REPUBLIC OF TÜRKİYE
KARADENIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL BIOCHEMISTRY

INVESTIGATION OF THE EFFECTS OF L-THEANINE ON KIDNEY TISSUE IN DIABETIC RATS

Sabrina AZI MOHAMED

MASTER'S THESIS

Prof. Dr. Birgül KURAL

TRABZON-2023

DECLARATION

I declare that this thesis was carried out and written in accordance with the standards of Karadeniz Technical University Graduate School of Health Sciences Thesis Preparation and Writing Guide, that the thesis is an original scientific research carried out by adhering to academic and ethical rules, that I have cited all the information and comments in the thesis that were not obtained by this thesis study and that the used citations are included in the list of references, and that I have not acted in violation of patents and copyrights during the study and writing of the thesis.

28/11/2022

Sabrina AZI MOHAMED

Dedication

I dedicate my work to my family and many friends. A special feeling of gratitude to my loving parents Azi and Hawa MOHAMED whose words of encouragement and push for tenacity ring in my ears. My brothers who supported me all along my journey. I also dedicate my work to my dear for all the emotional support and patience. Finally, big thanks to all the people that helped me even with a smile.

TABLE of CONTENTS

LIST of TABLES

LIST of FIGURES

LIST of PICTURES

LIST of ABBREVIATIONS, SYMBOLS and FORMULA

Abbreviations

ACE2	Angiotensin-Converting Enzyme 2
AD	Alzheimer's Disease
ADP	Adenosine Diphosphate
AGAL	L-Arginine Glycine Amidinotransferase
AGEs	Advanced Glycosylation End Products
AMP	Adenosine Monophosphate
Ang II	Angiotensin II
AT1R	Angiotensin II Type 1 Receptor
ATP	Adenosine Triphosphate
B.C.	Before Christ
BSA	Bovine Serum Albumin
BUN	Blood Urea Nitrogen
CLP	Cecal Ligation and Puncture
CST3	Cystatin 3 Gene
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
DNTB	5, 5'-Dithiol-bis-2-(Nitrobenzoic Acid)
DOX	Doxorubicin
EDTA	Ethylenediaminetetraacetic Acid
eGFR	Estimated Glomerular Filtration Rate
etc.	et cetera
G-6-P	Glucose-6-Phosphate
G-6-PDH	Glucose-6-Phosphate Dehydrogenase
GAMT	Guanidinoacetate N-Methyltransferase
GEDTA	Glycoletherdiamine-N, N, N', N'-Tetraacetic Acid
GFR	Glomerular Filtration Rate
GLUT2	Glucose Transporter 2
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione
H&E	Hematoxylin-Eosin

HFD-STZ	Streptozotocin High Fat diet
e.g.	For example
i.p.	Intraperitoneal
IBM	International Business Machines
IGFBP7	Insulin-Like Growth Factor-Binding Protein 7
IL-18	Interleukin-18
IQR	Interquartile Range
IRS	Insulin Receptor Signal
KIM-1	Kidney Damage Molecule-1
L-FABP	Liver-Type Fatty Acid-Binding Protein
LTEA	L-Theanine
MES	4-Morpholinethanesulphic Acid
MPA	Metaphosphoric Acid
NA	Nicotinamide
NAD^+	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
Na/K-ATPase	Sodium Potassium Pump
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NMDA	N-Methyl-D-Aspartate
pH	Potential Hydrogen
PTH	Parathyroid Hormone
RAAS	Renin-Angiotensin-Aldosterone System
ROS	Reactive Oxygen Species
SGLTs	Sodium-Glucose Cotransporters
SGLT3	Sodium-Coupled Glucose Cotransporter 3
SHAM	Control Group
SPSS	Statistical Package for the Social Sciences
STZ	Streptozotocin
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
T3DM	Type 3 Diabetes Mellitus
TEAM	Triethanolamine
TFF-3	Trefoil Factor-3

TGSH	Total Glutathione
TIMP2	Tissue Inhibitor of Metalloproteinase 2
TNB	5-Thiol-2-Nitrobenzoic Acid
TNB	5-Thiol-2-Nitrobenzoic Acid
TPTZ	2, 4, 6-Tri-(2-Pyridyl)-5-Triazine
UV	Ultraviolet
VC	Vasocongestion
VSMC	Vascular Smooth Muscle Cell

Symbols

%	Percentage
&	And
α	Alpha
β	Beta

Formula

Ca^{2+}	Calcium
Cl^{-}	Chloride
Fe^{2+}	Iron
K^{+}	Potassium
Mg^{2+}	Magnesium

ABSTRACT

Investigation of the Effects of L-Theanine on Kidney Tissue in Diabetic Rats

Diabetes mellitus (DM) is a common disease in the world, characterized by hyperglycemia that may result in damaging and dysfunctioning of kidney. The aim of this study was to determine whether L-Theanine (LTEA), an amino acid specific to green and black teas, which are widely consumed in the world, could be protective against damage in the kidney tissues of diabetic rats. All rats were separated into four groups (n=6/group): SHAM, LTEA, DM, and DM+LTEA. DM was induced by i.p. injections of 120 mg/kg nicotinamide (NA) and 60 mg/kg streptozotocin (STZ). LTEA treatment was administered intragastrically for 28 days. Serum levels of glucose, creatinine, blood urea nitrogen (BUN), albumin, and electrolytes were determined by an autoanalyzer. Serum and tissue cystatin C and angiotensin-converting enzyme 2 (ACE2) levels were determined by using ELISA kits, and oxidized and total reduced glutathione (GSSG and TGSH respectively) levels by using commercial assay kits. Kidney tissues were also evaluated histopathologically. LTEA treatment in diabetic rats decreased serum GSSG/TGSH ratio and iron levels, and increased renal TGSH levels, significantly ($p<0.05$). On the other hand, although this treatment decreased serum cystatin C levels insignificantly, it could not be effective in changing kidney cystatin C and serum/kidney ACE2 levels ($p>0.05$). Nevertheless, it was effective in reducing the histopathological complications seen in diabetic rat kidneys. In conclusion, it is possible to say that LTEA is an amino acid that may contribute to reducing complications related to diabetic nephropathy in rats. Nonetheless, the effectiveness of LTEA against damage to the diabetic human kidney needs to be investigated.

Keywords: Angiotensin-Converting Enzyme 2, Cystatin C, Diabetes Mellitus, Electrolyte, Glutathione, Kidney, L-Theanine

ÖZET

L-Theaninin Diyabetik Sıçanlarda Böbrek Dokusu Üzerine Etkilerinin İncelenmesi

Diabetes mellitus (DM) böbrek hasarına ve disfonksiyonu ile sonuçlanabilen hiperglisemi ile karakterize olan dünyada yaygın bir hastalıktır. Bu çalışmanın amacı dünyada oldukça çok tüketilen yeşil ve siyah çaya özgü bir amino asit olan L-Theaninin (LTEA) diabetik sıçanların böbrek dokularında oluşan hasara karşı koruyucu olup olamayacağını belirlemekti. Sıçanlar dört gruba ayrıldı (n=6/grup): SHAM, LTEA, DM ve DM+LTEA. DM, 120 mg/kg nikotinamid (NA) ve 60 mg/kg streptozotosin (STZ) i.p enjeksiyonları ile indüklendi. LTEA tedavisi 28 gün boyunca oral gavaj yoluyla uygulandı. Serum glukoz, albümin, kan üre azotu (BUN), kreatinin ve elektrolitlerin seviyeleri otoanalizörle belirlendi. Serum ve doku sistatin C ve anjiyotensin-dönüştürücü enzim 2 (ACE2) seviyeleri ELISA kitleri kullanılarak, oksitlenmiş ve toplam indirgenmiş glutatyon (sırasıyla GSSG ve TGSH) seviyeleri ise ticari deney kitleri kullanılarak belirlendi. Böbrek dokuları histopatolojik olarak da değerlendirildi. Diyabetik sıçanlara LTEA tedavisi, serum GSSG/TGSH oranını ve demir düzeylerini düşürmüş, böbrek TGSH düzeylerini ise anlamlı artırmıştır ($p<0.05$). Diğer taraftan, serum sistatin C düzeylerini istatistiksel olarak anlamsız düzeyde düşürmüş olmasına rağmen böbrek sistatin C ve serum/böbrek ACE2 düzeylerini değiştirmede etkili olamamıştır ($p>0.05$). Ancak, diyabetik sıçan böbreklerinde görülen histopatolojik komplikasyonları azaltmada etkili olmuştur. Sonuç olarak, LTEA'nın sıçanlarda diyabetik nefropatiye bağlı komplikasyonları azaltmaya katkı sağlayabilecek bir amino asit olduğunu söylemek mümkündür. Bununla birlikte, diyabetik insan böbreğinde oluşabilecek hasara karşı LTEA'nın etkinliğinin araştırılması gerekmektedir.

Anahtar Kelimeler: Anjiyotensin-Dönüştürücü Enzim 2, Diabetes Mellitus, Elektrolit, Glutatyon, Böbrek, L-Theanin, Sistatin C

1. INTRODUCTION and AIM

Diabetes mellitus (DM) is considered a metabolic disease characterized by abnormally elevated blood glucose. Therefore, it is seen as a grouping of chronic diseases identified with hyperglycemia originating from defects in either the secretion or action of insulin alone or together. There are multiple types of diabetes such as type 1 (T1DM), type 2 (T2DM), type 3 (T3DM), and gestational diabetes (1).

The kidneys are one the most vital organs of the body. The main role of the kidney is to maintain the homeostasis of water, electrolytes, and other solutes. It also contributes to glucose homeostasis by playing roles in the filtration and absorption of glucose. On the other hand, the kidney uses glucose for energy needs (2). However, hyperglycemia, mainly long-term, leads to kidney disease. Some pathologic conditions such as oxidative stress, inflammation and apoptosis induce the progressions of diabetic kidney diseases (3).

The main unit of the kidneys known as the nephron is responsible for the filtration and excretion of different elements (4). Kidney injury is evaluated by different parameters including cystatin C, creatinine, BUN, and albumin (5-7). Creatinine is a lactam form of creatine mostly found in skeletal muscles but eliminated via glomerular filtration (8). Cystatin C is a basic protein freely filtered by the glomerulus and is used for the evaluation of kidney function since it is less affected by external factors including race, gender and muscle mass than creatinine (9-11). ACE2 is an important enzyme responsible for converting angiotensin (Ang) I to Ang (1-9) and Ang II to Ang (1-7). It is a component of the renin-angiotensin-aldosterone system (RAAS). The location of ACE2 makes it a good candidate for evaluating kidney diseases (12-14). Electrolytes are very important for the body and mostly the kidneys control them. Thereby, a dysregulation of electrolyte homeostasis may lead to the diseases such as DM and nephropathy (15).

L-Theanine is a free amino acid richly found in tea trees. This amino acid has a similar structure to glutamine and has many positive effects on health such as relaxation, improvement of learning ability, preventions of many diseases including cancer and vascular diseases, contribution to weight loss. It has renal protective, lipid-lowering, antihypertensive, chemosensitizing, and hepatoprotective properties (16). Nephroportective effects of LTEA have been investigated by some studies (17-19). Nevertheless, as far as we researched, we could not find any study that investigate the effects it on any kidney related parameters in diabetic conditions.

Since tea is a widely consumed beverage and DM is a very serious disease for kidney disorders, we wanted to evaluate the effect of LTEA on kidney functions-related parameters including ACE2 and electrolytes in experimental induced diabetic rats.

2. LITERATURE REVIEW

2.1. Diabetes Mellitus

The word diabetes mellitus is a combination of the Greek word “diabetes” which means passing through and the Latin word “Mellitus” which means sweet. The word diabetes was first used by Apollonius of Memphis around 250-300 (1). This disease was known as « great emptying of the urine » in the ancient Egyptian manuscripts around 1500 B.C. John Rollo British surgeon-general used the Latin word “Mellitus” (which means sweet like honey); in 1798, he used it to make difference between different diabetes types (20).

Diabetes is a metabolic disease caused by the inability to control the concentration of glucose in the blood (1). A combination of genetic and non-genetic changes leads to a disturbance in glucose metabolism (21). The cause of diabetes is generally defined as disordered insulin or glucagon secretion and sometimes both lead to interrupted homeostasis (20). Increased glucose and lipid levels can cause damage to beta-cell function and apoptosis. Oxidative stress, endoplasmic reticulum stress, alternations in beta cell metabolism, low levels of insulin receptor signal (IRS), and induction of pro-apoptotic signals have been reported to be the main participants in beta cell failure (22). Diabetes affects vital organs by causing micro-and macro-vascular complications via various mechanisms (23). Subjects with diabetes for a prolonged time develop different complications such as vision loss, nephropathy with risk of foot ulcers, autonomic neuropathy effecting gastrointestinal, genital and cardiovascular systems (21).

