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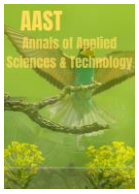

Type of the Paper (Research Article)

Ameliorative effect of Quercetin and Alpha-lipoic acid on insulin hormone in diabetic mice

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Abstract: Diabetes is a chronic metabolic disease and is considered as the major cause of death among the diseased persons. Antioxidants such as quercetin and alpha-lipoic acid when used in combination provide amelioration against metabolic illness. Current study aimed to evaluate the effect of quercetin and alpha lipoic acid for the treatment of diabetes in mice. For this purpose, forty mice were divided into eight groups. Diabetes was induced by using alloxan and confirmed by fasting blood glucose monitoring. Mice were sacrificed after 45 days by using cardiac puncture method. Plasma was separated and was used for the estimation of biochemical assay (Total proteins, Albumin, globulins), hormonal assay (Insulin), HOMA-IR for insulin resistance. Data was analyzed to evaluate significant difference between control and all treated groups. Total proteins decreased when compared with diabetic control and increased when compared with control group. Albumin decreased when compared with diabetic control and increased when compared with control group. Globulins decreased when compared with diabetic control and increased when compared with control group. Insulin increased significantly. HOMA-IR decreased. Both quercetin and alpha lipoic acid significantly ameliorate the diabetic symptoms caused by alloxan.

Keywords: Alloxan, Quercetin, Alpha lipoic acid, Insulin

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease which causes abnormal glucose homeostasis (Imam, 2012). It is characterized by increased blood glucose level also associated with disturbance in carbohydrates, proteins and fats metabolism. It is an autoimmune disorder resulting either from insulin secretion leading to the deficiency of pancreatic beta cells resulting in the production of diabetes mellitus type I (DM1), or by insulin resistance (IR), which alters insulin secretion and hyperglycemia, results in diabetes mellitus type II (DM2) (Wolpin et al., 2013). Harmful effects of hyperglycemia include coronary artery disease, microvascular complications like neuropathy, retinopathy and diabetic neuropathy, peripheral arterial disease (Michael and Fowler, 2018). Other complications of diabetes include depression, (Nouwen et al., 2011), dementia and sexual dysfunction (Thorve et al., 2011). In 2016, WHO estimated that 58% of all deaths are caused by non-communicable diseases (NCDs) of which diabetes is one. According to the data collected in 2016, 3% deaths are caused by diabetes (Hasan and Siddiqui, 2024). Alloxan (ALX) is 2nd most widely used drug to induce diabetes experimentally in animal models like rat, mice, rabbits and dogs etc (Etuk, 2010; Iranloye et al., 2011). Alloxan's cytotoxic action is due to reduction by interaction with intercellular glutathione (Elsner et al., 2006) which results in the formation of ROS.

In recent time, the use of alternative medicines has increased which leads to the use of herbal medicines having less adverse effects and high cure. Flavonoids are commonly used in this aspect. Alpha lipoic acid (ALA) is one of the coenzymes also has anti-diabetogenic activities. It is involved in energy

metabolism of fats, proteins and carbohydrates, mainly function in the disposal of blood glucose and clean up the free radicals. In animal tissue, ALA are found in the heart, liver, and kidney in higher amount while Brussel, broccoli, tomato, spinach, garden peas, sprout and rice bran have rich amount among plant sources. Quercetin is a flavonol compound from one of the subclass of flavonoid. Flavonol compounds are present in variety of vegetables and fruits. Among all the flavonol 13.82rag/day are in form of quercetin

Materials and Methods

Animals used

This study was conducted in the animal house of University of the Punjab Lahore. Forty albino mice weighing 25-35 grams were used. Animals were kept in clean cages provided with food in pallet form and water ad libitum.

Pharmacological agents

- 0.6ml Sodium Chloride from Otsuka Pakistan Ltd, F/4-9 H.I.T.E, Hub, Balochistan.
- Carboxymethyl cellulose (CMC) from Sigma- ALDRICH.
- Alloxan obtained from Sigma- ALDRICH, Co.
- Quercetin obtained from General Nutrition Cooperation.
- Alpha lipoic acid obtained from Puritan's Pride.

