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AMELIORATIVE EFFECT OF QUERCETIN ON LIPID, ENZYMATIC, HORMONAL AND BIOCHEMICAL PROFILING OF STZ-INDUCED DIABETIC RATS.

Farah Naz Tahir[1], Asma Ahmed[1]*, Gul-e-Rana[2], Khadija Kiran[3], Rehana Badar[1,4]

[1]*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Punjab, Islamic Republic of Pakistan

[2]Department of Biochemistry, Sharif Medical and Dental College, Lahore, Punjab, Islamic Republic of Pakistan

[3]Department of Physiology, Gujranwala Medical College, Gujranwala, Punjab, Pakistan

[4]Department of Biological Sciences Superior University Lahore Punjab, Islamic Republic of Pakistan

***Corresponding Author: -** Asma Ahmed

* Email: asma.ahmed@imbb.uol.edu.pk

ABSTRACT

Herbal medicine was commonly used before the development of other medicine with an increase in their scope of use each year. *Camellia sinensis* (L.) leaves important phyto-constituents like quercetin. In current study, therapeutic effects of intraperitoneally induced quercetin (10mg/mL/Kg b.w.) was checked to cure streptozotocin-induced (55mg/mL/Kg b.w.) diabetes in albino Wistar rats of both genders (300- 400 g), by keeping metformin (250mg/mL/Kg b.w.) as positive control, followed by the estimation of total lipids, cholesterol, triglycerides, HDL, LDL, VLDL, AST, ALT, ALP, , amylase, lipase, LDH ,urea, creatinine, uric acid, bilirubin, albumin, globulin, A/G ratio, total proteins, sodium, potassium, calcium, magnesium, chloride and HbA1clevels by kit methods. Statistically analyzed results (at $p \leq 0.05$) revealed that quercetin have improved the levels of cholesterol (88.25±10.47 and 47.3±3.0 mg/dL), triglycerides (46.00±5.72 and 50±8.0 mg/dL), HDL (57.50±1.29 and 26.3±2.0 mg/dL), LDL (29.75±6.95 and 16.3±1.0 mg/dL), VLDL (16.50±1.29 and 32.3±1.2 mg/dL), Urea (3.1±0.8 and 1.26±0.2 mg/dL), creatinine (0.7±0.1 and 1.26±0.2 mg/dL), uric acid (1.6±0.16 and 1,56±0.08 mg/dL), bilirubin (0.43±0.02 and 0.3±0.05 mg/dL), AST (77.14±7.0 and 59±4.5 U/L), ALT (31.2±4.8 and 34.3±2.0 U/L), ALP (85±2.5 and 56.6±2.5 U/L), albumin (2.3±0.9 and 3.9±0.08 mg/dL), globulin (3.82±0.4 and 2.82±0.4 mg/dL), A/G ratio (1.01±0.6 and 2.0±0.5 %), total proteins (4.9±0 and 3.3±0.03 mg/dL) and HbA1C (3.7±0.89 and 3.9±0,01 %) in both male and female rats respectively while sodium (138.14±6.76 mmol/L), potassium (4.5±0.33 mmol/L), calcium (9.9±1.72 mg/dL), magnesium (1.7±0.44 mmol/L), chloride (106.50±8.5 mmol/L), amylase (443.79±29.28 U/L), lipase (18.6±4.1 U/L), LDH (688.86±13.54 U/L) were same in both genders, as compared to negative control [cholesterol (95.25±10.47 and 105±7.0 mg/dL), triglycerides (87.00±5.72 and 82.3±2.0 mg/dL), HDL (57.00±5.36 and 64.3±4.0 mg/dL), LDL (28.75±5.56 and 25.3±1.0 mg/dL), VLDL (13.25±1.71 and 12.6±0.5 U/L), Urea (80±6.0 and 67.5±3.8 mg/dL), creatinine (1.31±0.1 and 0.8±0.02 mg/dL), uric acid (1.6±0.10 ad 1.8±0.1 mg/dL), bilirubin (2.06±0.3 and 1.26±0.05 mg/dL), AST (199.83±8.3 and 155.5±5 U/L), ALT (72.3±5.6 and 77.1±4.8 U/L), ALP (95±3 and 89±6.3 U/L), albumin (2.9±0.5 and 3.36±0.04 mg/dL), globulin (1.03±0.03 and 2.4±0.2 mg/dL), total protein (6.56±0.7 and 6.64±2 mg/dL), A/G

ratio (1.1±0.1 % in both), sodium (144.14±8.76 and 132.6±12 mmol/L), potassium (7.25±3.38 and 5.9±0.03 mmol/L), calcium (9.25±1.7 and 10.5±4.0 mmol/L), magnesium (4.01±0.448 and 2.5±0.2 mmol/L), chloride (101.50±0.58 and 106±10 mmol/L), amylase (312.6±10.28 and 418±13 U/L), lipase (180± 9.2 and 29.4±4.0 U/L), LDH (1654±164 and 1771±98 U/L) and HbA1c (4.4±0.29 and 3.2±0.4%)] while effect of quercetin extract was almost similar to positive control drug or metformin. Histopathological analysis showed recovery of renal, hepatic, and pancreatic tissues, along with a reduction in mononuclear cell infiltrate and improvement in steatosis in the liver, while pancreas showed mild restoration of pancreatic beta cells. Current results can be used for the isolation and hepato-renal safety profiling of quercetin from *C. sinesnsis* (L.) via its bioactivity guided isolation.

Keywords: *Camellia sinensis* (L.), Quercetin, diabetic hyperlipidemia, hepato-renal and pancreatic protective, electrolytes, inflammatory markers.

INTRODUCTION

Many medicinal plants have been used traditionally in the management of diseases like diabetic hyperlipidemia, diabetes mellitus, and its hepatic, renal and pancreatic complication along with management of inflammatory markers that are raised in uncontrolled diabetes due to damage in the pancreatic beta cells (Yakubu et al. 2007) in both developed and developing countries due to their higher efficacy, fewer side effects and very less toxicity (Amalraj et al. 2017; Orekhov et al., 2017). Hyperlipidemia is a group of disorders characterized by abnormally increased levels of cholesterol, triglyceride, LDL, and VLDL in the blood and a decrease in HDL-C. It may be due to the abnormal metabolism of fats that results in the overproduction of lipoproteins or defects in various stages of their degradation (Sithu et al.2017). Predisposing risk factors for the development of hyperlipidemia, and hyperglycemia includes physical inactivity, obesity or overweight, low HDL cholesterol, cigarette smoking, diabetes mellitus and hypertension. These risk factors if remain uncontrolled lead to the development of cardiovascular diseaseas well (Ullah et al. 2017).