The incidence of diabetes has increased and continued to rise, especially in high-population and developing countries (23). Regions like the middle east and north Africa seem to have the highest frequency of diabetes in adults (10.9%), while other regions like the western pacific have the highest number of adults identified as diabetic with a percentage of 37.5 (21).

2.1.1. Type 1 Diabetes

Type 1 DM (T1DM) is identified with the depletion of insulin-producing β cells and accounts for approximately 10% of DM cases. Genetic and environmental factors are involved in the pathology of T1DM. T cell-induced immune attack destroys β cells (24). Therefore, T1DM is a chronic autoimmune pathological condition that requires lifelong insulin dependence (25, 26).

T1DM was formerly called "childhood diabetes" or "insulin-dependent diabetes" (25). The disease is mostly seen in younger groups of people starting from children to young adults. In other words, it can be seen at any age (24, 26). These patients have a high cardiovascular risk (24).

2.1.2. Type 2 Diabetes

Type 2 DM (T2DM) is the most frequent (90%) type of DM. Hyperglycemia develops due to insulin deficiency or insulin insensitivity. It is also often referred to as "insulin resistance". Although it is mainly seen in older adults, it can now be seen at any age due to nutritional problems and lack of movement. The incidence of T2DM is increasing day by day (24). The organs that play a role in T2DM are the pancreas (β-cells and α-cells), brain, liver, kidneys, small intestine, skeletal muscle, and adipose tissues. Moreover, the abnormal changes in gut microbiota, immune dysregulation and inflammation may be important pathophysiological factors of T2DM (27).

2.1.3. Type 3 Diabetes

The term "Type 3 DM" is used for the insulin-resistant milieu in the neuropathogenic state of Alzheimer's disease (AD). Insulin resistance plays an important role in AD progression. The incidence of AD is high in patients with T2DM (28). Amyloid β, tau, and amylin can accumulate in the brain in both T2DM and AD (29). Insulin helps break down, transport, and modify amyloid-β from the brain. Defective insulin signaling leads to abnormal accumulation of these misfolded peptides in the brain (28). Amylin released from pancreatic β cells regulates blood sugar. However, in AD, oxidative stress and inflammation develop with the accumulation of amylin in the neurons. Advanced glycosylation end-products (AGEs) induce neurotoxicity by promoting the aggregation of amyloid oligomers (29).

2.1.4. Gestational Diabetes

Gestational diabetes, the most common cause of pregnancy complications, is an important health problem characterized by hyperglycemia affecting the mother, fetus, and newborn (30). Glucose tolerance usually returns to normal after delivery, but it does not return to normal in some women, increasing the risk of T2DM (31). In the second trimester of pregnancy, there is an increase in placental hormones and this causes insulin resistance (24, 32). The pancreas tries to compensate for maternal insulin resistance by producing more

insulin. Excessive insulin secretion leads to beta-cell dysfunction (32). Hyperglycemia, insulin resistance, and hyperinsulinemia bring some complications. Fetoplacental endothelial dysfunction develops due to maternal hyperglycemia (30).

2.2. Kidneys

The kidneys are oval bean-shaped organs (Figure 1) (33, 35, 36). The primary role of the kidney is to preserve the homeostasis of the body by controlling the selective retention or elimination of water, electrolytes, and other solutes (34). The structural and functional units of the kidney, nephrons, play many roles including the regulation of electrolyte, acid-base and volume homeostasis, the filtration and excretion of toxins, xenobiotic and metabolic wastes, the reabsorption of nutrients, water, and electrolytes, the endocrine activity, and interaction with the sympathetic nervous system (4). The time-adjusted glomerular filtration volume is defined as the glomerular filtration rate (GFR) (34).

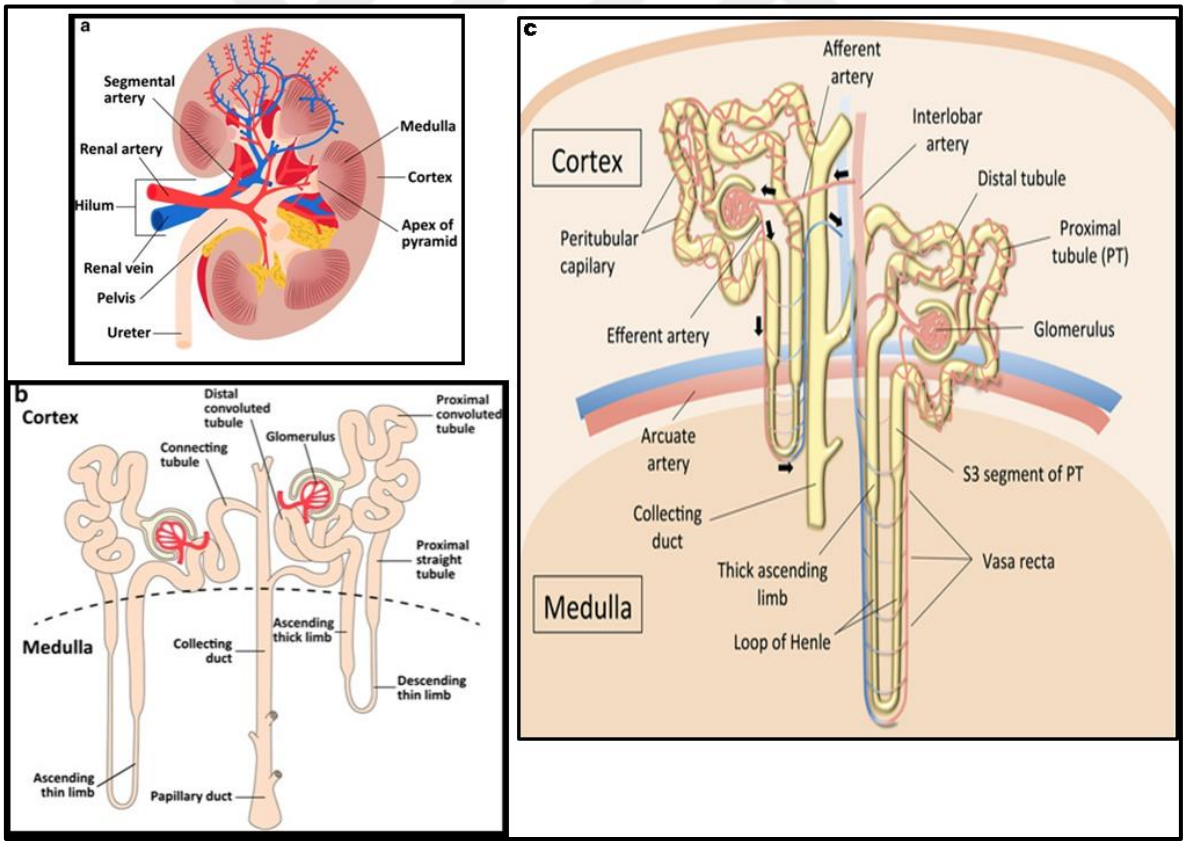


Figure 1. Kidney structure and parts, a) Kidney structure b) The parts of a nephron and c) The microvasculature of a nephron (From Ishimoto, 35 and de Boer, 36)

2.2.1. Kidney Function Biomarkers

There are different parameters in the evaluation of kidney functions (Figure 2): Cystatin C, β2-microglobulin (B2M), kidney damage molecule-1 (KIM-1), clusterin, netrin-1, trefoil factor-3 (TFF-3), lipocalin-2 (NGAL), interleukin-18 (IL-18), tissue inhibitor of metalloproteinase 2 (TIMP2), liver-type fatty acid binding protein (L-FABP), insulin-like growth factor-binding protein 7 (IGFBP7), albumin, urinary exosomes, etc. (5-7).

Blood urea nitrogen is a low molecular weight serum byproduct of protein metabolism (37). Although BUN and serum creatinine are the most commonly used markers for the clinical detection of kidney injury, the level of these parameters can also change independently of renal function (7, 38). Another parameter is serum and urine albumin (39). Albumin is considered one of the most abundant circulating proteins in the plasma. It is produced by the liver cells and rapidly sent to the bloodstream (40). Because, albumin is reabsorbed, processed, and catabolized from the glomerular filtrate by the proximal tubule (41). The decrease in serum albumin may reflect the decrease in eGFR (39).

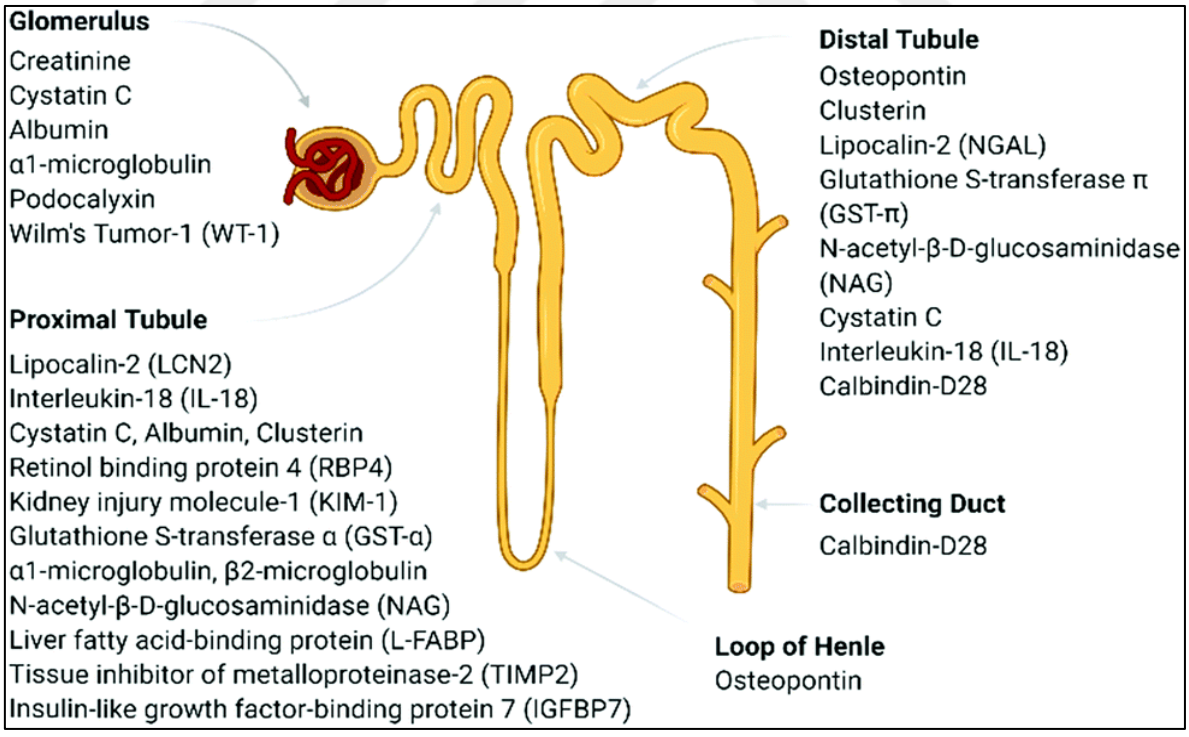


Figure 2. Biomarkers for kidney functions (From Yao, 5)

2.2.1.1. Creatinine

The amino acids glycine, arginine, and methionine are needed for the synthesis of endogenous creatine (2-[Carbamimidoyl(methyl)amino]acetic acid, 131.13 g/mol) It is synthesized by the action of L-arginine glycine amidinotransferase (AGAT) and guanidinoacetate N-methyltransferase (GAMT)] enzymes in the kidney and liver, respectively (42, 43). Creatinine (2-Imino-1-methyl imidazolidine-4-one, 113.12 g/mol) is a lactam form and a metabolite of the creatine molecule (44). The skeletal muscle is considered the main detainer of creatine and creatine phosphate (8). Serum creatinine is produced by a non-enzymatic spontaneous anhydration of creatine in the muscle cells. This process depends respectively on pH and temperature levels (8). Serum creatinine exposes to passive clearance by glomerular filtration and active clearance by tubular secretion (45). Since it is a small molecule, it is almost eliminated via glomerular filtration. Creatinine is one of the most frequently evaluated parameters for kidney function. (46). Creatinine clearance is better for estimating glomerular filtration rate (GFR) but has some disadvantages when assessing renal function (45, 46). Changes in GFR levels are reflected in creatinine levels late (7). Moreover, creatinine is affected by many factors including muscle mass, protein intake, gender, time of day, certain medications, hyperglycemia, and pregnancy. In addition, the gut microbiota breaks down creatinine in advanced chronic kidney disease. Therefore, it does not fully reflect kidney function (45, 46).

Since the skeletal muscle is one of the main target organs of insulin, there may be a link between serum creatinine and T2DM. A decrease in skeletal muscle may lead to the elevation of the risk of T2DM (47).