Experimental design

Animals were divided into eight groups each group having five samples (n=5).

Group I Control saline group (C): This group contained five mice which were given 0.6ml saline solution orally for 45 days.

Group II Control carboxymethyl cellulose group (CMC): This group contained five mice which were given 0.3ml of carboxymethyl cellulose orally for 45 days.

Group III Control alpha lipoic acid group (ALA): This group includes five mice were given alpha lipoic acid (150mg/kg body weight) for 45 days (Ko et al., 2021).

Group IV Control quercetin group (QR): This group includes five mice which were given of quercetin (50mg/kg body weight) for 45 days (SO et al., 2020).

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Group V Control diabetes mellitus group (DM): Diabetes was induced in mice by alloxan injection given intraperitoneally (200mg/kg body weight) for one day (Aljazzaf et al., 2023).

Group VI Treatment alpha lipoic acid group (DM + ALA): Diabetes was induced in mice by alloxan 200mg/kg body weight received alpha lipoic acid (150mg/kg body weight) for 45 days (Ko et al., 2021).

Group VII Treatment quercetin group (DM + QR): Diabetic mice received quercetin (50mg/kg body weight) for 45 days (SO et al., 2020).

(Kelly et al., 2011). Quercetin lowers the amount of glucose level in plasma in T2DM (Chen et al., 2016). Previous studies showed the diabetogenic role of quercetin (Ali et al., 2024). Quercetin and alpha lipoic acid both helps in decreasing the oxidative stress also inhibits the cell apoptosis caused by alloxan (Budin et al., 2006). The current study was intended to evaluate the effect of quercetin and alpha lipoic acid on diabetes mellitus.

Group VIII Treatment combination group (DM + ALA + QR): Diabetic mice received both quercetin (100mg/kg of body weight) and alpha lipoic acid 150mg/kg of body weight for 45 days.

Blood sampling

Blood glucose level was checked at regular intervals after two weeks till the end of protocol. Mice were euthanized and their blood sample was collected by cardiac puncture technique using heparin coated syringes from the tip of heart. Blood was centrifuged by the use of micro-centrifuge machine (WiseSpin® CF-10) at 3000rpm for 20 minutes; serum was separated which was stored at -200C until analyzed.

Biochemical estimation, Organosomatic index and HOMA-IR

- Globulins level was calculated by using the equation: Globulins = Total proteins – Albumin. Albumin test was conducted by using Crescent Diagnostics kit (Cat. No. CS. 600) kit, Total protein was conducted by using kit of Crescent Diagnostic kit (Cat. No. CS610) and Insulin (Catalog No. IN374S) was estimated.
- Organosomatic index was calculated by using organosomatic index formula: Organosomatic index = organ weight / body weight × 100.
- HOMA-IR was measured by using following formula: HOMA– IR = Fasting insulin level (microU/L) × fasting glucose (nmol/L) / 22.5.

Statistical analysis

SPSS and Microsoft Excel were used to do analysis of data. One way analysis of variance (ANOVA) followed by Tukey's and Dunnett's test was done and all results was illustrated in tabular and graphical form. A value of $p < 0.05$ considered as significant and $p < 0.001$ considered as highly significant.

Results

Body weight of control diabetic group showed non-significant increase when compared to control group. Group given ALA showed non-significant increase when compared with diabetic control group. Quercetin treated group also showed non-significant increase when compared with diabetic control group. Treatment group given alpha lipoic acid and quercetin in combination increased non-significantly when compared with diabetic control group (Table 1). Organosomatic index of control diabetic group showed significant increase when compared with control

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group. Treatment group given alpha lipoic acid showed non-significant decrease when compared with diabetic control group (Table 1). Blood glucose level of control diabetic group showed highly significant decline when compared with control group. Treatment group given alpha lipoic acid showed highly significant decrease when compared with diabetic control group. Group treated with quercetin also showed highly significant decrease when compared with diabetic control group. Treatment group given alpha lipoic acid and quercetin in combination showed highly significant decrease when compared with diabetic control group (Table 1).