Current drug therapy available for the management of diabetic hyperlipidemia is associated with severe adverse effects, development of more resistance and increased levels of HbA1c (Barrios et al.2017), while diabetes itself is one of the most alarming disease regarding health issues on a worldwide basis as most common complications due to uncontrolled diabetes are retino- nephro and neuropathy, cardiovascular (CVD), cerebrovascular (CVA), and peripheral vascular disease (PVD), which causes morbidity and mortality all over the world. Current treatment option available for diabetes is metformin and is considered as one of the first-line agents for the treatment of type 2 diabetes. But this drug also causes hyperlipidemia in long-term use. Chronic inflammation is also associated with hyperglycemia, hyperlipidemia, hypertension, and pancreatic damage by releasing many inflammatory markers along with serum amylase, lipase, and LDH, all of which further worsen insulin resistance and lead to the development of metabolic disorders (Zhang et al.2022). Abnormal glucose levels also disturb electrolyte levels, thus effects acid-base balance (Mao et al. 2022), and this electrolytes distribution leads to hyperglycemia-induced osmotic fluid shifts or of total-body deficits due to osmotic diuresis. Complications from end-organ injury and therapies used in the management of diabetes may also contribute to electrolyte disturbances. Increased levels of plasma glucose changes sodium concentration by the movement of water from the intracellular space to the extracellular space, thereby diluting the extracellular concentration of sodium and thus causes plasma tonicity. Increased plasma sodium concentrations in the presence of hyperglycemia indicate a clinically significant deficit in total body water (Goguen et. al 2013).

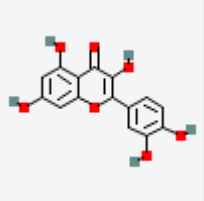
Diabetes is also linked with increased concentration of potassium. In patients with type 2 diabetes, the insulin-mediated uptake of glucose is impaired, but the cellular uptake of potassium remains normal. Efflux of potassium from the cell is due to intracellular dehydration, which results from the administration of dextrose in water as a short-term therapy for hyperkalemia, while absence of concomitant administration of insulin worsen hyperkalemia in patients with diabetes, since the

endogenous secretion of insulin in these patients may be insufficient or unpredictable and may thereby result in increases in plasma tonicity (Yasir et.al.2022). So multi-drug treatments through allopathic and medicinal plants become essential in medical research. A number of natural medicine when taken with allopathic medicine show synergetic effects (Sial et al.2021).Quercetin is one of the major and important tea polyphenols which is present in tea(*Camellia sinensis* L.) and is associated with hypolipidemia, hypoglycemia, and treatment of metabolic syndrome due to its strong antioxidant tumor-suppressive, scar de-pigmentation, hepato protection, platelet aggregation and thrombus formation inhibition, suppression in immune system, anti-inflammatory, anticholinergic, protective for nervous system, hyperuricemic, antibacterial, and antifungal properties (Shams-Ghahfarokhi et al.2018).

MATERIAL AND METHODS

In current study, therapeutic effects of intraperitoneally induced quercetin (10mg/mL/Kg b.w.) was checked to cure streptozotocin-induced (55 mg/mL/Kg b.w.) diabetes in albino Wistar rats of both genders (300- 400 g), by keeping metformin (250 mg/mL/Kg b.w.) as positive control, followed by the estimation of total lipids, cholesterol, triglycerides, HDL, LDL, VLDL, urea, creatinine, uric acid, bilirubin, AST, ALT and ALP, albumin, globulin, A/G ratio, serum total proteins, sodium, potassium, calcium, magnesium, chloride, amylase, lipase, LDH and HbA1c levels by kit methods.
Quercetin was purchased from the Sigma Aldrich(SKU: quercetin-PEC) due to its outstanding physiochemical properties (Table 1) to prepare its aqueous solution and given to at the dose of 10mg/ml/ kg body weight of male and female albno Wistar rats (300- 400 g).

Table 1 Physicochemical properties of quercetin

PubChem CID	5280343	Hydrogen Bond Acceptor Count	7	Undefined Atom Stereocenter Count	0
Structure		Rotatable Bond Count	1	Defined Bond Stereocenter Count	0
Molecular Formula	$C_{15}H_{10}O_7$	Exact Mass	302.04265265	Undefined Bond Stereocenter Count	0
Synonyms	Quercetin 117-39-5 Meletin Sophoretin Quercetine	Monoisotopic Mass	302.04265265	Covalently-Bonded Unit Count	0
Molecular Weight	302.23	Topological Polar Surface Area	127 Å²	Compound Is Canonicalized	1
IUPAC Name	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	Heavy Atom Count	22	Isotope Atom Count	0
XLogP3	1.5	Formal Charge	0	Defined Atom Stereocenter Count	0
Hydrogen Bond Donor Count	5	Complexity	488		

Animal grouping:

Healthy and disease free albino Wistar rats(300- 400 g)were divided into five groups (Table 2) (each having half male and half female) in animal house of IMBB, The University of Lahore in standard lab conditions (at 20-26 ^{0}C/ 68-78.8 ^{0}F, 30%–70% humidity)and fed with standard diet (mixture of 23 % crude protein,3.0% crude fat, 7.0% crude fiber, 8% acid insoluble ash, 1-2.5 % calcium, 0.9% Phosphorus, 0.5-1% sodium and 12% moisture) during the period of whole experiment.

Table 2 Animal groups

Animal groups		**Treatments**
Control groups	Vehicle	Only normal saline
	Negative/ High fed diet treated	High fed diet treated
	Negative/ Hyperlipidemic	Only 55 mg STZ /mL/ Kg b.w. of rats
	Positive/ Hypolipidemic	250 mg/mL/ Kg b.w. metformin*
Experimental group	Treated with quercetin	10 mg/mL/ Kg b.w in distilled water*

* Drug has been induced after the induction of diabetic hyperlipidemia via 55 mg STZ/mL/Kg b.w. of rats

Induction of diabetic hyperlipidemia, Quercetin, and Hypolipidemic drug

Before the induction of diabetic hyperlipidemia and to check which animal can become diabetic rapidly after the induction of STZ, Oral Glucose Tolerance Test (OGTT)was performed in 8-10 hour fasted rats, by the administration of 10% glucose solution, followed by the measurement of blood glucose level after 0, 30, 60, 90, and 120 minutes and those animals were considered to develop diabetes whose blood glucose level was more than 200mg/dL(Asma et al., 2016).

Rats were made hyperlipidemic by intraperitoneal induction of 55mg/mL/Kg body weight of streptozotocin (STZ), followed by the oral administration of 10%glucose solution to prevent severe hypoglycemic effect of STZ (Brian et al., 2021).

10mg/ml/Kg b.w. of aqueous solution of quercetin (in experimental group) and 250 mg/mL/kg b.w. metformin (in positive control group) had been injected intraperitoneally in rats after STZ induction (Hou et al.2023).

Effect of drugs on hormonal, biochemical and histological profile of rats

Rats were anesthetized by giving inhalant anesthesia (chloroform, halogenated ether and placed in closed container for 2 to 3 minutes). After sedation,rats were removed from container,placed on a slab(pre-cleaned with spirit, to avoid from any contamination of skin) and made a small cut in the middle of abdomen to the snout anteriorly and the genital opening posteriorly by making transverse incisions along the length of the limbs. Blood sample were taken in EDTA and non-EDTA blood vacutainers, centrifuged at 2000 rpm for 5 minutes to separate the serum and blood cells for the estimation of cholesterol, triglycerides, HDL, LDL, VLDL,urea, creatinine, uric acid(Bamanikar et al., 2016), bilirubin(Jendrassik and Grof, 1938), albumin, globulin total protein, A/G ratio (Yokozawa et al., 2005), alkaline aminotransferase (ALT), aspartate transferase(AST)(Pezeshki et al., 2016), Alkaline phosphatase (ALP)(Sundaramet al., 2013), sodium, potassium, magnesium, chloride, calcium (Zhao et al.2018), amylase, lipase (Hirano et al., 2004) and LDH (Antinoneet al., 2009), HbA1c(Little et al., 2009) by using standard kit methods.