2.2.1.2. Cystatin C

Cystatin C (13.3 kDa) is a nonglycosylated protein in all nucleated cells, generated at a constant rate by the *CST3* gene (9, 11, 48, 49). It is a potent competitive extracellular inhibitor of cysteine proteinases; mainly controls extracellular protease activity (50). It is involved in the inflammatory cascade (45). Because of its basic pH, cystatin C is filtered from the glomerulus freely but is completely reabsorbed in the proximal tubules. It is not reabsorbed into the bloodstream (51). Cystatin C is an alternative endogenous parameter for the assessment of GFR (10). Since it is less affected by external factors (i.e. gender, race, muscle mass), it is a more reliable marker than serum creatinine in reflecting kidney functions (11, 52). Contrary to creatinine cystatin C is not released through the proximal

tubules, and its creation is not affected by the muscle mass or any other variables like dietary meat intake (51). This molecule is diffused in the extracellular fluid. On the other hand, creatinine is diffused in the internal environment of the whole body, which means that in diminished glomerular filtration rate, serum cystatin C rises faster than creatinine (38). In addition to evaluating kidney disease, serum cystatin C can also be evaluated as a marker to reflect problems that cause cardiovascular diseases such as insulin resistance, metabolic syndrome, obesity and essential hypertension (53).

2.2.1.3. Angiotensin-Converting Enzyme 2

Angiotensin-converting enzyme 2, which converts Ang I to Ang (1-9) and Ang II to Ang (1-7), is widely distributed throughout the body organs such as lungs, heart and kidneys (Figure 3). It is an important counter-regulatory of RAAS (12, 14). By activating the signaling pathways, RAAS balances fluids and natrium, and modulates the homeostasis of cardiovascular circulation and blood pressure. The physiological balance of sodium and fluid is sustained by the activation of the signaling pathways by RAAS along with regulating the homeostasis of blood pressure and cardiovascular circulation (54). Enzymatic reactions within the renin-angiotensin system produce Ang II, which promotes vasoconstriction and inflammation and detrimental cardiovascular effects. ACE2 cleaves angiotensin II, balancing RAAS (55). It is an important counter-regulatory enzyme by converting Ang II to Ang (1–7), that reduces blood pressure and inflammation by controlling vasodilation, increasing renal excretion of water and natrium ion, and producing nitric oxide (12). Plasma ACE2 may be a marker of RAAS (55). Therefore, dysregulation of the renin-angiotensin system and elevated ACE2 are associated with cardiovascular diseases (12, 56). ACE2 is found at the brush border of the proximal tubule and glomerular podocyte in the kidneys. Ang II promotes the progression of kidney disease, while Ang (1-7) promotes regression. Therefore, ACE2 is important for the kidneys. Loss of ACE2 can cause inflammation and fibrosis in the tubulointerstitium and glomerulus (13). ACE2 levels vary with age, with the highest levels in young animals and the lowest in older animals, with lower levels in older males and females (14, 57).

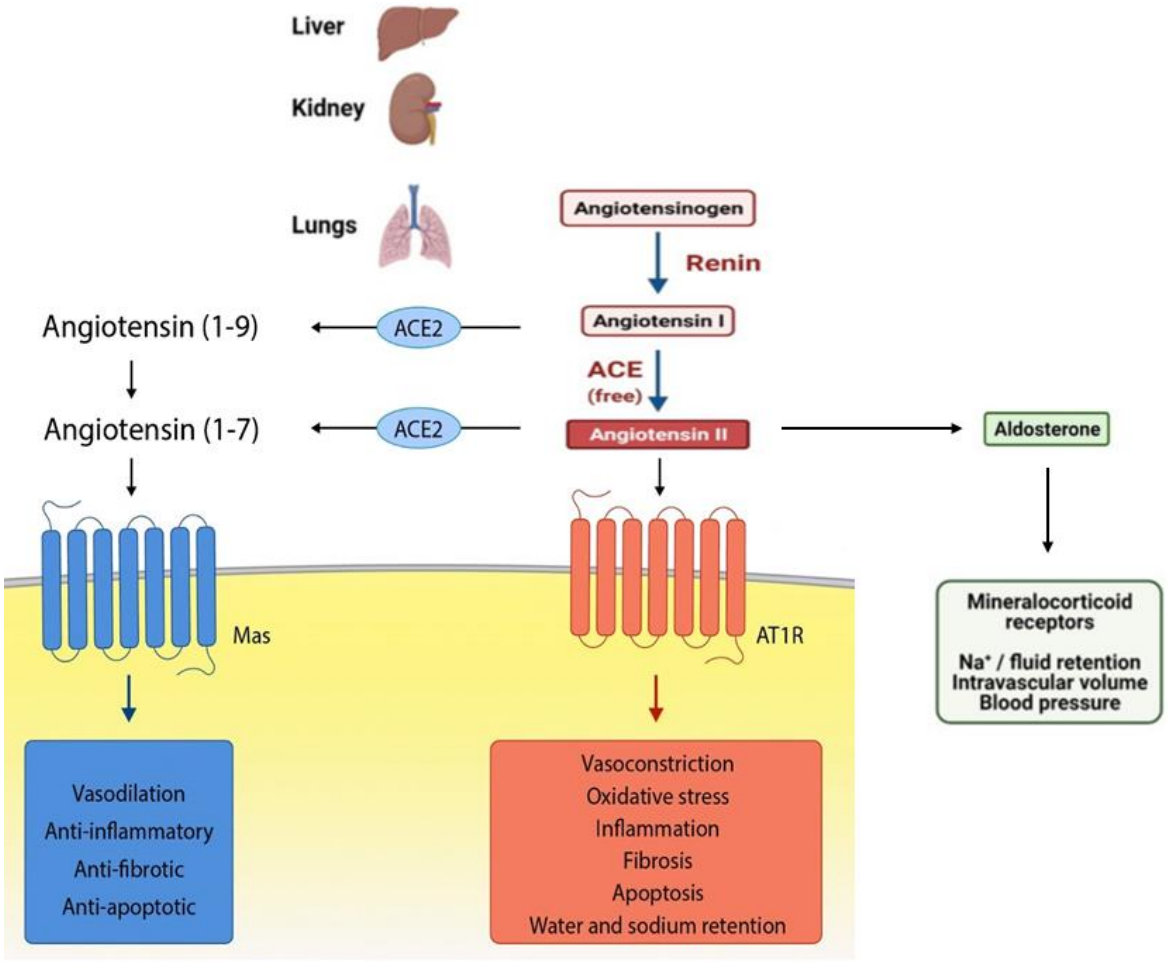


Figure 3. The effects of ACE2 and the renin-angiotensin-aldosterone system (Modified from Beyerstedt, 58 and Cooper, 59) ACE2: Angiotensin-converting enzyme 2; AT1R: Angiotensin II type 1 receptor.

2.2.2. Kidneys and Diabetes Mellitus

Kidneys play an important role in maintaining glucose homeostasis. Glucose is freely filtered in the glomerulus and undergoes tubular filtration by sodium-glucose cotransporters (SGLTs). Filtered glucose is reabsorbed in the proximal tubule. Although the low GFR seen in kidney disease reduces the filtered glucose load, the reabsorption capacity decreases due to the decrease in nephrons, and glucose excretion increases. While the kidneys use 10% of the plasma glucose as an energy source, they contribute about 25% to the blood glucose in the fasting state. On the other hand, it also enacts the metabolism of insulin. Briefly, the

kidneys play a role in the filtration and absorption of glucose, the regulation of its metabolism, and its own energy needs (2).

Diabetes Mellitus leads to kidney disease, vascular endothelial damage, mesangial cell proliferation and matrix expansion, advanced protein glycosylation, and the release of cytokines. It continues with some problems including metabolic acidosis, electrolyte imbalance, edema, excessive protein degradation, body weight, and muscle loss. As a result, insulin resistance, advanced glycation products, and lipid accumulation can be seen (54). Hyperglycemia in DM triggers GLUT1/2-dependent basolateral glucose uptake causing to increase in total kidney mass by remodeling epithelial cells and resulting in tubular hypertrophy. The progression of lesions in the diabetic kidney increases with some conditions including hypoxia, oxidative stress, inflammation and apoptosis (3).

2.3. Electrolytes, Kidney and Diabetes Mellitus

Electrolytes are very important for different body processes such as controlling fluids, maintaining acid-base balance (pH), blood clotting, muscle contraction and nerve conduction (15). Electrolyte disorders are often multifaceted. It contains multiple physiologic abnormal states that act in combination or alone (i.e. coexistent acid-base defects, gastrointestinal absorption capacity and other co-occurring health conditions) (60).

Electrolyte imbalance is more common in diabetic patients, especially in those with kidney disease. This imbalance is more common in hospitalized diabetics (15). Diabetic ketoacidosis, one of the most common complications of DM, is triggered by electrolyte imbalance. Hyperosmolality develops in hyperglycemia (24). Glucose, an osmotically active molecule, causes the movement of water out of the cells, increasing serum osmolality (60, 61). Hyperglycemia leads to cellular dehydration by disrupting cell surface receptors and transport channels that carry fluid and electrolytes from the intracellular space to the extracellular space (15). This can cause hypotension and shock (24).

Sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) are electrolytes found in serum. These electrolytes are responsible for the sustainment of mediating metabolism and cellular function, as well as the enzyme activities and maintenance of electrical gradients (15). The kidneys functions in regulating electrolytes and fluid balance (Figure 4) (62).

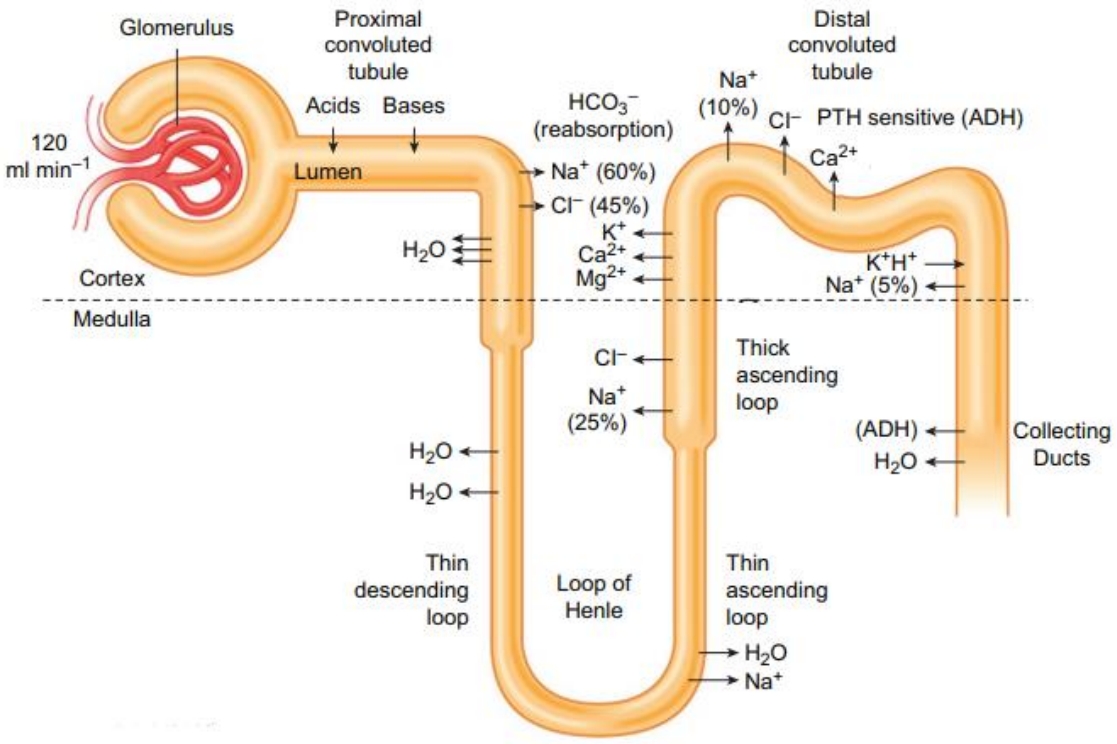


Figure 4. The fluxes of electrolytes along the nephron (Modified from Bell, 62)

Sodium is an osmotically active cation. It is considered one of the most important electrolytes. It plays a role in the maintenance of the extracellular fluid volume and can regulate the membrane potential of cells. This electrolyte is exchanged with potassium across the cell membrane by active transport. Sodium is regulated and mostly absorbed in the distal convoluted tubule of the kidneys (63).

In a diabetic coma, the intracellular movement of electrolytes is impaired due to impaired insulin action. This leads to the deterioration of serum Na^+ and K^+ levels (15). Hyponatremia (low Na^+) develops due to intracellular dehydration, especially in uncontrolled DM (60, 61). Increased ketone bodies in diabetic ketoacidosis induce sodium loss via urinary electrolyte increase. In addition, hypernatremia may develop due to osmotic diuresis. Hypernatremia may occur in cases of greater water loss from sodium and potassium (60). In DM patients with hyperlipidemia, Na^+ may also be artificially decreased (pseudohyponatremia) due to the decrease in water in the serum. Even if patients have normal serum sodium levels (pseudonorrmonatremia), hypernatremia should be considered. In diabetes, changes in the renin-angiotensin system also cause changes in serum sodium concentration (61).