Total protein of control diabetic group showed highly significant increase when compared to control group. Treatment group given alpha lipoic acid showed highly significant decrease when compared with diabetic control group. Treatment group given quercetin showed highly significant decrease when compared with diabetic control group. Treatment group given alpha lipoic acid and quercetin in combination showed highly significant decrease when compared with diabetic control group (Table 2).

Albumin level of control diabetic group showed non-significant increase when compared to control group. Treatment group which given alpha lipoic acid showed non-significant decrease when compared with diabetic group. Quercetin treated group showed non-significant decrease when compared with diabetic group. Treatment groups which given alpha lipoic acid and quercetin in combination showed non-significant decrease when compared with diabetic group (Table 2). Globulins level of control diabetic group showed significant increase when compared with control group. Treatment group given alpha lipoic acid showed significant decrease when compared with diabetic group. Treatment group given alpha lipoic acid and quercetin in combination showed significant decrease when compared with diabetic group (Table 2).

Insulin level of control diabetic group showed highly significant decrease when compared with control group. Treatment group given quercetin showed significant increase when compared with diabetic group. Treatment group given alpha lipoic acid and quercetin in combination showed significant increase when compared with diabetic group (Figure 1). HOMA-IR of control diabetic group showed significant increase when compared with control group. Treatment group given alpha lipoic acid and quercetin in combination showed significant decrease when compared with diabetic group (Figure 2).

Table No. 1. Percentage increase or decrease in body weight, blood glucose level and organosomatic index of control, diabetic and treatment groups before and after treatment for 45 days in albino male mice (n = 5)

Groups	Percentage Increase in Body weight before & after treatment	Organosomatic index	Percentage increase or decrease in blood glucose level before & after treatment
C	↑ 6.985%	0.50 ± 0.06	↓1%
CMC	↑ 7.52%	0.43 ± 0.05	↑9%
ALA	↑ 7.38%	0.39 ± 0.04	↑4%
QR	↑ 6.5%	0.41 ± 0.03	↓13%
DM	↑ 7.26%	0.76 ± 0.05*	↓26%**
DM + ALA	↑ 6.98%	0.76 ± 0.32	↓146%**/
DM + QR	↑ 6.19%	1.05 ± 0.37	↓278%**/
DM + ALA + QR	↑ 6.85%	0.70 ± 0.21	↓311%**/

C: Control; CMC: Carboxymethyl cellulose; ALA: Alpha lipoic acid; QR: Quercetin; DM: Diabetes mellitus; DM + ALA: Diabetes mellitus + alpha lipoic acid; DM + QR: Diabetes mellitus + quercetin; DM + Alpha lipoic acid + QR: Diabetes mellitus + alpha lipoic acid + quercetin

(Post-hoc Tukey's and Dunnett's test)

$p < 0.05$* = Significant, $p < 0.001$** = Highly Significant

*,** = compared with control, */,**/ = compared with diabetic control

Table No. 2. Comparison of the total proteins, albumin and globulins of control, diabetic and treatment groups after 45 days of treatment (n =5)

Groups	Total proteins (g/dL)	Albumins (g/dL)	Globulins (g/dL)
C	5.3 ± 0.16	1.66 ± 0.06	3.64 ± 0.15

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CMC	5.06 ± 0.10	1.68 ± 0.05	3.38 ± 0.12
ALA	5.04 ± 0.32	1.56 ± 0.10	3.48 ± 0.27
QR	4.5 ± 0.11	1.62 ± 0.05	2.88 ± 0.11
DM	6.98 ± 0.66**	2.0 ± 0.07	4.98 ± 0.08**
DM + ALA	4.52 ± 0.16**/	1.78 ± 0.08	2.74 ± 0.16*/
DM + QR	5.16 ± 0.12**/	1.54 ± 0.25	3.56 ± 0.32
DM + ALA + QR	4.46 ± 0.12 **/	1.86 ± 0.09	2.6 ± 0.15*/