Liver, kidney and pancreas were preserved in 10% neutral formalin for the histopathological analysis of hepatic and renal tissues at 400 um (Alkiyumi et al., 2012).

Statistical analysis:

The data was analyzed by using Two-Way ANOVA by consideringp<0.05 through GarphPad prism 8.0.2 while results has been expressed as mean± SD.

RESULTS

Effect of quercetin onlipid profile

Statistically observed results showed that in both male and female rats of negative control group, concentration of cholesterol (88.25±10.47 and 105±7.0 mg/dL), triglycerides (94.00±5.72 and 82.3±1.0 mg/dL), HDL (57.00±8.36 and 64.3±3 mg/dL), LDL (29.75±5.56 and 26.1±2.0 mg/dL) and VLDL (14.25±1.71 and 16±2.0 mg/dL) respectively was almost similar to that of animals treated with high fed diet {cholesterol (137.3±30.54 and 134±6.5 mg/dL), triglycerides (50.25±1.16 and 55±3.0 mg/dL), HDL (80.75±5.38 and 65±5.2 mg/dL), LDL (47.25±4.92 and 92±9.02 mg/dL) and VLDL (40.25±1.71 and 45±0.2 mg/dL) respectively, while in male and female rats of positive control group, concentration of total cholesterol (285±19.94 mg/dL and 334.6±11.0), triglycerides (273.60±32.65 and 262.3±22 mg/dL), HDL (60.50±1.96 and 53.3±4.3 mg/dL), LDL (65.5±6.81 and 105.3±12 mg/dL respectively) and VLDL (38.3±3.32 and 40.3±1.1 mg/dL) respectively were nearly equal to the animals treated with aqueous solution of quercetin (Figure 1a- e).

Effect of quercetin on kidney and liver metabolites

Statistically analyzed results showed that urea content in both male and females of negative control group (193.6±6 and 195.6±14mg/dL) and high fed diet treated groups (67.6±4 and 40.3±3mg/dL) respectively were higher as compared to positive control group (34±1 and 32.6±1mg/dL respectively) and vehicle (46.3±1and 45.3±4 mg/dL respectively), while it decreased in animals treated with quercetin solution (36.6±5 and 32±2mg/dL respectively), (Figure 1, f).

Creatinine content in males and females of the negative control group has been same (0.7±0.1 mg/dL in both) as compared to vehicles (0.63±0.08 and 0.8±0.2mg/dL respectively), but it decreased as compared to animals treated with high-fed diet (1.5±0.2 and 2.8±0.1 mg/dL respectively). Male rats of positive control group has less amount of creatinine (0.56±0.06 mg/dL) as compared to female rats (2.8±0.1 and 0.7±0.1mg/dL) and rats treated with aqueous solution of quercetin (1.7±5 and 1.26±2mg/dL respectively) (Figure 1, g).

Uric acid content was least in both genders of vehicle (1.6±0.1 and 1.8±0.1mg/dL respectively) as compared to animals in negative control group (3.03±0 and 3±0mg/dL respectively) and high-fed diet treated rats (2.16±0.1 and 2.83±0.16 mg/dL respectively), while it was almost similar to animals treated with metformin (1.5±0.26 and 1.33±0.16mg/dL respectively) and quercetin solution (1.6±5 and 1.5±2mg/dL respectively)(Figure 1, h).

Statistically analyzed results of bilirubin content showed its high concentration in male and females of the negative control group (2.3±0.1 and 2.6±0.2mg/dL respectively) as compared to vehicle (0.2±0 and 1.6±0 mg/dL respectively). It has been increased in males and females treated with metformin (1.6±0 and 1.1±0 mg/dl respectively) and quercetin solution (0.43.6±5 and 0.3±2mg/dl respectively), as compared to animals treated with high-fed diet (1.83±0.1 and 2.2±0.05mg/dl) and vehicle (Figure 1, i).

Effect of quercetin on proteins

Albumin content was same in males and females of high fed diet treated group (2.66±0.3 and 3.76±0.1 g/dL respectively), hyperlipidemic (2.9±0.5 and 3.36±4 g/dL respectively), hypolipidemic control groups (2.6±0.3 and 3.7±0.2g/dL respectively) and in experimental group (3.5±5 and 3.9±2mg/dL respectively)(Figure 1, j), while globulin content has been raised slightly in both males and females rats treated with high-fed diet (2.6±0.1 and 1.8±0.1 g/dL respectively) and decreased in rats of experimental group (2.3±5 and 2.2±2mg/dL respectively) and these results were significantly ($p \leq 0.05$) similar to that of hyperlipidemic (1.03±0.03 and 2.4±0.2g/dL respectively)and positive (1.8±0.2 and1.7±0.1g/dL respectively) control groups as compared to vehicle (2±0.05 and 2.06±0.2g/dL respectively) (Figure 1, k). Total protein content in males and females in negative control group has been significantly increased (6.56±7and 6.46±.2mg/dL respectively) as compared to vehicle (6±0 and5.16±0 mg/dL respectively) and like rats treated with high-fed diet (6.16±0.4 and5.23±0.1 mg/dL respectively) and in positive control group (6.56±7and 6.46±.2 mg/dL

respectively). But it has been decreased significantly in rats treated with aqueous solution of quercetin (3.82±5 and 3.3±2mg/dL respectively) (Figure 1, l). Similarly A/G ratio of males and females rats treated with high-fed diet (1.23±0.03 and 1.33±0.03 % respectively) has been increased as compared to vehicle (1.26±0.1 and 1.4±0.2 % respectively), and slightly decreased in rats of hyperlipidemic (1.1±0.1 and 1.1±0.08 % respectively) and positive control groups (1.1±0.5 and1.1±0.09 % respectively), while it has been decreased significantly in rats treated with quercetin solution (1.01±5 and 1.03±2 % respectively)(Figure 1, m).

Statistically observed results showed that HbA1c level in both males and females of high fed diet treated group (3.6±0.030 and 3.13±0.8 %respectively), hyperlipidemic (4± 0.5% and4.9±0.2% respectively) and anti-hyperlipidemic controls (5.68±0.29% and 4.9±0.2%respectively) has been increased as compared to vehicle (3.9±0.05 and 3.4±0.20 % respectively), while its levels in male and females rats treated with aqueous solution of quercetin was3.76±0.1 and 3.9±0.01 %respectively (Figure 1, n).