Potassium is one of the most abundant ions in the human body, it is mostly found intracellularly. Potassium intake is made by the Na/K-ATPase pump and flows through the potassium channels. Intracellular potassium distribution is influenced by different intra/extracellular factors such as acid-base status, hormones, and osmolality / osmotic pressure, and potassium concentrations (64). The kidneys are the main filtrating point for the potassium ion specifically at the glomerulus. Potassium is reabsorbed at the proximal convoluted tubule and thick ascending loop of Henle. However, its secretion takes place at the distal convoluted tubule (63).

Hypokalemia in DM is seen due to the redistribution of K^+ into the extracellular fluid compartment due to insulin administration, and gastrointestinal and renal potassium loss. Insulin deficiency leads to the development of hyperkalemia. The decrease in pH seen in acidosis increases the K^+ concentration. Hyperkalemia may develop due to decreased tubular secretion of K^+ in hyporeninemic hypoaldosteronism syndrome in diabetics (60). Hyperkalemia increases plasma tonicity (61).

Chloride (Cl^-) is an important anion involved in the regulation of cell volume and acid-base homeostasis. It is abundant in extracellular fluid and accounts for a significant portion (about one-third) of extracellular fluid tonicity (65, 66). This ion regulates renin secretion, renal sodium utilization, blood pressure, oxygen transportation, and muscular electrical activity (65, 67). The kidney, which is an important regulator of chloride homeostasis, provides chloride reabsorption and maintains extracellular fluid volume. Filtration of chloride happens at the glomerulus and the reabsorption at mainly proximal and secondly distal tubules by both active and passive transport (64).

In the case of hyperchloremia, kidney damage may develop (66). In DM ketoacidosis, keto acids reduce the blood pH and disrupt the acid-base balance, which increases the serum chloride level (61). Therefore, serum chloride level has been an important laboratory parameter to be considered in cases of metabolic acidosis (67).

Magnesium is essential for life, takes place in numerous (>300) enzymatic reactions to maintain cell function, and is involved in the carbohydrate, lipid, protein, and nucleic acid metabolism, ATP utilization, mitochondrial function, regulation of ion channels, neurotransmitter releasing, oxidative stress, immunity, cell proliferation, etc. (60, 68, 69). Magnesium is needed as a cofactor in the drift of glucose into the cell and for carbohydrate metabolism. It is involved in the cellular activity of insulin. Insufficient magnesium intake

is considered a risk factor for diabetes. (70). Magnesium plays a role in glutathione synthesis as a cofactor, which is believed to be linked to its antioxidant capacity and may upregulate antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase. Moreover, magnesium is capable of protecting against oxidative damage and inactivating lipid peroxidation (71).

Most of the magnesium is found in bone and muscle. The main excretion route of magnesium is the kidneys. Most of the filtered magnesium is reabsorbed in the loop of Henle. The final site of Mg reabsorption is in the distal convoluted tubule (68). Renal hypomagnesemia may lead to an augmentation of the glomerular filtration and insufficient reabsorption in proximal tubular cells. In addition, it may lead to the thickening of the ascending limb of the loop of Henle and the distal convoluted tubule (69). Magnesium deficiency exacerbates tubular cell death and phosphate-induced inflammation it causes the progression of chronic kidney disease (72).

Osmotic diuresis also causes hypomagnesemia in diabetic patients. Hyperglycemia leads to renal Mg^{2+} loss. The incidence of hypomagnesemia is higher in diabetic acidosis. Gastrointestinal system disorder also increases magnesium loss. In addition, hypoalbuminemia in DM may give the appearance of false hypomagnesemia. Serious pathological problems may develop with electrolyte disturbances such as hypokalemia, hypocalcemia, and hypophosphatemia accompanied by hypomagnesemia. Therefore, hypomagnesemia in patients with diabetic nephropathy reflects advanced kidney disease. Hypomagnesemia worsens DM by impairing carbohydrate metabolism (60). In patients with T2DM, hypomagnesemia reflects renal dysfunction (68). Tubular dysfunction may contribute to urinary magnesium loss by impairing magnesium absorption (72). Hypocalcemia is a manifestation of severe hypomagnesemia (68).

Calcium is an important electrolyte with many functions including bone and tooth structure, muscle contraction, blood coagulation, cardiac contractility, normal neurological function, and some hormones (73). Ca^{2+} is an electrolyte mostly found in extracellular fluid. It is mostly absorbed in the intestine under the control of the hormonally active form of vitamin D. Moreover, Ca^{2+} secretion is regulated by the parathyroid hormone in the distal tubule of the kidneys (63). Kidney disease causes imbalances in renal calcium transport. Hypercalcemia develops when the absorption of calcium in the nephrons is low and its urinary excretion is high. Hypercalcemia causes renal vasoconstriction (73). Cytolytic

calcium is involved in insulin secretion in β cells. The rapid increase of Ca^{2+} in the cell promotes insulin secretion and calcium homeostasis is widely impaired in diabetics (74). In DM, calcium absorption deficiency due to intestinal disorder may result in hypocalcemia. Because, hyperglycemia may lead to calcuresis or hypercalciuria (75). The changes in urine pH may cause hypercalciuria (when metabolic acidosis) or a decrease in calcium excretion (when metabolic alkalosis) (73).

Iron (Fe^{2+}) is an indispensable element for life, which has many functions, mainly carrying oxygen (76). Cells that have high metabolic rates need more iron and are at a major risk for dysfunction during iron deficiency (77). It is also necessary for the metabolic needs of kidney cells. The kidney prevents iron loss from the body by absorbing filtered iron. In kidney dysfunction and/or damage, blood iron levels may decrease due to iron transport disorder or increased urinary excretion. On the other hand, disruption of iron homeostasis can cause kidney damage. Because, Fe^{2+} causes some pathological conditions (via the Fenton reaction), such as oxidative stress, inflammation, and mitochondrial dysfunction. Therefore, an increase in blood iron or an accumulation of iron in the kidney, or excess iron excretion in the urine may reflect kidney damage problems (76).

Iron has a role in the effect and secretion of insulin (78). Insulin contributes to iron metabolism by affecting the distribution of transferrin on the cell surface and increasing iron uptake into tissues. Insulin sensitivity increases in iron deficiency (79). On the other hand, Fe^{2+} causes tissue damage by producing reactive oxygen species (ROS) with its pro-oxidant property. Moreover, an increase in iron levels increases the risk of DM. Iron accumulation in pancreatic β cells may impair insulin secretion (79).

2.4. L-Theanine

Tea is one of the most consumed beverages on the planet, especially in Türkiye. Teas produced from *Camellia Sinensis* are the unfermented type green tea, the semi-fermented type oolong tea, and entirely fermented and dried black or red tea (80). LTEA is viewed as the most abundant amino acid in these teas. It gives a unique taste and smell to the tea (81). Theanine synthetase is responsible for the ligation of ethylamine and L-glutamate to form LTEA in tea plants. Moreover, theanine amidohydrolase has the potential to hydrolyze LTEA, as a result, making L-glutamic acid and ethylamine that is homogenous to the one catalyzed by glutamine amidohydrolase (82). LTEA is mostly found in the cotyledons, shoots, and roots of the tea plant seedling. Additionally, in mature plants, its biosynthesis

takes place in the roots and is transferred to the phloem via the stem to attend to the growing shoots. LTEA abundance in tea trees depends on different factors such as the method of cultivation, tea grade and variety, time of harvest, and exposure to sunlight (16). It is considered that one cup (250 mL) of adequately brewed green may provide around 40 mg of LTEA (83).

L-Theanine (MW. = 174.2 g/mol) also has known different names such as 5-N-ethylglutamine, γ-ethylamino-L-glutamic acid, γ-glutamylethylamide, γ-L-glu-ethylamide, and γ-glutamyl-L-ethylamide (16). It shares a similar structure with L-glutamine as a result LTEA has the potential to compete with L-glutamine for its receptors on the surface of cell membranes (84).

Orally induced LTEA is rapidly immersed into the bloodstream via intestinal absorption, which will be transferred to the body's major organs such as the brain. The concentration of LTEA attains its maximum level in the bloodstream about 30 min to 2 hours from the administration. It is removed from the body via urine in which the amino acid will be catabolized by amide hydrolysis resulting in glutamic acid and ethylamine (16).

L-Theanine has different positive effects on health such as relaxation effects, learning ability improvement, cancer prevention, and prevention of vascular diseases, improvement of the immune system, effects on weight loss, lipid-lowering, antihypertensive, chemosensitizing, and hepatoprotective (16).

This amino acid penetrates the blood-brain barrier (85). It may reduce the stimulating effect of caffeine on the central nervous system. Moreover, it has a neuroprotective effect to its capability of inhibiting neuronal cell death after cerebral ischemia such as stroke (86). It has the ability to reduce neurodegeneration and improve neuronal survival (87). LTEA may also act as an attenuator of stress-related symptoms such as depression, and anxiety, and it may improve cognitive function (88).

L-Theanine may act as a biochemical modulator for chemotherapeutics and may suppress tumor cell invasion. The antitumor effect of this amino acid has been investigated in many studies, which conclude that LTEA is strongly effective as an antitumor component (89). In addition, LTEA deploys an anti-inflammation and extracellular matrix-protection effect and may display a positive effect on damaged- cartilage (90).

Moreover, LTEA is considered an anti-obesity component and may be beneficial for weight loss (91). It may have the ability to protect partially the pancreatic β-cells in an oxidative stress environment and may be considered an insulinotropic agent, which may be utilized as a preventive treatment for diabetes (92). It has the ability to initiate the insulin pathway. It can regulate the metabolisms of biomolecules (carbohydrate, lipid, protein…) via the signaling of insulin and AMP-activated protein kinase, as well as their downstream signaling pathways. LTEA promotes glycogen synthesis and conceals gluconeogenesis. Moreover, it has been suggested that this amino acid stimulates glycolysis (93). It can also affect the regulation of the metabolism by reducing glucose uptake via SGLT3 and GLUT5, and regulating the intestinal microflora (19).

Studies have shown that LTEA has protective effects against kidney damage in rats with experimental sepsis (17) and doxorubicin (DOX)-induced nephrotoxicity (18, 94). In acute nephrotoxic rats, LTEA may have the ability to decrease creatinine clearance, and inhibit the lipid peroxidation process and the reduction of glutathione content. Therefore, LTEA may play a role in protecting the kidney by modifying the levels of glutathione (19).

3. MATERIALS and METHODS

3.1. Materials

The list of devices, tools, and materials used for this study and their manufacturers are in Table 1; the chemicals are in Table 2, and the commercial kits are in Table 3.