C: Control; CMC: Carboxymethyl cellulose; ALA: Alpha lipoic acid; QR: Quercetin; DM: Diabetes mellitus; DM + ALA: Diabetes mellitus + alpha lipoic acid; DM + QR: Diabetes mellitus + quercetin; DM + Alpha lipoic acid + QR: Diabetes mellitus + alpha lipoic acid + quercetin
(Post-hoc Tukey's and Dunnett's test)
p< 0.05* = Significant, p< 0.001** = Highly Significant
*,** = compared with control, */,**/ = compared with diabetic control

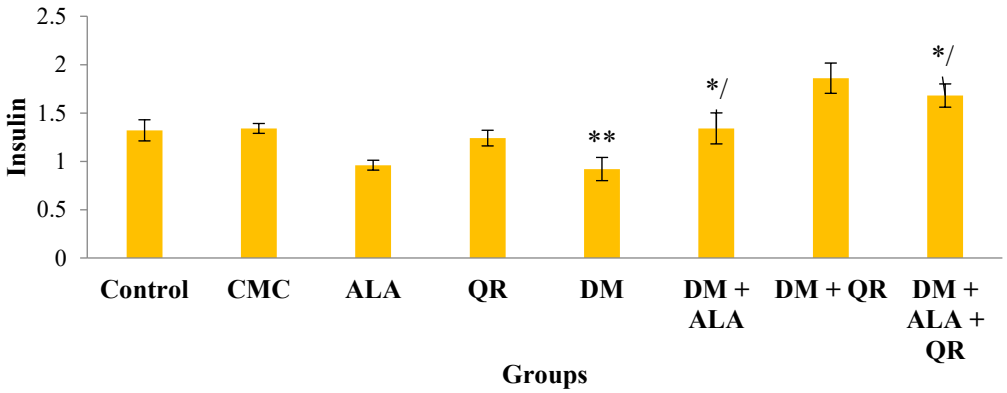

Figure No. 1. Comparison of Insulin of control, diabetic and treatment groups after 45 days of treatment in albino mice (n= 5)
C: Control; CMC: Carboxymethyl cellulose; ALA: Alpha lipoic acid; QR: Quercetin; DM: Diabetes mellitus; DM + ALA: Diabetes mellitus + alpha lipoic acid; DM + QR: Diabetes mellitus + quercetin; DM + Alpha lipoic acid + QR: Diabetes mellitus + alpha lipoic acid + quercetin
(Post-hoc Tukey's and Dunnett's test)
p< 0.05* = Significant, p< 0.001** = Highly Significant
*,** = compared with control, */,**/ = compared with diabetic control

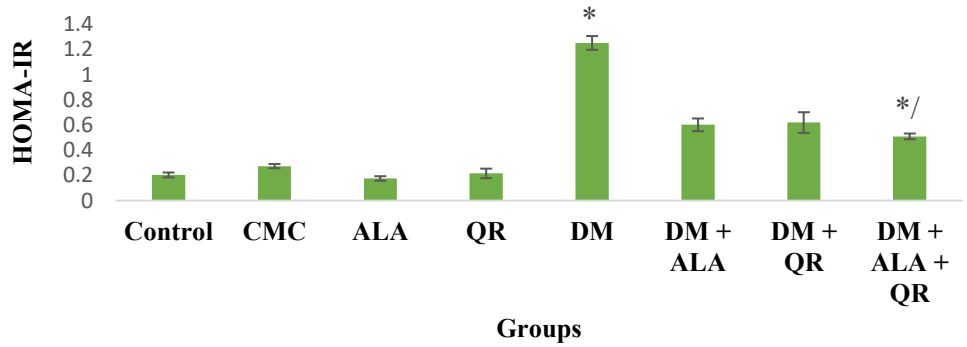

Figure No. 2. Comparison of HOMA-IR of control, diabetic and treatment groups after 45 days of treatment in albino mice (n= 5)
C: Control; CMC: Carboxymethyl cellulose; ALA: Alpha lipoic acid; QR: Quercetin; DM: Diabetes mellitus; DM + ALA: Diabetes mellitus + alpha lipoic acid; DM + QR: Diabetes mellitus + quercetin; DM + Alpha lipoic acid + QR: Diabetes mellitus + alpha lipoic acid + quercetin
(Post-hoc Tukey's and Dunnett's test)
p< 0.05* = Significant
*= compared with control, */ = compared with diabetic control