Effect of quercetin onelectrolyte balance of animals

After statistical analysis of serum electrolytes, results showed that the electrolytes of males and females in negative control {Na (144.14 ± 8.7 and 141.1 ±1mmol/L respectively), k (7.25 ± 3.338 and 6.5± 2 mmol/L respectively), Ca (9.25 ± 1.728 and 10± 1 mmol/L respectively) , Mg (4.01 ± 0.448 and 4.3 mmol/L respectively), Cl (144.50 ± 0.58 and 145 mmol/L respectively)} has been significantly increased as compared to vehicle {Na (138.6±1 and 140.6±0.3 mmol/L), K (4.5±0.2 and 4.8±0.4 76mmol/L respectively), Cl (113±3 and113±2 mmol/L respectively), Mg (1.5±2 and 2.1±1 mmol/L respectively), Ca(10.5±0 and 10.6±2 mmol/L respectively)} and it slightly elevated in rats treated with high fed diet {Na(149±3 and 120.6±9 mmol/L respectively), K (5.5±0.2 and 5.43±0.2 mmol/L respectively), Cl (104±5 and 113±1 mmol/L respectively), Mg(2.2±6 and 5.3±0.3 mmol/L respectively), Ca (11.5±2 and 10.5±2mmol/L respectively)} and in positive control group {Na ((141.14 ± 6.76 and 143 mmol/L respectively), K (4.25 ± 3.33 and 4.6 mmol/L respectively), Ca (13.15 ± 1.1 and 13.2 mmol/L respectively), Mg (3.1 ± 0.44 and 3.3 mmol/L respectively), Cl (141.50 ± 0.5 and 142 mmol/L respectively)}. But it has been decreased significantly in rats treated with aqueous extract of quercetin {Na(138.14 ± 6.76 and 139.2 mmol/L respectively),K (4.5 ±3.33 and 4.1 mmol/L respectively), Ca (9.9 ± 1.72 and 10 .2 mg/dl respectively), Mg (1.7 ± 0.44 and 1.5 mmol/L respectively), chloride (106.50 ± 0.5 and 101 mmol/L respectively)} (Figure 1, o-s).

Effect of quercetin on enzymes in animals

Statistically observed results showed that AST level in both males and females of high fed diet treated group (63±2.5 and 73±3.1 U/L respectively), hyperlipidemic control (72.3±2.9 and 77±2.5 U/L respectively), anti-hyperlipidemic control (88.4±1.99 and 94±2.2 U/L respectively) and experimental groups (77±5.1 and 59±2.8 U/L respectively) has been increased as compared to vehicle (40±3.5 and 43±2.8 U/L respectively)(Figure 1, t)

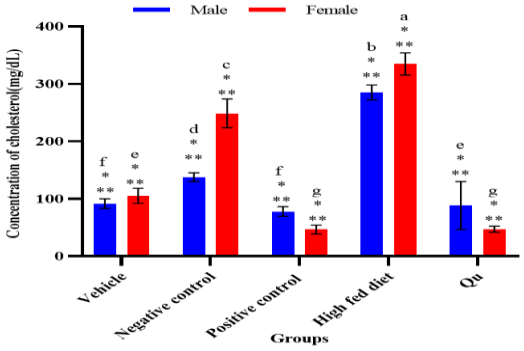
ALT content in both males and females animals of high fed diet treated group (63±1.2 and 73±3.1 U/L respectively), hyperlipidemic (72.3±2.2 and 77±2.1 U/L respectively) and positive control (88.4±1.1 and 94±2.1 U/L respectively) has been increased as compared to vehicle (40±2.5 and 43±2.99 U/L respectively), while it has been decreased in male and females rats treated quercetin solution (31±1.5 and 36 ±2.7 U/L respectively) (Figure 1, u).

Statistically analyzed results of ALP content showed that in both males and females of rats treated with high fed diet (108.3±11.1 and 122.3±12.1 U/L respectively) has been decreased as compared to vehicle (111±15.1 and 128±4.9 U/L respectively) and but still it was more than the animals in negative (95±3.6 U/L and 89±8.1 U/L respectively) and positive control (95.4±9.2 U/L and 104±9.3 U/L respectively) groups. Aqueous solution of quercetin has decreased ALP content (77±5.9 and 59 ±2.8 U/L respectively) as compared to negative control group (85±4.8 U/L and 56±3.9 U/L respectively) (Figure 1, v).

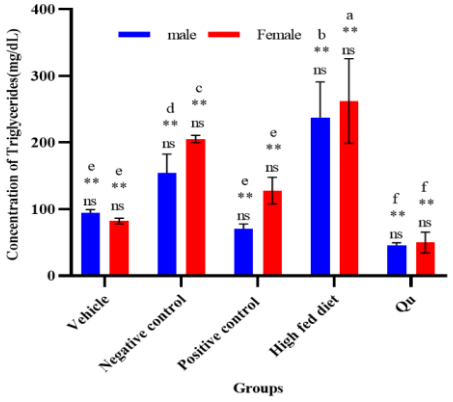
Amylase content in both males and females animals of high fed diet treated group (628±12 and 704±30.5 U/L respectively), hyperlipidemic (742.79±29.6 and 740±21.11 U/L respectively) and positive control (353.9±94 and 345.1±22.5 U/L respectively) groups has been increased as compared to vehicle (559±6 and 581±30 U/L respectively), while it has been decreased in male and females rats treated with quercetin solution (443.79±26.28 and 440.1± 26.5 U/L respectively) (Figure 1, w).

Lipase content in both males and females animals of high fed diet treated group (222±9.5 and 219±2.6 U/L respectively), hyperlipidemic (55.56±4.8 and 56.2±1 1/L respectively) and positive control (28.3±5 and 31±2.8 U/L respectively) has been increased as compared to vehicle (559±6 and 581±30 U/L respectively), while it has been decreased in male and females rats treated with aqueous solution of quercetin (18.6±4.1 and 19.6± 0.1 U/L respectively) (Figure 1, x).

LDH content in both males and females animals of high fed diet treated group (2305±121 and 2657±103 U/L respectively), hyperlipidemic (3000.86±121 and 1354.32±201 U/L respectively) and positive control (1200.8±13 and 1010±52.2 U /L respectively) groups has been increased as compared to vehicle (559±16 and 581±30 U/L respectively) and decreased in rats treated with aqueous solution of quercetin (688.86±13 and 671.2±0.6 U/L respectively) (Figure 1, y).



(a)



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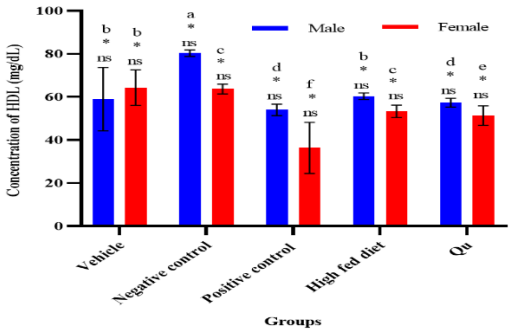

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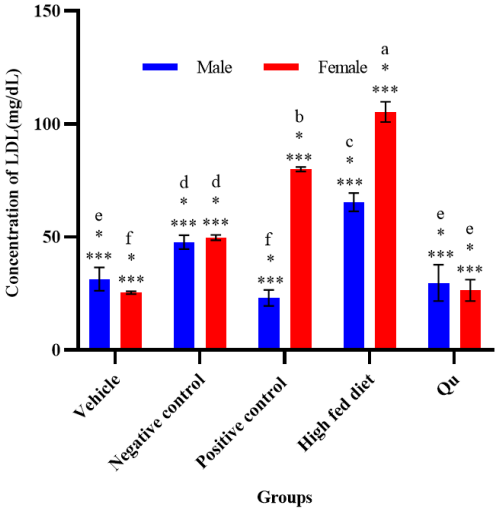