Table 1. Devices, tools, and materials used in the laboratories, and their manufacturers

Devices, Tools and Materials	Manufacturer
Autoanalyzer	Beckman Coulter AU5800 (USA)
Automatic Pipettes	Socorex (Swiss); Isolab (Germany); Ependorf (Germany)
Centrifuge	Eppendorf, Centrifuge 5810 (Germany)
Deep Freezer (-80 °C)	Thermo Electron Corporation (USA)
ELISA Washer	Biotek ELx 50 (USA)
Hand Homogenizer	OMNI-Tissue Master 125 (USA)
Incubator	Galenkamp (England)
Lamella	Marienfield, Lot No: 37393 017 (Germany)
Light Microscope	Olympus BX-51, Olympus Optical Co. (USA)
Magnetic Stirrer	Termal (Türkiye)
Microcentrifuge	Thermo IEC Micromax (USA)
Microcentrifuge Tube	Iso Lab, Lot No: MTPPN7015002 (Germany)
Microplate Reader	Versa Max, Moleculer Devices (USA)
Microplate Washer	BioTek ELx50 (USA)
Microtome	Leica RM2255 (USA)
Microtome Knife	Therma, Lot No: 9G302226 (USA)
pH-meter	Hanna Instruments, HI 9321(UK)
Pipette Tips	Kartell Spa (Italy), Lot No: 4806; Iso Lab (Germany), Lot No: PTPPN19010002
Positively Charged Slide	HDA, 20181122 (China)
Precision Balance	Mettler Toledo AB 204-S (Sweden)
Refrigerated Centrifuge	Beckman-Coulter (USA)
Refrigerator (+4 °C)	Arçelik, Vestel (Türkiye)
Rolled Lam	Marienfield, Lot No: 37909306 (Germany)
Shaker Incubator	ShelLab/Sheldon S14-2 (USA)
Sonication Device	Sonics Vibra-Cell, 42174L (USA)
Vortex	IKA Vortex, Genius 3 (Türkiye)
Water Purifier	Kros (Türkiye)

Table 2. Chemicals

Chemicals	Manufacturer, Code and Purity
Absolute Ethanol	Smyras, Lot No: 305240919060 (Türkiye)
Entellan	Merck, Lot No: HX378130 (Germany)
Eosin	Biognost, Lot No: E0YA-13/18 (Belgium)
Formaldehyde	Sigma, Lot No: SZBE2450V (Germany)
Phosphate Buffer (PBS)	VWR Life Science, Cat No: E404 (USA)
Granular Paraffin	Tekkim, Lot No: 240215224 (Türkiye)
Crystal Violet	ZAG Chemicals, Cas No: 548-62-9 (Türkiye)
Xylene	Tekkim, Lot No: 090709134001 (Türkiye)
L-Theanine	Chem-Impex Intl, Cat No: 14293, (%99) (USA)
Nicotinamide	Sigma, Cat No: 102116371, (≥ %99.5) (Germany)
Streptozotocin	Cayman, Lot No: 1.800.364.9897 (≥ %95) (USA)
Trichrome Stain (Masson)	Histomed, Lot No: 252017.002 (Türkiye)
Triton X-100	Merck, K2351869, (> %95) (Germany)
Ethyl Alcohol	Aytaş, Lot No: 200-578-6 (%96) (Türkiye)

Table 3. Commercial kits

Kits	Trademark, Product code, Lot No, City, Country
ACE2	Elabscience, E-EL-R2453, 7LKEIMPXYD, Texas, USA
GSH	Cayman Chemical, 703002, Michigan, USA
GSSG	Cayman Chemical, 703002, Michigan, USA
Cystatin C	Elabscience, E-EL-R0304, ZF69MCGLQ2, Texas, USA

3.2. Methods

This study was fulfilled with the support of the Karadeniz Technical University Scientific Research Project (BAP) named “Investigation of the Effects of L-Theanine on Kidney Tissue in Diabetic Rats” (Project No: TYL-2021-9925). In addition, the approval (No: 2021/50, date 24.09.2021).

3.2.1. Experimental groups

In this study, 24 Wistar male rats (2-3 months old, 150-250 g), obtained from KTU Surgical Practice and Research Center were used. Feed and water were given *ad libitum* by providing 12 hours of darkness and 12 hours of daylight. These animals were arbitrarily separated into 4 experimental groups (n=6/group).

Group I (SHAM): Healthy rats given drinking water

Group II (LTEA): Healthy rats given 200 mg/kg/day of L-Theanine

Group III (DM): Diabetic rats given drinking water

Group IV (DM+LTEA): Diabetic rats given L-Theanine

To induce DM, the rats will fast overnight and 60 mg/kg STZ prepared in 0.1 M citrate buffer (pH 4.5) was applied by i.p. To reduce pancreatic cell damage, 15 min before STZ injection, 120 mg/kg NA was given by i.p. After 2 days, blood glucose levels in tail veins were measured with a glucometer (Vitals G400, Ankara, Türkiye). The animals with a fasting glucose concentration >250 mg/dL (13.875 mmol/L) were considered diabetic rats (95).

Drinking water and LTEA (freshly dissolved in water) were given by gastric gavages for 28 days (96, 97).

At the end of the 28-day experiment period, the animals were sacrificed by the decapitation method. The blood of the rats was taken into tubes without anticoagulant and with EDTA and centrifuged at 3000 rpm for 10 min to obtain serum and plasma samples, respectively. After the kidney tissues of the rats are removed, half of them were used for histopathological examinations and the other half for biochemical examinations. Samples taken for biochemical analysis were stored at -80 °C until the day of analysis.

3.2.2. Biochemical Analysis

3.2.2.1. Parameters Analyzed by the Autoanalyzer

Serum glucose, albumin, BUN, creatinine, Na, K, Cl, Ca, Mg, and Fe levels in serum samples were analyzed in Karadeniz Technical University Farabi Hospital Biochemistry Laboratory using a Beckman Coulter Autoanalyzer AU5800.

Glucose concentrations have been analyzed with the enzymatic (hexokinase) method. Glucose-6-phosphate produced by hexokinase is oxidized by the glucose-6-phosphate dehydrogenase enzyme and reduces NAD^+ to NADH. The increase in NADH absorbance at 340 nm reflects glucose level.

Albumin level analysis was based on the photometric method. When bromcresol green (BCG) reacts with albumin, a colored complex is formed in this method. The absorbance at 600/800 nm of the formed complex is proportional to the albumin level.

Creatinine amounts were evaluated with the kinetic color test. This test takes place in an alkaline environment. Creatinine and picric acid sculpt a yellow-orange composite. The rate of change at 520/800 nm is proportional to the creatinine level.

BUN was gauged with the kinetic UV test by hydrolyzing urea with the urease enzyme forming ammonia and carbon dioxide. The formed ammonia fuses with 2-oxoglutarate and NADH while using glutamate dehydrogenase. BUN levels were determined by measuring the decrease in NADH absorbance.

Sodium, **potassium,** and **chloride** levels were measured in the ISE module of Beckman Coulter analyzers. The reagents were used in conjunction with the ISE module for the quantitative gauging of Na^+, K^+, and Cl^- levels. The module using crown ether membrane electrodes measures both sodium and potassium. On the other hand, chloride was analyzed with a molecularly based PVC membrane electrode. Conform to the Nernst Equation, each ion has a specific electrical potential and is compared to an internal reference which is converted to a voltage and finally to an ion concentration of the sample.

Total calcium levels were measured by a photometric color test. This test is based on calcium ions (Ca^{2+}) which react with Arsenazo III (2,2'-[1,8-Dihydroxy-3,6-disulfonophthylene-2,7-bisazo]-bisbenzenarsonic acid) to form a dense purple (Ca-Arsenazo III) complex. The absorbance of the complex are analyzed at 660/700 nm biochromatically. The calcium levels and the augmentation of the absorbance are directly proportional.

Magnesium levels were found by photometric color test. The test takes place in a very acidic environment, in which magnesium ions fuse with xylidyl blue making a colored compound. This formed compound is gauged biochromatically at 520/800 nm. The magnesium concentration and the colored complex are proportional. Calcium interference is eliminated with glycoletherdiamine-N,N,N',N'-tetraacetic acid .

Iron analyzing principle is based on the photometric color test. In the Beckman Coulter method, TPTZ [2,4,6-Tri-(2-pyridyl)-5-triazine] is used as a chromogen. In an acidic environment, iron bound to transferrin is broken down into free valent iron ions and apotransferrin. Hydrochloric acid and sodium ascorbate reduce trivalent iron ions to bivalent. The bivalent iron ions react with TPTZ to form a blue-colored, bichromatic measurable at 600/800 nm. The iron concentration is directly proportional to the rise of the absorbance.

3.2.2.2. Determination of Cystatin C Levels

Cystatin C levels were determined in serum and in kidney tissues of rats by using a commercial cystatin C kit (Elabscience, Item no: E-EL-R0304, Texas, USA) based on Sandwich-ELISA. Tissue homogenate preparation and cystatin C measurement were performed according to the kit protocol described below.

Preparation of Homogenates

100 mg of tissue was placed in 1 mL of PBS buffer. Tissues were homogenized in an ice-cold environment with a homogenizer at 5000 rpm for 30 s. After homogenization, 15 s sonication (130 Watt, 20 Khz) was applied. The homogenates were centrifuged at 5000 xg for 5 min and the supernatant was collected.

Experimental Procedure

1. Sample supernatants prepared for cystatin C measurement, standards, and reagents available in the kit were applied to the microplates following the kit protocol. Absorbance was read at 450 nm using a microplate reader.

2. The sample concentrations were calculated in ng/mL according to the standard graph shown in Figure 5.

3. Cystatin C levels in serum were given as ng/mL but in the tissues were given per protein levels (ng/mg protein).

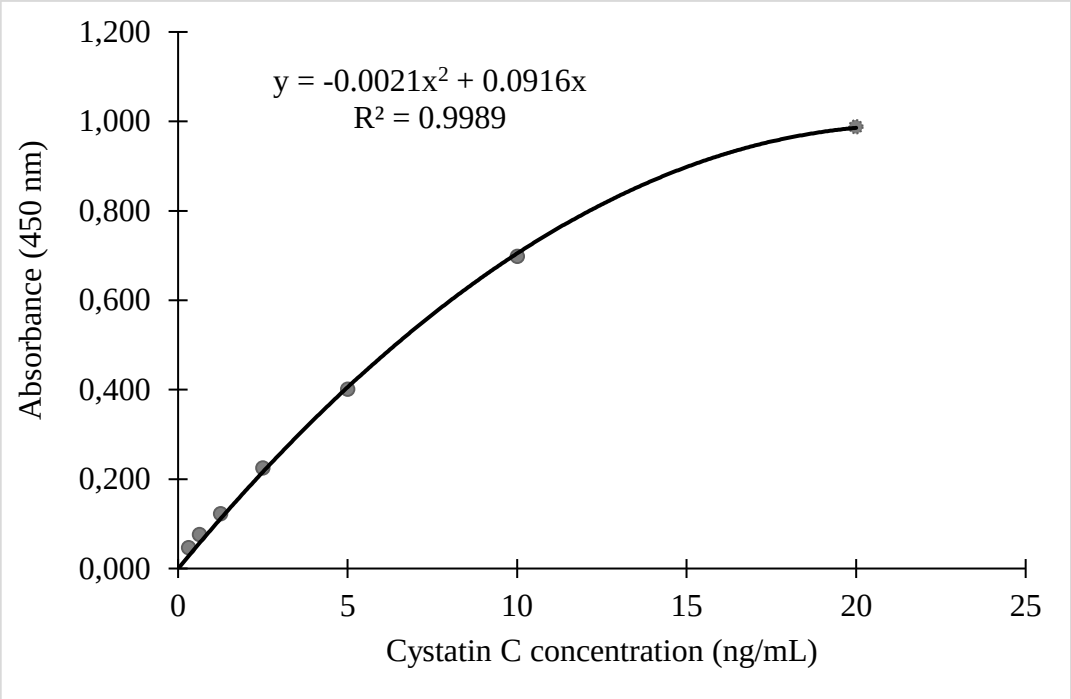


Figure 5. Standard curve for cystatin C concentration

3.2.2.3. Determination of ACE2 Levels

To evaluate the kidney function, ACE2 levels in serum and kidney tissues of rats were measured using a commercially purchased ACE2 ELISA kit (Elabscience, Item no: E-EL-R2453, Texas, USA). Tissue homogenate preparation and ACE2 measurement were performed by the kit protocol.

Preparation of Homogenates

100 mg of tissue was placed in 1 mL of PBS buffer. Tissues in an ice-cold environment were homogenized at 5000 rpm for 30 s. After homogenization, 15 s sonication (130 Watt, 20 Khz, Sonics Vibra-Cell, 42174L) and then centrifugation at 5000xg for 5 min were applied. The obtained supernatants were used.

Experimental Procedure

1. Sample supernatants, standards, and reagents were applied to the microplates according to the kit protocol. Absorbance was read at 450 nm using a microplate reader.

2. The sample concentrations were calculated in ng/mL according to the standard chart in Figure 6.

3. ACE2 levels in serum were given as ng/mL but in the tissues were given per protein levels (ng/mg protein).

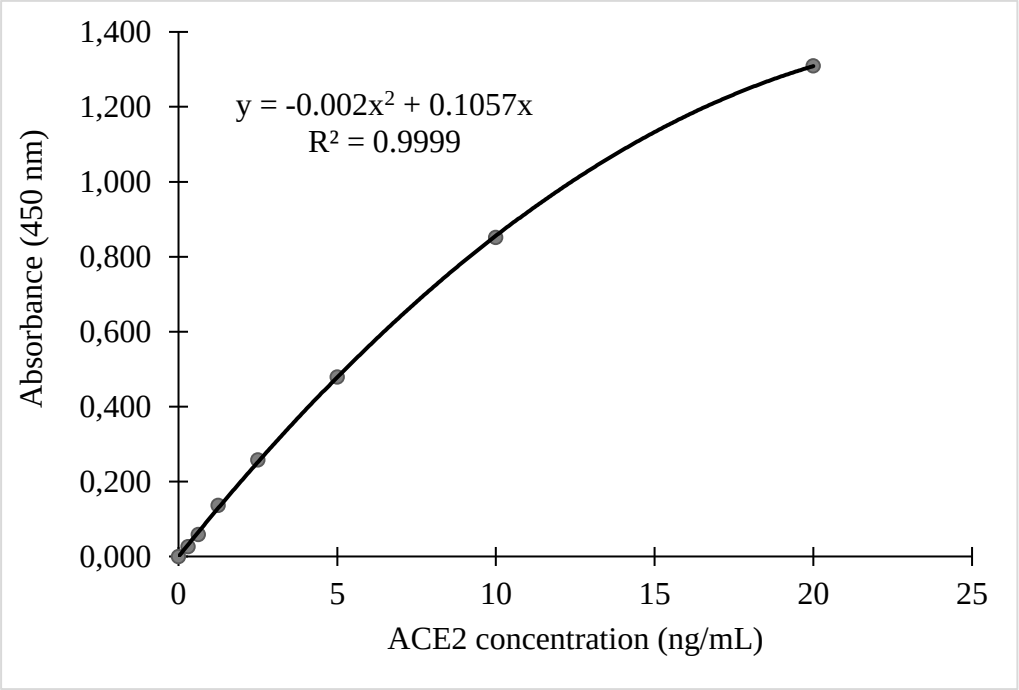


Figure 6. Standard curve for ACE2 concentration

3.2.2.4. Determination of Glutathione Levels

To evaluate the oxidant-antioxidant balance levels, GSH and GSSG levels in serum and kidney tissues were determined using, a commercial glutathione kit (Cayman Chemical, Item No: 703002, Michigan, USA). This kit is based on an enzymatic method using glutathione reductase. As a result of the reaction of the sulfhydryl group of GSH with DNTB (5,5'-dithiol-bis-2-(nitrobenzoic acid), Ellman's reagent), the yellow 5-thio-2-nitrobenzoic acid (TNB) is formed. Measurement of the resulting TNB absorbance (405 nm) gives the amount of GSH in the sample.