Discussion

In the current work, less elevation in the body weight has been seen in our experimental groups (ALA, QR, DM + ALA, DM + QR, DM + ALA + QR) as compared to the control diabetic group. Less increase in the body weight has been seen in QR group, same

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conclusion previously given by Miana et al., (2011). Slight increase in the body weight was documented previously in another study (Ferrazzano et al., 2009). Antioxidant lower adipose tissue synthesis, as reported in the case of diabetic group treated with alpha lipoic acid, quercetin and their co-administration in diabetic model. Borissova, et al., (2004); and Kabir, et al. (2005), also documented that weight gain play main role in causing T2DM, also reflects the development of IR. Correlation present between obesity and IR proposed that a major mediator, FFAs is secreted by adipocytes (Grundy, 2004) plays a key role in formation of ROS.

The Organosomatic index (OSI) is a measure used in biology and environmental sciences to assess the health and condition of an organ relative to the entire organism. The OSI provides valuable information about the relative size and development of organs in different physiological or environmental conditions. Diabetes can impact the beta cell's structure and function due to factors like insulin resistance. An altered OSI may indicate changes in pancreas size and health. Our research findings reveal increase and decrease in the Organosomatic index (OSI), indicating a complex and varied impact of the treatment or condition under study on different organs relative to the overall body weight. The observed variations in OSI suggest that the treatment or condition has diverse effects on various organs within the study.

In recent research work, glucose level of blood of control group was measured to be in normal range. On the other hand, in the diabetic control group, increase in the glucose level in blood was noted. During the present investigation, alloxan was used to artificially induce diabetes in albino mice, results in the elevation of glucose levels in blood. Increased level of blood glucose may be caused as a result of partial damage of the pancreatic β-cells. Alloxan, a β-cytotoxin, used to induces diabetes by destroying the insulin secreting β-cells (Jelodar et al., 2003). Similar conclusion was given by Vuksan and Sievenpiper (2005); Sharma et al., (2013) who reported, that the use of alloxan significantly elevates the level of glucose is due to the alloxan's cytotoxic effect on pancreatic beta cells. Alloxan is somehow toxic to pancreatic β- cells which produces insulin because it compiles in the β- cells through uptake via the glucose transporter-2. This cytotoxic action is carried out by the production of ROS. ROS action massively increases in the calcium concentration of cytosol, which further results in the rapid damage of beta cells, thereby decline in the secretion of insulin, as a result, increase the glucose level of blood. When the resistance of insulin is combined with pancreatic beta-cell malfunction, blood glucose levels are unable to be controlled (Kahn et al., 2006).

After the treatment of experimentally induced diabetic mice with quercetin and alpha lipoic acid, decline in blood glucose level was observed possibly because alpha lipoic acid increase the transportation of Glucose transporter type 4 (GLUT-4) in fat and muscle cell membranes it also increase glucose transport of skeletal muscles. Our findings corresponds with the findings documented by Elbadawy et al., 2021. Diabetes caused due to hyperglycemia-induced oxidative stress by pancreatic beta cells apoptosis quercetin might increase the activity of GPX4 in pancreas which results in reduction of oxidative stress which increased the production of beta cells and insulin secretion. Ansari et al., 2022 work supported our findings. Alpha lipoic acid and quercetin act synergistically and might act the same way as alpha lipoic acid and quercetin acted separately against diabetes. In the current findings, diabetic animals treated with different hypoglycemic agents showed reduction in the blood glucose level. Antioxidants (superoxide dismutase and catalase) constitute the most effective defense set-up which limits the free radical's toxicity. The oxidative stress which is caused in diabetes is combined with decline in the antioxidant status, which might increase the free radical's deleterious effect (Atli et al., 2004). A decrease in the activities of the antioxidant enzymes might result in the presence of O2 and H2O2 in excess, which further generate OH, results in the propagation and initiation of LPO. Grundy (2004) showed that the ROS production reduced the antioxidant enzyme. Impairment in the uptake of glucose in muscles and adipose tissues is because of the oxidative stress, which reduces the secretion of insulin from the pancreatic beta cells as reported by Park et al., (2009), documented that oxidative stress mediated IR and diabetes mellitus by effecting the glucose consumption and secretion of glucose.