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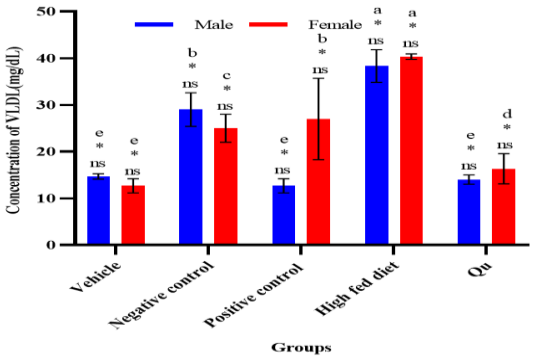
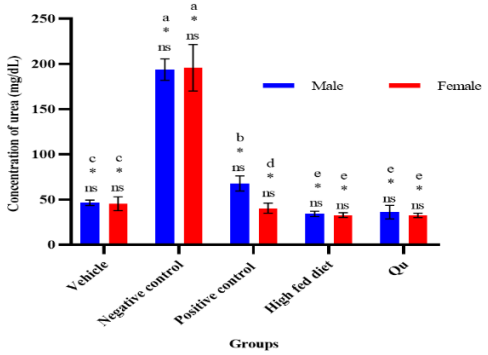
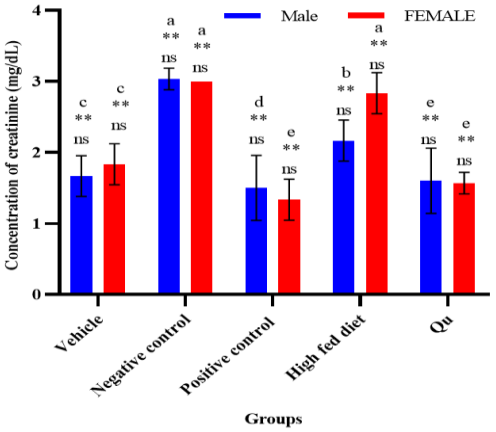

Male
Female
Concentration of VLDL(mg/dL)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

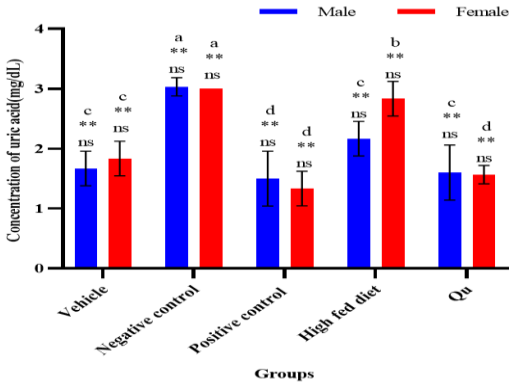
(e)


Male
Female
Concentration of urea (mg/dL)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

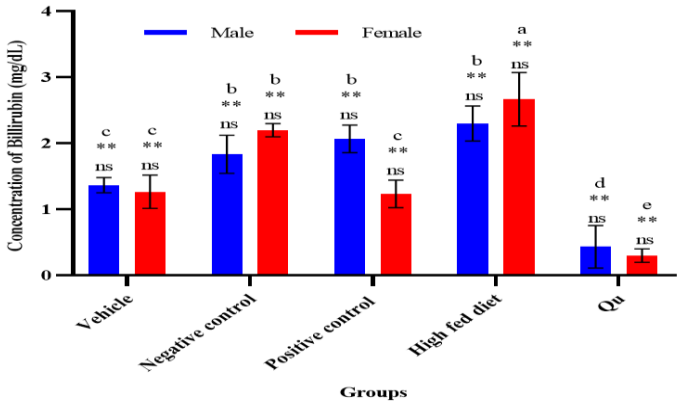
(f)


Male
FEMALE
Concentration of creatinine (mg/dL)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

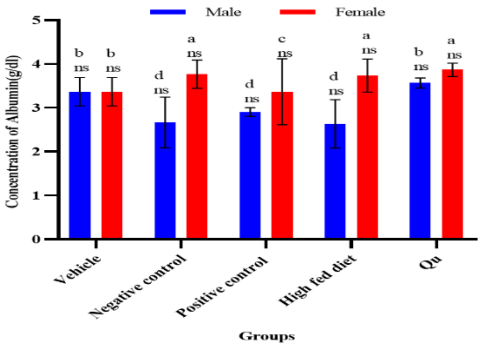
(g)


Male
Female
Concentration of uric acid(mg/dL)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

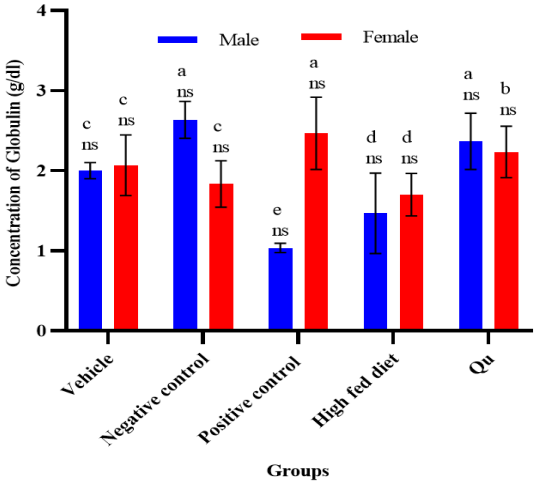
(h)


Male
Female
Concentration of Billirubin (mg/dL)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

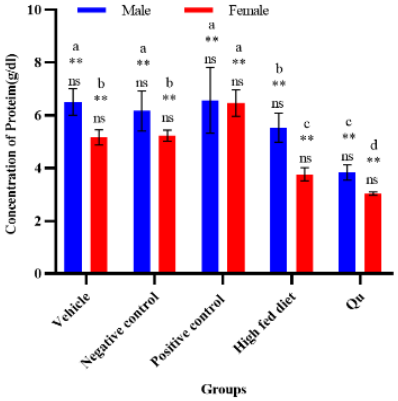
(i)


Male
Female
Concentration of Albumin(g/dl)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

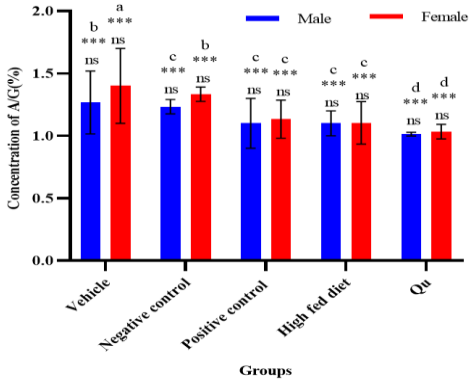
(j)


Male
Female
Concentration of Globulin (g/dl)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

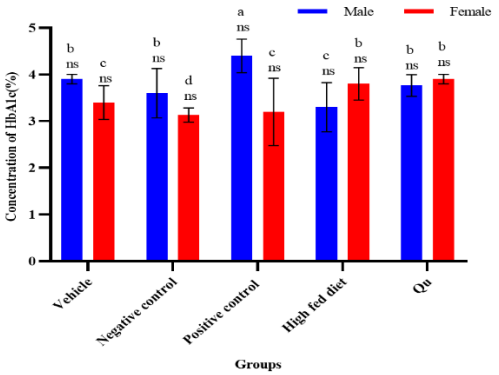
(k)


Male
Female
Concentration of Proteim(g/dl)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

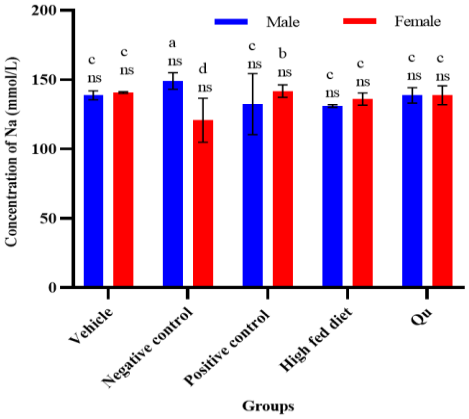
(l)