Used solutions

1. MPA reagent (10 %): 5 g of metaphosphoric acid was diluted in 50 mL of distilled water.

2. TEAM reagent (4 M): 531 µL of triethanolamine was diluted in 469 µL of distilled water.

3. 2-vinylpiridin (1 M): 108 µL of 2-vinylpiridin was diluted in 892 µL of ethanol.

Preparation of Homogenates

In accordance, 100 mg of tissue was weighed and homogenized in 1 mL of cold 4-morpholinethanesulfic acid (MES) buffer at 5000 rpm for 30 s. It was centrifuged at 10000×g at 4°C and the supernatant was taken.

Deproteinization

Before the GSH and GSSG measurements, deproteinization was applied to the supernatant. An equal volume of 10 % metaphosphoric acid (MPA) reagent was added to the samples, vortexed, and incubated at room temperature for 5 min. It was then centrifuged at 2500×g for 5 min. The supernatant was taken and 50 µL of 4 M triethanolamine (TEAM) reagent per mL supernatant was added and vortexed. The prepared samples were used for the determination of total GSH levels.

Derivatizing

Before GSSG measurement, the deproteinized supernatant was derivatized with 2-vinylpyridine. 10 µL of 1 M 2-vinylpyridine was added per mL of deproteinized samples and vortexed. It was incubated for 60 min at room temperature. This process, which was applied to the samples, was also applied to the standards. Samples are ready for GSSG analysis. With the kit for determining GSH and GSSG levels, derivatization of GSH with 2-vinylpyridine is required to determine GSSG levels.

Experimental Procedure

GSSG and GSH standard graphs were drawn (Figure 7, and Figure 8, respectively) and total GSH (TGSH) and GSSG concentrations were calculated with the help of the standard graphs. Tissue GSH and GSSG levels were given in nmol/mg protein units to standardize.

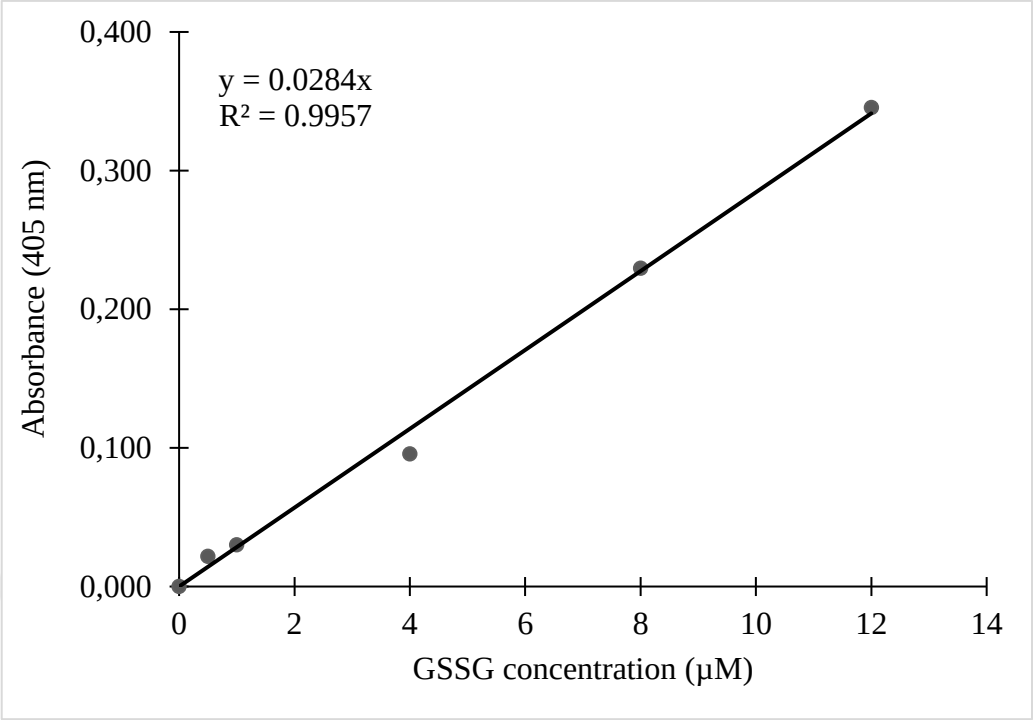


Figure 7. Standard curve for GSSG concentration

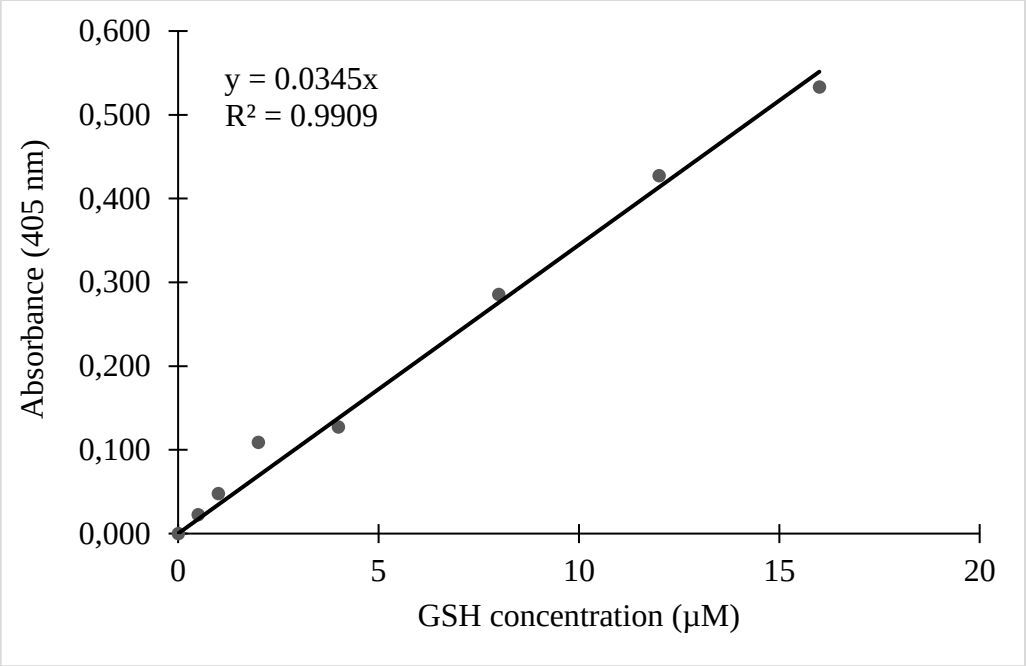


Figure 8. Standard curve for GSH concentration

3.2.2.5. Determination of Protein Levels in Kidney Tissues

It is based on the binding of the proteins in samples to an organic dye, Coomassie Brilliant Blue G250, in a phosphoric acid medium. The formed blue complex gives the maximum absorbance at 600 nm.

Reagents:

1. Bradford Reagent: 10 mg of Coomassie Brilliant Blue G250 was diluted in 5 mL of 95 % ethanol, and 10 mL of 85 % H_3PO_4 was added. The final volume was completed to 100 mL with distilled water.

2. Standards: Bovine serum albumin (BSA) solutions (0.5, 0.4, 0.3, 0.2, 0.1, 0.05 mg/mL) were prepared.

Experimental Procedure:

10 µL of the sample and standard were added to the microplate.100 µL of the Bradford reagent was added and shaken for 10 min in a dark place. The absorbance was read at 600 nm.

The experimental procedure shown in Table 4 was applied.

Table 4. Experimental steps for the protein level determination

	Blank (µL)	Standard (µL)	Sample (µL)
Distilled Water	10	-	
Standard	-	10	-
Sample	-	-	10
Bradford Reagent	100	100	100
Incubation at room temperature and in a dark environment for 10 min.			
Reading of the absorbance at 600 nm.			

By using the protein standard graph (Figure 9), the concentration of the proteins in kidney tissues was calculated as mg/mL.

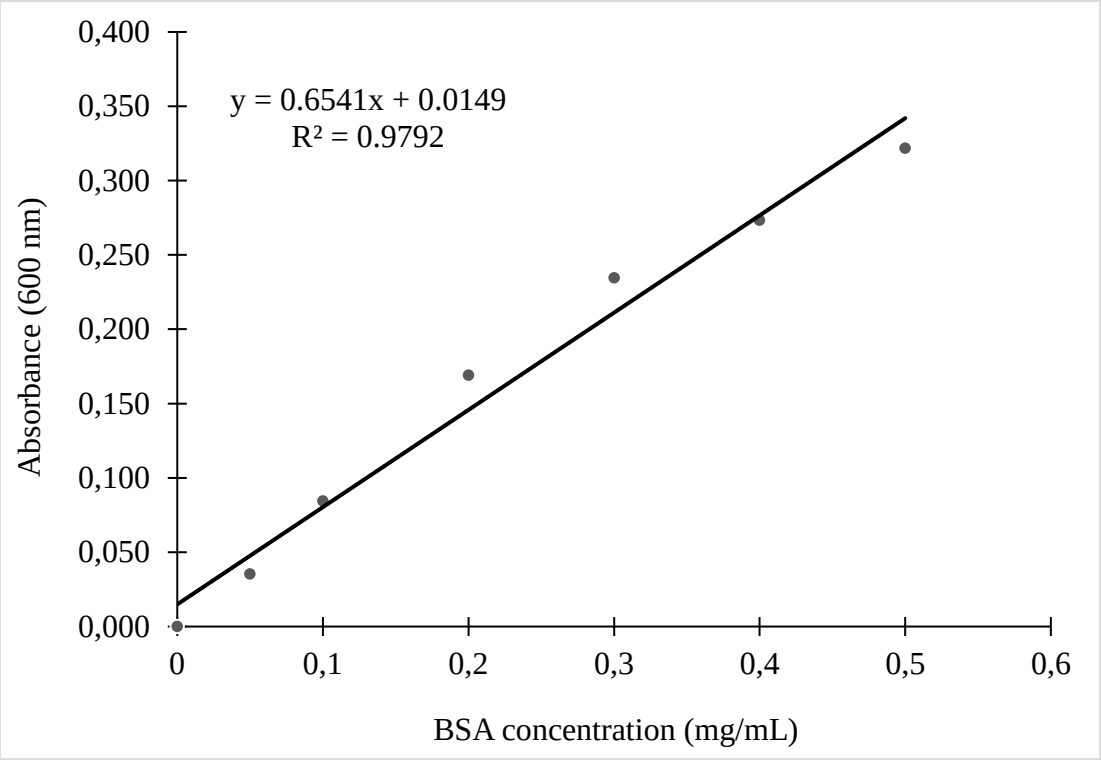


Figure 9. Standard curve for protein concentration

3.2.3. Histopathological Analysis

After fixing of kidney tissues in a 10 % formaldehyde solution for 48 hours, they were dehydrated by passing through a series of increasing degrees of alcohol. Paraffin-embedded tissue sections at 5 μm thickness taken with a fully automated microtome (Leica RM2255, Japan) were stained with hematoxylin-eosin (H&E) for histopathological evaluation. An Olympus BX51 light microscope (Tokyo, Japan) was used to examine and the results were photographed by using the Olympus DP71 (Olympus, Tokyo, Japan) camera attachment at different magnifications. Preparations belonging to all groups were made by an experienced histologist. In particular, the renal cortex and outer medullary area were evaluated under high magnification (400X) in 5 different areas. According to the findings obtained in the evaluation of the preparations, they were examined in terms of focal glomerular necrosis, Bowman's capsule dilatation, degeneration of tubular cells, vacuolization of tubular cells, shedding of tubular epithelial cells, intertubular vascular congestion, tubular enlargement, and intratubular hyaline deposition.

