight of the diabetic animals were identified to assess the beneficial effect of the resveratrol-quercetin combination where they help to improve liver glycogen content and body weight of diabetic animals.

Overall, resveratrol-quercetin combination was advantageous in the treatment of diabetes and its complication such as nephropathy and hyperlipidemia support its claim as a possible alternative or as an adjuvant therapy in diabetes.

6. List of abbreviations

DM: Diabetes mellitus

HDLs: High density lipoproteins

IL-1beta: Interleukin 1 beta

LDLs: Low density lipoproteins

NADP: Nicotinamide adenine dinucleotide phosphate

OECD: Organization for Economic Cooperation and development

TCA: Trichloroacetic acid

Q: Quercetin

R: Resveratrol

ROS: Reactive oxygen species

RQ: Resveratrol-quercetin combination

7. Declarations

7.1. Ethics approval

All the experimental animals were procured from and study protocol was approved by the Institutional animal ethical committee (Approval No.: SNIOP/CPCSEA/IAEC/CP-PL/20/2022, dated 18-04-2022), animal care and handling were followed as per the CPCSEA guidelines from ministry of animal husbandry in India.

7.2. Consent for publication

Not Applicable

7.3.Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

7.4.Competing interests

The authors declare that they have no competing interests.

7.5.Funding

This research did not receive any specific grant from funding agencies in the public, commercial, ornot-for-profit sectors.

7.6.Author's contributions

DSM carried out all experimental work, analysis and interpretation of study results, and inscribed the major part of manuscript. SCD was associated in supervising and advising experimental work. All authors go through the manuscript in detail and approved the final manuscript.

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