According to the results of our model, mice who were given alpha-lipoic acid (ALA) and quercetin together gained weight. This raises the possibility that ALA and quercetin treatment may cause an elevation in body weight. In diabetic mice, the declined insulin secretion worsens hyperglycemia. Insulin resistance, in which the liver becomes less receptive to the hormone insulin, is a feature of diabetes. There is a noticeable difference between normal mice and diabetic mice in terms of their organ function and overall health. Diabetes is a type of metabolic disorder caused either due to high blood glucose levels or due to impaired insulin function or inadequate insulin production. In the context of diabetes, mice are often used as models for studying the disease and developing potential treatments. Grinberg-Bleyer et al., (2010) documented same results, who documented that diabetic mice are induced with diabetes-like symptoms, typically through genetic modifications or chemical interventions (Grinberg-Bleyer et al., 2010). The chemical combinations that we are using in our model inhibit certain enzymes involved in glucose production and increase glucose uptake in peripheral tissues, contributing to improved glycemic control.

In the present findings, serum parameter i.e., Total protein, albumin and globulin levels were low in the control diabetic group indicating the hypoprotonemia and hypoalbuminemia. Shradha and Sisodia, (2010);

documented that oxidative stress plays a main role in the development of both types of diabetes. Total proteins level in the control diabetic group increased sufficiently as compared to the control saline group. While in treatment groups, it increases a little. Our findings reveal an increase in total protein and a slight rise in total protein in the treatment groups (DM + ALA and DM + ALA + QR). Toghyani et al., (2011), also showed same conclusions, documented that rise in blood total protein and globulin concentration in birds fed diets enriched with thyme power. Globulins are thought to be the source of nearly all the proteins which are immunologically active and present in the blood (Jha et al., 2007). Because albumin is vital in the transfer of various chemicals, such as medicines, in the blood. Albumin levels did not increase much in most of the diabetic treatment groups (DM + QR) in the study, same results made by Ahmadi et al., (2012), where less increase in the albumin level was seen as compared to the other treatment groups. Globulin supposed to respond similarly to total protein, which increased significantly in all experimental groups on 15 to 30 days of sampling. Similar conclusions were documented by Ahmadi et al., (2012). Insulin level decreased in the diabetic control group. While in the treatment groups it increased to a sufficient level. Similar findings were made in diabetes research (Jeong et al., 2012). HOMA-IR is a kind of surrogate index calculated for the resistance of insulin (Muniyappa et al., 2008). The administration of antioxidant decreases the value of HOMA- IR. Similar findings were made in diabetes research on HOMA- IR (Jeong et al., 2012).

Conclusion

To conclude, the protective role of quercetin and alpha lipoic acid on insulin hormone in diabetic mice shows great promise in reversing the effect of alloxan and treating the damaged beta cells of pancreas. Recent study concludes that quercetin and alpha lipoic acid has an anti-inflammatory properties and potent antioxidant, minimizing the damage caused to pancreatic beta cells. Their ability to increase insulin sensitivity and regulate the level of glucose homeostasis offers potential benefits in managing diabetes related hyperglycemia and its effect on beta cells. Quercetin and alpha lipoic acid act synergistically and protect the pancreatic beta cells from apoptosis might increase regeneration of beta cells which showed results against diabetes.

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