Each section was scored semi-quantitatively between 0-3. Accordingly, (0: no damage; 1: mild=<25% damage, focal and mild changes; 2: moderate=25-50% damage, multifocal, significant changes, and 3: severe=>50% damage, diffuse changes) (98)

3.2.4. Statistical Analysis

The data were statistically evaluated in the "SPSS (IBM SPSS Statistics 23)" program. The conformity of the parameters to the normal distribution was checked with the “Kolmogorov-Smirnov” test. The "Kruskal-Wallis" test was used to compare more than two variables that did not fit the normal distribution, and the "Mann-Whitney U" test was used for binary variables. Results are given as “Median (interquartile range (IQR)) and 25^{th}-75^{th} percentiles”. The “One-Way ANOVA” test was used to evaluate the histological analysis results for more than three independent groups. Tukey test was used for post hoc evaluations. Histological results were given as “mean ± standard error”. $p<0.05$ was considered statistically significant.

4. RESULTS

4.1. Animal Weights

Initiation weights of the study groups, before the treatments with LTEA or drinking water, were initially kept close to each other. However, after 28 days, the DM rats gained less weight and LTEA treatment was not significantly effective to change the diabetic rat weights (Table 5).

Table 5. Body weights and glucose levels of the study groups

Parameters	SHAM	LTEA	DM	DM+LTEA
Initial Weight (g)	[197 (12)] (193-204)	[195 (27)] (182-209)	[197 (16)] (189-205)	[198 (16)] (188-204)
Final Weight (g)	[240 (11)] $^{\#}$ (234-245)	[233 (10)] $^{\#}$ (230-240)	[206 (25)] ^{b} (188-213)	[196 (19)] ^{a,b} (189-208)
Initial Glucose (mmol/L)	[5.42 (0.46)] (5.18-5.64)	[5.51 (0.74)] (5.15-5.88)	[22.12 (9.22)] ^{a,b} (18.18-27.39)	[22.48 (8.16)] ^{a,b} (17.72-25.88)
Final Glucose (mmol/L)	[6.05 (1.38)] * (5.68-7.05)	[6.35 (0.35)] * (6.25-6.60)	[10.95 (3.68)] ^{a,b,*} (9.58-13.25)	[12.55 (7.18)] ^{b,*} (7.93-15.10)

Results were given as "[Median (IQR)] and 25th-75th percentiles".
P values according to 'Mann Whitney U' test.
p<0.05 was accepted statistically significant according to ^{a}: Control; ^{b}: LTEA; ^{c}: DM; $^{\#}$: initial weight;
**: initial glucose level.*

4.2. Biochemical Results

In glucose levels, there was no significant difference in LTEA group compared to SHAM group, and DM+LTEA group to DM goup ($p>0.05$) after 28 days (Table 5). But, a significant elevation was observed in DM group compared to SHAM and LTEA groups. When comparing DM+LTEA and LTEA groups, we identified a bordeline higher level in the DM+LTEA group but not significant ($p=0.09$).

Albumin and creatinine levels were not significantly different among groups ($p>0.05$) (Table 6). However, BUN and serum cystatin C levels were higher in DM groups than SHAM and LTEA groups. Moreover, LTEA treatment decreased blood cystatin C levels in diabetic rats insignificantly ($p>0.05$). On the other hand, although the elevated level in kidney tissue was seen in DM group compared to SHAM, this was not statistically significant ($p=0.180$) (Figure 10).

Table 6. The levels of **s**erum/plasma biochemical parameters in the study groups

Parameters	SHAM	LTEA	DM	DM+LTEA
Albumin (g/L)	[31.90 (2.70)] (30.30-33.00)	[32.05 (4.97)] (29.40-34.37)	[31.50 (6.47)] (27.35-33.83)	[30.40 (2.08)] (29.40-31.48)
BUN (mg/dL)	[18.00 (1.75)] (17.75-19.50)	[20.00 (19.00)] (13.50-32.50)	[31.50 (5.75)] [a] (28.50-34.25)	[31.00 (14.75)] [a] (26.25-41.00)
Creatinine (mg/dL)	[0.22 (0.05)] (0.19-0.24)	[0.24 (0.05)] (0.22-0.27)	[0.24 (0.03)] (0.24-0.27)	[0.24 (0.07)] (0.20-0.27)
Cystatin C (ng/mL)	[0.37 (0.36)] (0.23-0.58)	[0.54 (0.43)] (0.34-0.77)	[1.75 (1.64)] [a,b] (0.87-2.51)	[0.79 (1.46)] (0.43-1.90)
ACE2 (ng/mL)	[0.34 (1.32)] (0.17-1.49)	[0.44 (0.55)] (0.18-0.73)	[1.34 (1.81)] (0.34-2.17)	[1.14 (1.43)] (0.48-1.90)
Sodium (mEq/L)	[140 (6.0)] (138-144)	[125 (23)] (122-145)	[138 (27)] (122-149)	[137 (15.8)] (131-147)
Potassium (mEq/L)	[5.90 (0.52)] (5.68-6.20)	[9.60 (6.90)] (6.10-13.00)	[6.10 (0.42)] (5.95-6.38)	[6.40 (1.38)] (5.93-7.30)
Chloride (mEq/L)	[105 (3.8)] (103-107)	[100 (17)] (92.00-109)	[108 (39)] (82-121)	[98 (14)] (92-106)
T. Calcium (mg/dL)	[10.10 (0.50)] (9.92-10.42)	[9.80 (1.30)] (9.20-10.50)	[10.10 (0.72)] (9.85-10.57)	[10.20 (0.75)] (9.97-10.72)
Magnesium (mg/dL)	[2.01 (0.14)] (1.91-2.04)	[2.02 (0.88)] (1.82-2.70)	[2.14 (0.53)] (1.97-2.49)	[2.02 (0.48)] (1.79-2.28)
Iron (µg/dL)	[185 (76)] (165-246)	[190 (130)] (116-246)	[214 (85)] (171-256)	[154 (80)] [c] (93-172)
GSSG (µM)	[15.3 (5.2)] (13.5-18.3)	[18.6 (2.73)] (16.97-19.70)	[19.2 (8.5)] (15-23.5)	[11.5 (4.50)] [b,c] (11.3-15.8)
TGSH (µM)	[18.2 (5.6)] (15.8-21.4)	[23.2 (4.9)] (20.3-25.3)	[22 (9.07)] (17.3-26.4)	[15.3 (6.5)] [b] (13.6-20)
GSSG/TGSH	[0.85 (0.10)] (0.79-0.88)	[0.79 (0.09)] (0.76-0.85)	[0.88 (0.09)] [b] (0.85-0.94)	[0.80 (0.12)] [c] (0.74-0.86)

Results were given as "[Median (IQR)] and 25th-75th percentiles". P values according to 'Mann Whitney U' test. p<0.05 was accepted statistically significant according to [a]: Control; [b]: LTEA; [c]: DM.
BUN: Blood urea nitrogen; ACE2: Angiotensin-converting enzyme 2; GSSG: Oxidized glutathione; TGSH: Total glutathione.

The levels of ACE2 in serum (Table 10) and kidney tissues (Figure 11) in DM group were higher insignificantly than SHAM group (p=0.177 and p=0.310, respectively). LTEA

treatment was not effective to change ACE2 levels in both serum and kidneys of rats with and without DM ($p>0.05$).

Sodium, potassium, chloride, calcium and magnesium levels were not statistically different in study groups ($p<0.05$). But iron levels showed differences. Borderline insignificant higher levels ($p=0.093$) was observed in DM group than SHAM group. LTEA treatment decreased iron levels significantly in diabetic rats (DM+LTEA group vs DM group, $p<0.05$) (Table 6).

According to the assessment of glutathione levels, serum GSSG and TGSH levels were slightly higher in the DM groups than in the SHAM group ($p>0.05$). LTEA treatment of diabetic rats significantly decreased serum GSSG levels and GSSG/TGSH ratio ($p<0.05$) (Table 6). When the kidney tissues of DM and SHAM groups were compared, we observed an increase in GSSG levels and GSSG/TGSH ratio, but only the difference in GSSG levels was significant ($p<0.05$). LTEA treatment increased GSSG levels and GSSG/TGSH ratio in non-diabetic rats and TGSH in diabetic rats (Figure 12).