Male
Female
Concentration of A/G(%)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

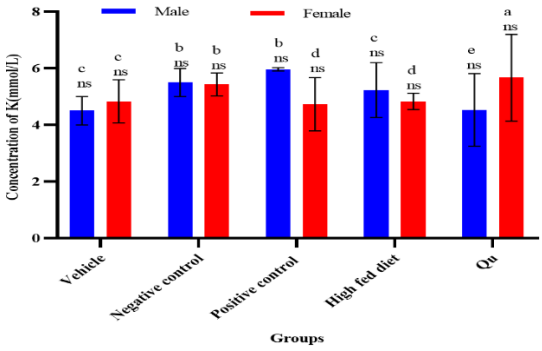
(m)


Male
Female
Concentration of HbA1c(%)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

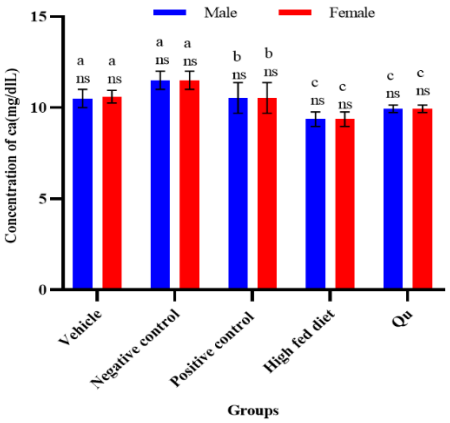
(n)


Male
Female
Concentration of Na (mmol/L)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

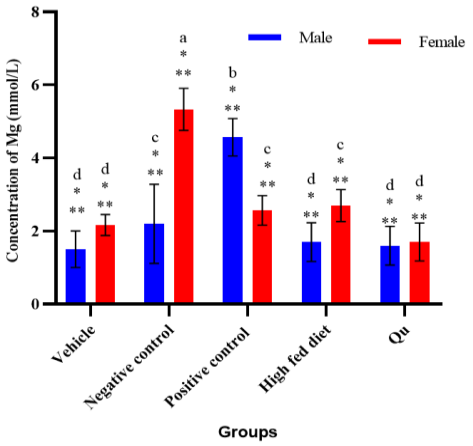
(o)


Male
Female
Concentration of K(mmol/L)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

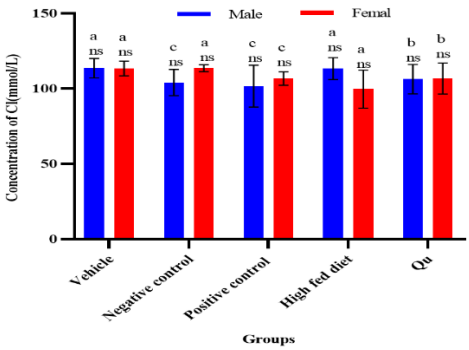
(p)


Male
Female
Concentration of ca(mg/dlL)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

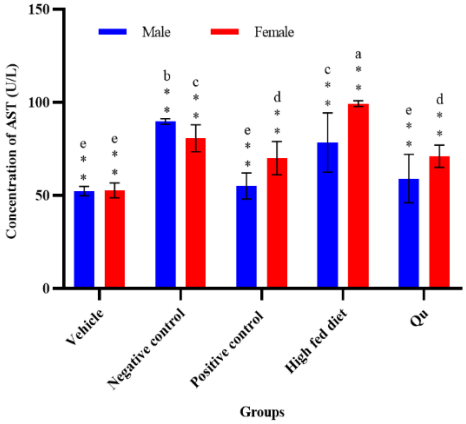
(q)


Male
Female
Concentration of Mg (mmol/L)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

(r)


Male
Femal
Concentration of Cl(mmol/L)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

(s)


Male
Female
Concentration of AST (U/L)
Vehicle
Negative control
Positive control
High fed diet
Qu
Groups

(t)

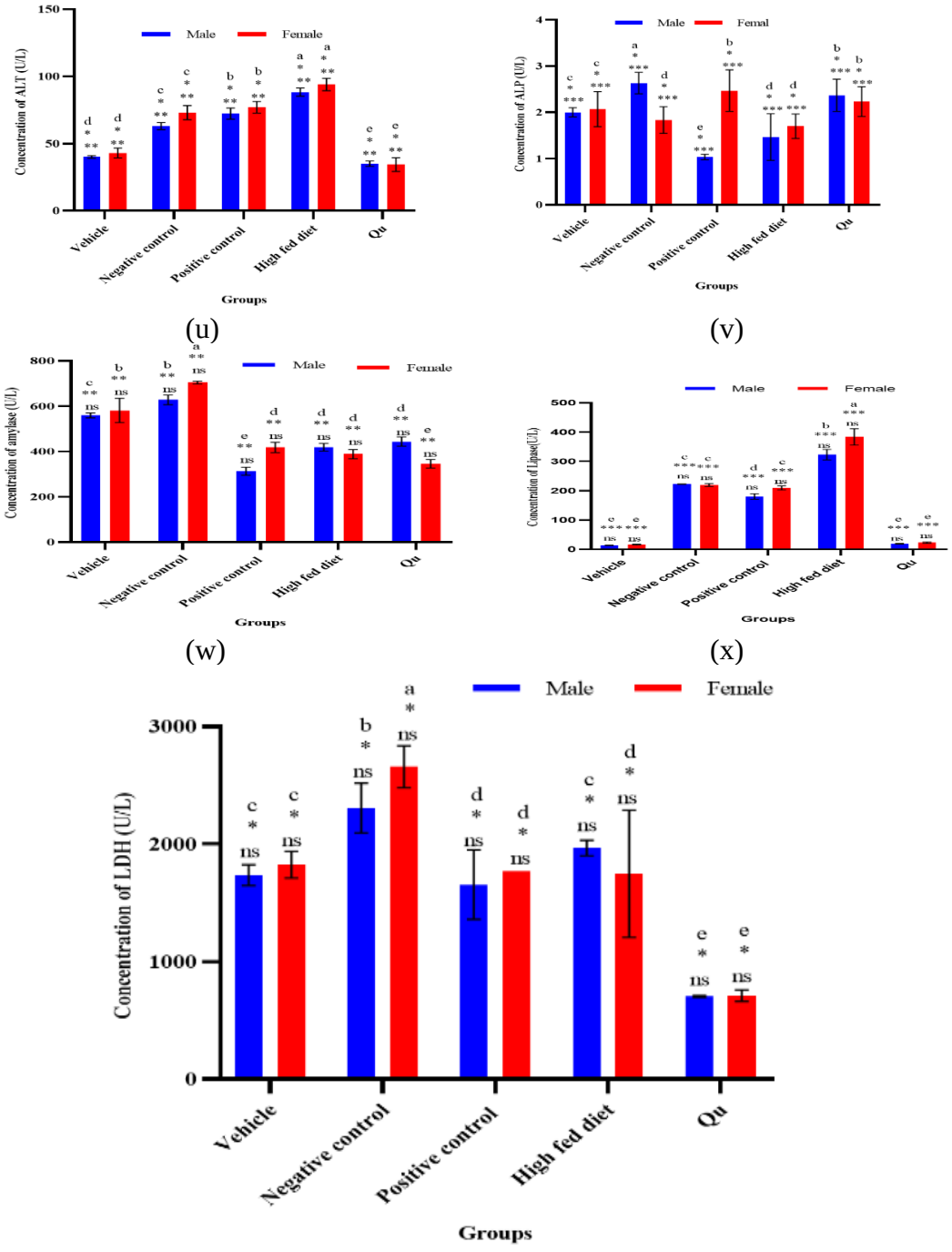


Figure 1: Concentration of (a) cholesterol, (b) triglycerides, (c)HDL, (d) LDL, (e) VLDL, (f) urea, (g) creatinine, (h) uric acid, (i) bilirubin, (j) albumin, (k) globulin, (l) total protein, (m) A/G ratio,(n) HbA1C, (o)Na, (p) K, (q) Ca, (r) Mg, (s) Cl, (t) AST, (u) ALT, (v) ALP, (w) Amylase (x) Lipase (y) LDH

Qu=-quercetin a-g =Comparison of animal groups from most significant results to less significant. Highly significant ***= P- 0.0001, Most significant**= P<0.01(0.0010-0.0092), Significant*= P<0.05(0.0392-0.0471), ns=non significant

Histopathological profile of renal, hepatic and pancreatic tissues

In vehicle, histological features of renal tissue showed normal glomerular structure, and tubules, while vascular hemorrhage, congestion, hyperplasia, and edema were unremarkable. Renal tissues in

high fed diet group showed mild dilation in blood vessels and increased space in Bowman capsule. Mononuclear cells infiltrate was present, while increased glomerular sclerosis was seen in very few segments, along with increased connective tissue. Hyperlipidemic control group showed abnormal kidney structure in the form of mesangial cell expansion with dilated glomerular blood capillaries, while main pathology was tubular regeneration. Hypolipidemic group showed recovery of pathological changes in a few segments of renal tissues. Experimental group showed normalization of glomerular sclerosis index. Glomuular tubules were normal in histological appearance. Mild glomerular volume atrophied is seen. Another section showed an increased mesangial extracellular matrix {Figure 2 (i- x)}.

Similarly, histological features of hepatic tissue showed normal lobular structure, while fatty changes, sinusoidal dilation, chronic venous congestion, and fragments of lobules were maintained and were unremarkable. Hepatic tissues of high fed diet treated group showed appearance of typical liver steatosis with lipid droplets, while in negative/hyperlipidemic control group, abnormal interruption in the radiating pattern of hepatocytes cords has been observed. Hepatocytes showed degeneration with loss of architecture and cytoplasmic vacuolation, while nuclei were heterogeneous in shape and size along with blood vessel hemorrhages in multiple hepatic segments. Hepatic tissue of positive/ hypolipidemic group showed interruption in radiating pattern of the hepatocytes cord, but with reduced cytoplasmic vacuolation and fatty changes as compared to only STZ treated group, which shows mild recovery in pathological aspects, with no vascular hemorrhages. Rats treated with quercetin solution showed hepatocytes a distinct outline, and evenly distributed polygonal cells. The Portal canal showed a layer of fenestrated endothelial cells. Kupper cells showed normal architecture with mild infiltration of lymphocytes and few macrophages in the peripheral zone. Apoptosis was not seen {Figure 2 (xi- xx)}.

In vehicle, histological features of the male pancreas showed normal structure of pancreatic cells, with normal beta cells and septa. Pancreatic tissue treated with high fed diet showed dilated interlobular and intralobular ducts with few secretions in the lumen. Blood vessels showed thickness with the periphery surrounded with eosinophilic material. Severe fatty infiltration had been seen, while female pancreas treated with a high-fed diet showed an increase in Islet mass and Islets lesion appeared as an irregular contour, with reduced Islets cells and vacuolated cytoplasm, pyknotic, and karyolysis nuclei. Many cells showed degeneration and were disrupted. The Islet’s blood sinusoids showed significant dilation and congestion. Few inflammatory cells were present and invaded the pancreatic lobule. STZ treated group showed a reduction in the number of normal islets cells and the destruction of beta cells. Septa were completely distorted. Blood vascular hemorrhages was seen in different segments of cells and females showed a complete destruction of beta cells. Septa were completely distorted. Blood vascular hemorrhages are seen in different segments of cells. Pancreas treated with quercetin solution showed regeneration of pancreatic islets and an increased number of Langerhans cells in a specific region. Blood vessels showed mild hemorrhage in a few areas, while evidence of cell infiltration was absent {Figure 2 (xxi- xxx)}.

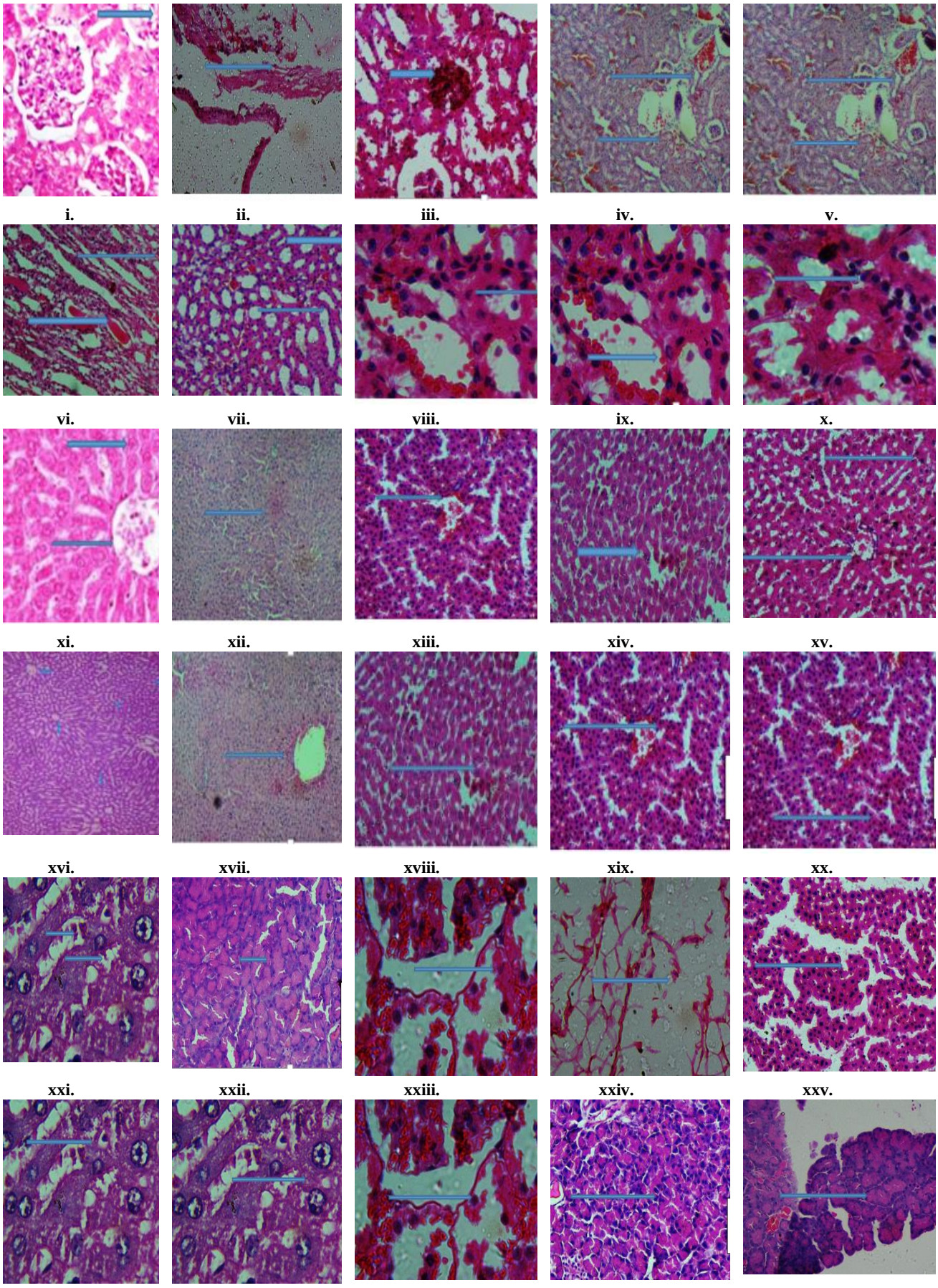


Figure 2: Histological features kidney/ renal(i- x),liver/ hepatic (xi- xx) and pancreatic(xxi-xxx) tissues of at400 µm

K= Kidney/ Renal, L= Liver/ Hepatic, P= Pancreatic, G I= Vehicle, G II= High fed diet group, G III= Negative control group, G IV= Positive control group, G V= Rats treated with quercetin solution♂ =Male, ♀=Female

Discussion

Flavanols present in *Camellia sinensis* L. leaves have been quantified as quercetin, myricetin, and kaempferol. Presence of these compounds was also reported in fruits and vegetables and they show a strong association in the prevention and treatment of many diseases including hyperlipidemia, diabetes mellitus, and hepato- renal derangements. Ming-Zhi et al., (2020) had described strong hypolipidemic effects of quercetin due to the change of microbial composition of gut microorganisms and the change in gut permeability, which are attributed by Que et al., (2017), Li et al., (2018) and Zhang et al., (2017) to an increased level of quercetin in the gut after the flowering process and continuous degradation of galloylcatechin. Moreover quercetin has strong antioxidant (Lee etal.,2012), tumor-suppressive (Ren et al., 2018), scar depigmentation (Wang et al., 2018), hepato-protective (Draeloset al.,2008), platelet aggregation inhibitor (Peng et al., 2017), anti-thrombotic (Ro et al.,2015), immune system suppresser (Lee et al.,2013), anti-inflammatory (Kumar et al.,2016), anti chlethiogenic (Khajahet al., 2019), nervous system protector (Reddy et al.,2007), hypouricemic (Singh et al.,2017), antibacterial (Kim et al.,1997), and antifungal properties (Shams-Ghahfarokhiet al.,2007). Its hypolipidemic effect is due to an increased level of PPAR-γ, LXRα, and ABCA1 gene and increased cholesterol efflux to apo-A1 and HDL (Galaviet al., 2021). Quercetin is also reported to increase liver and pancreatic LDLR receptor and PCSK 9 activity and its secretion. Quercetin, catechins, and epicatechin of *C. sinensis* (L.) extracts binds with HMGR (3-hydroxy-3-methylglutaryl coenzyme A reductase) at the same point with amino acid where hypolipidemic drug (simvastatin) binds to be inhibited, followed by the inhibition of cholesterol synthesis steps. Franco et al., (2020) confirmed that quercetin controls hyperlipidemia and hyperglycemia by inhibiting lipase and amylase respectively. Moreover it also act as free radical scavengers, decrease glycation and inhibit lipase and glucosidase.

Li et al. (2020) investigated that quercetin controlled hyperlipidemia by inhibiting the proliferation and differentiation of premature adipocytes into mature one, by decreasing lipid accumulation in adipose cells and by decreasing the expression of adipogenic factors and PPARγ protein. Zulfiqar et al., (2020) also reported the hypolipidemic, hypoglycemic, and antioxidant effects of methanolic and ethanolic extracts of quercetin. Swierczewska et al., (2019) studied that hypolipidemic and hypoglycemic activity of quercetin is due to inhibition of pancreatic lipase and alpha-amylase respectively.

Wei et al. (2020) investigated that quercetin has strong anti-inflammatory activity and inhibit NF-κb and TNF signaling pathway, thus decreasing the expression of TNF-α, VCAM-1 and IL-1 β at mRNA levels, which ultimately inhibits the progression of plaque in blood vessel.

There is a strong relationship between quercetin intake and cardiovascular protective disease. Recent studies have shown that those subjects who drink two cups of *C. sinensis* (L.) had lower plasma cholesterol concentration and their incidence of death from cardiovascular disease is reduced to 22% to 33 % and this important role is attributed to catechins (epigallocatechin-3-gallate) and quercetin. Both of these compounds are reported to be cardioprotective as they inhibit thrombin formation, oxidation process and main enzyme for lipid synthesis and thus decrease intestinal absorption of lipid. Increased intake of tea (*C. sinensis* L.) is also associated with a lower risk of hyperlipidemia and its related disorders (Hill et al.2021) as its phenolic compounds have chlorogenic acid effects on carbohydrate metabolism, which have antihyperglycemic, antihyperlipidemic, hepatoprotective, and anti-atherogenic activity. Moreover green tea phenolics also decrease amylase and glucosidase levels in STZ-induced diabetic hyperlipidemic rats (Amit et al.2021, Anouar et al.2019).

Amel at al. (2017) and Koch et al. (2018) reported the protective effects of butanolic and ethanolic extracts of quercetin on oxidatively damaged (to induce hyperlipidemia) heart, kidney, and lipid

profile of rats and results hsowed that there was marked improvement in serum transaminases, cholesterol, triglycerides, lipid peroxidation (MDA), reduced glutathione (GSH), and glutathione peroxidase (GPx), which suggested that butanolic and ethanolic extracts of quercetin can be used for the treatment of hyperlipidemia, liver injury, and renal injury, along with improved antioxidant levels to protect against cancer development.

CONCLUSION

In current study the quercetin-treated group AST, ALP, neutrophils, platelets, TLC, and lipase levels increased in both gender, and total lipids, creatinine, A/G ratio, RBC, magnesium, and lymphocytes increase only in males and HbA1c only in females. On the other hand cholesterol, HDL, urea, bilirubin, ALT, globulin, total protein, HCT, chloride, amylase, and LDH levels decreased in both gender, and total lipids, creatinine, uric acid, A/G, RBC, Mg, and HbA1c decreased only in males and albumin, MCHC, MCH decreased only in females. So it is concluded that quercetin has positive effect for the regulation of enzymatic, hormonal, metabolic and mineral profile in hyperlipidemic rats like metformin but metformin not only played a role for the cure of diabetic hyperlipidemia but it also has produced pathological symptoms in hepatic and renal tissues of rats. Along with quercetin, high fed diet also have shown more therapeutic effects for the reduction or elimination of diabetic hepato-renal toxicity, although it can produce diabetic nephropathy in streptozotocin induced diabetic rats through the generation of free radicals. So it is recommended that instead of using synthetic medicine, there is more chances for the reduction or elimination of toxicity by the use of pure phytocompounds along with high-fed diet formula.

COMPETING INTREST

Authors have declared that no competing interest exist.

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