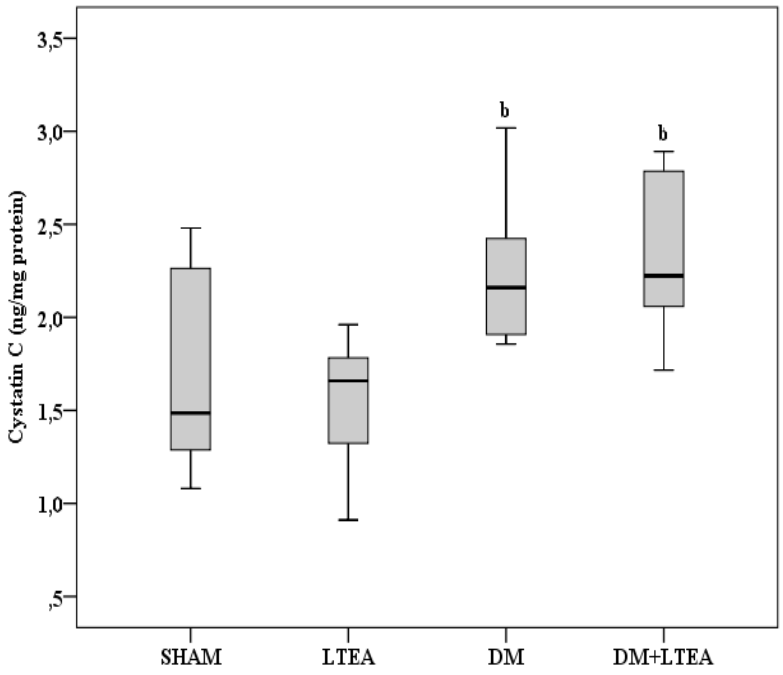


Figure 10. Kidney tissue cystatin C levels in the study groups

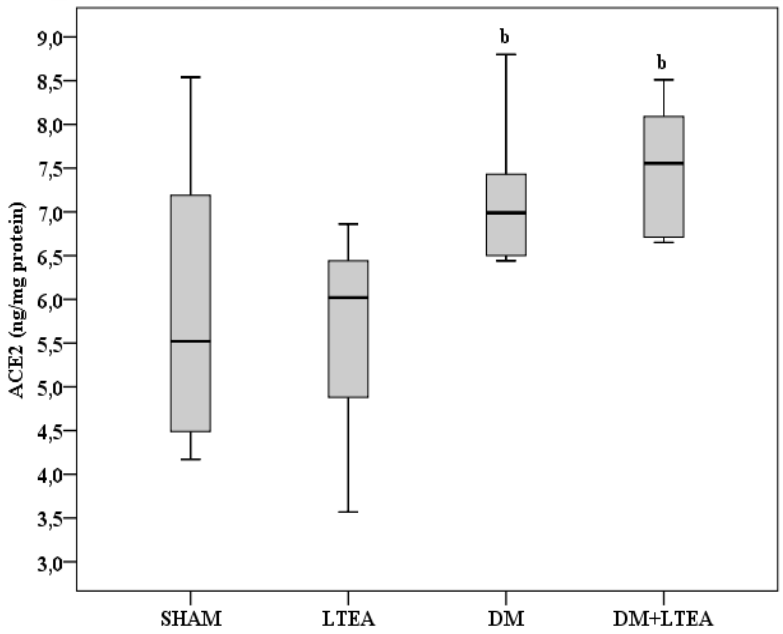


Figure 11. Kidney tissue ACE2 levels in the study groups

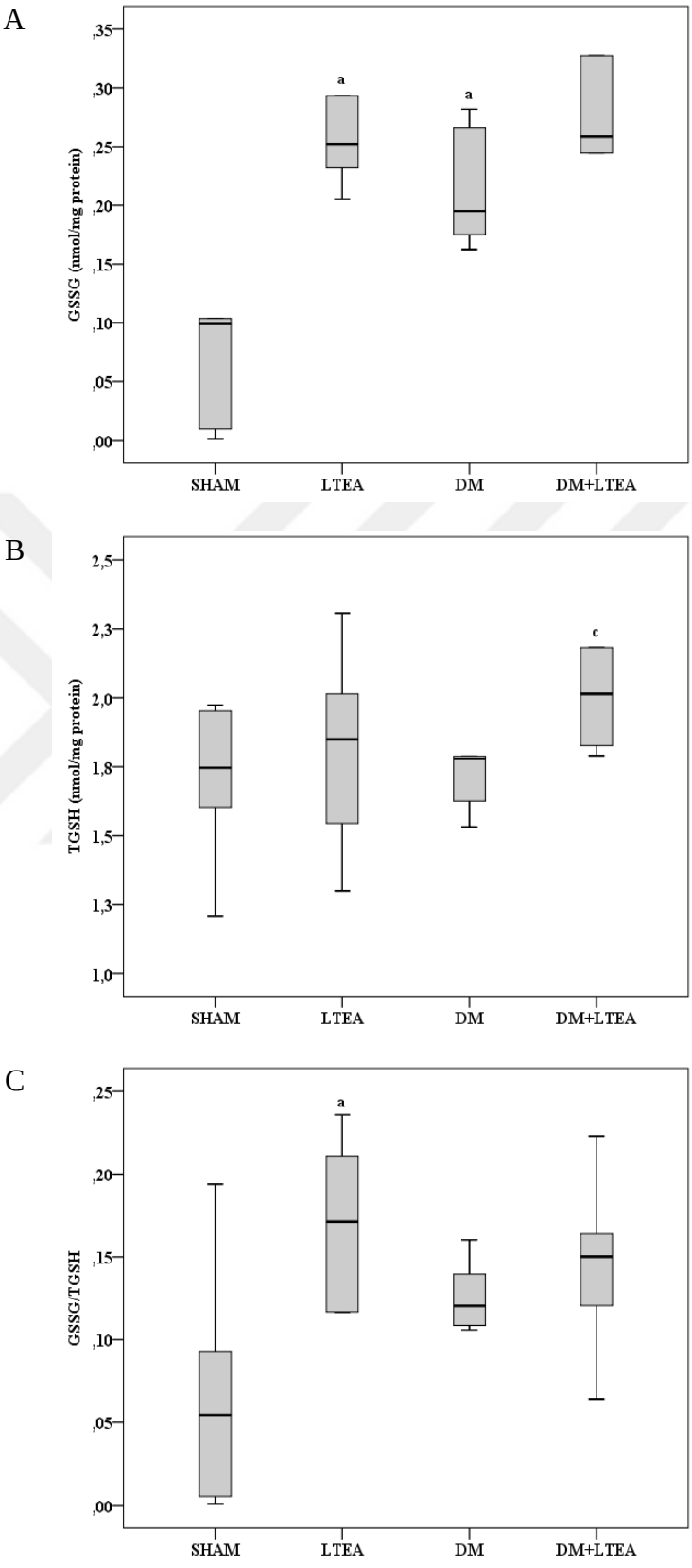
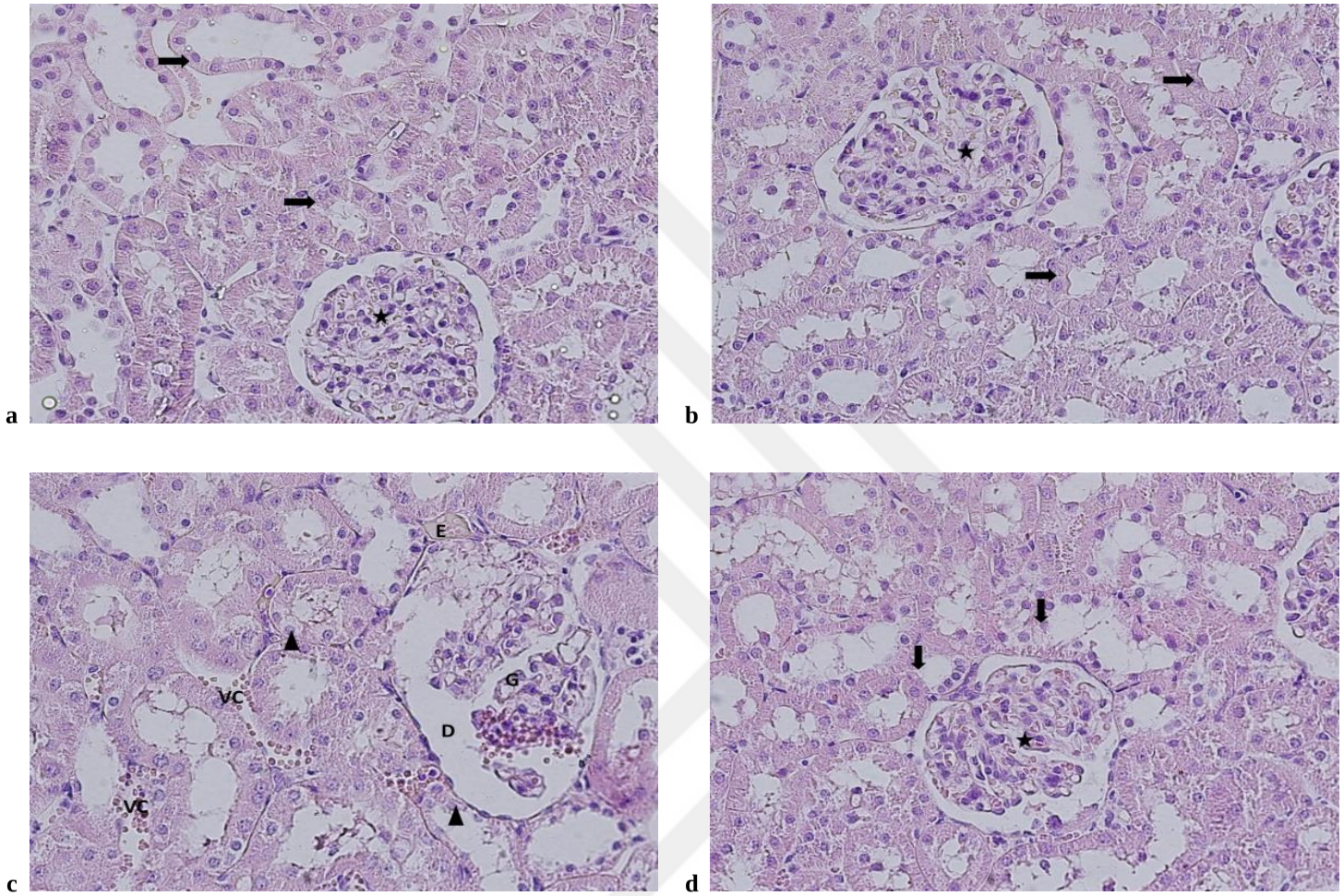


Figure 12. Kidney tissue glutathione (A:GSSG; B:TGSH; C:GSSG/TGSH) levels in the study groups



Picture 1. Photomicrograph of kidney tissues (H&E X 400). a: SHAM, b: LTEA, c: DM and d: DM+LTEA group.
➡ : Normal epithelial cell; ★ : Normal glomerular structure; ▲ : Degenerated tubular epithelial cells;
D: Dilatation in Bowman's space; **E** : Intertubular edema; **G** : Glomerular shrinkage; **VC** : Vasocongestion
⬇ : Mildly degenerated tubule epithelial cell.

4.3. Histopathological Results

In sham group, normal tubule and glomerular structures were determined in the renal cortex. Rarely, mild vacuolization was observed in 1-2 tubular epithelial cells (Picture 1a). In LTEA group, although a normal histological structure of the renal cortex and medulla were detected, vacuolization and degeneration were detected in mild tubule epithelial cells in a few places (Picture 1b). On the other hand, in DM group, moderate and severe dilatation in the Bowman's space, shrinkage in the glomerular structure, vacuolization in the tubular epithelial cells, degeneration in the tubular epithelial cells, and intertubular vasocongestion were observed in the renal cortex. In addition, mild hyaline deposition was seen in the intertubular area (Picture 1c). However, vasocongestion in the renal cortex was significantly decreased in DM+LTEA group. Vacuolization and degeneration of tubular epithelial cells were mild and occasionally moderate. The glomerular structure was close to normal (Picture 1d).

As seen in Figure 13 and Table 7 that show damage scores, the kidney was severely damaged in DM group ($p<0.05$). However, DM+LTEA group showed significantly decreased damage with respect to DM group.

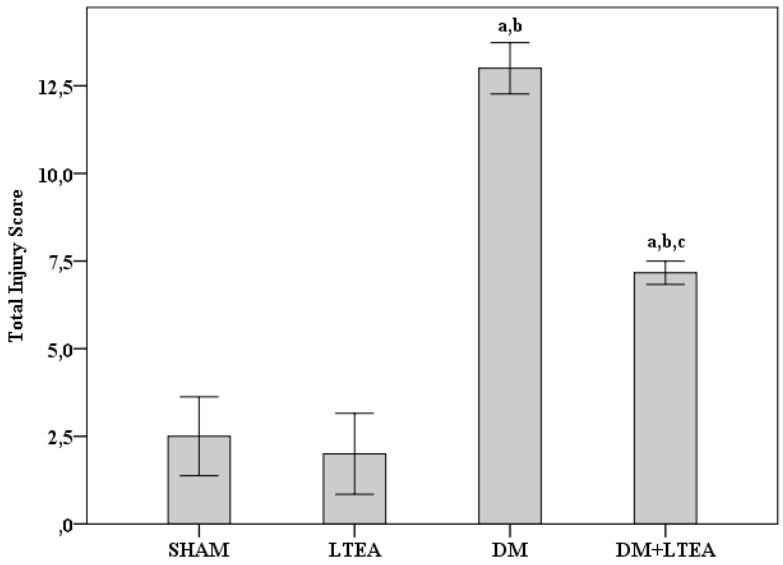


Figure 13. Total damage scores of kidney tissues in the study groups

Table 7. The histological damage scores of kidney tissues in the study groups

	Rat No	SHAM	LTEA	DM	DM+LTEA
Dilatation of Bowman's Space	***1***	1	1	3	2
	2	0	0	2	1
	3	1	1	2	1
	4	0	0	1	1
	5	1	0	2	1
	6	1	0	2	1
Shrinkage in Glomerular Structure	***1***	0	1	3	1
	2	0	0	3	1
	3	0	0	2	1
	4	0	0	2	1
	5	1	0	3	1
	6	0	0	2	2
Vacuolization in Tubular Epithelial Cells	***1***	1	1	2	1
	2	0	0	3	2
	3	1	1	2	2
	4	0	1	3	1
	5	0	0	2	2
	6	1	0	2	2
Degeneration of Tubular Epithelial Cells	***1***	1	0	1	1
	2	0	0	2	2
	3	1	1	2	1
	4	0	1	3	1
	5	0	0	2	1
	6	0	0	2	1
Intratubular Hyaline Deposition	***1***	0	0	2	1
	2	1	1	1	1
	3	0	0	2	1
	4	0	0	2	1
	5	0	0	2	1
	6	0	0	1	1
Vasocongestion	***1***	0	1	3	1
	2	1	1	2	1
	3	1	0	2	1
	4	0	0	3	2
	5	1	1	2	1
	6	1	0	3	1

Score 0: no damage; 1: mild=<25% damage, focal and mild changes; 2: moderate=25-50% damage, multifocal, significant changes; 3: severe= >50% damage, diffuse changes.

5. DISCUSSION

In this study, we evaluated the effects of oral LTEA administration on kidney function-related parameters including, cystatin C, ACE2, and electrolytes in NA/STZ-induced diabetic rats.

In our study, it was decided to intragastric administration of LTEA at 200 mg/kg/day for 28 days, considering some studies. 28-day treatments of various active agents to STZ-induced diabetic rodents have been identified in many literatures (99-102). Moreover, there are some researchers studying with 200 mg/kg LTEA (23, 103-106). LTEA is absorbed from the small intestine and reaches peak plasma level within 30 minutes after oral taking. The rat plasma concentrations of LTEA and ethylamine reached their highest levels at about 0.5 and 2 h, respectively, after 200 mg LTEA administration (107).

In our study, to create DM, a single dose of NA (120 mg/kg, in saline) and STZ (60 mg/kg, in 0.1 M citrate buffer, pH=4.5) were given to animals, i.p. at 15 min intervals, respectively (108-110). STZ (a glucosamine derivative of nitrosourea) is an antibiotic widely used to create an experimental model of T1DM by causing pancreatic β-cell destruction (95,109). This agent enters beta cells via GLUT2, causes DNA damage through alkylation, induces cytotoxicity and autoimmunity, and ultimately leads to necrosis of these cells (111, 112). In the presence of insulin deficiency in animals, pathological conditions such as hyperglycemia and polyuria may develop (95). Injection of NA prior to STZ partially protects β-cells from severe damage by STZ. This type of application is used to create the experimental T2DM model (95, 108). Because, this model shows the characteristics of human T2DM, accompanied by hyperglycemia and insulin deficiency (110). With the application of NA + STZ, inflammation increases and diabetes-related kidney damage develops (113). STZ has a short half-life and is eliminated by the kidney. In this stage, STZ can enter the renal cells also by GLUT2 and thereby can show its toxicity to the kidney (114). Hyperglycemia and its subsequent complications lead the kidneys to injury. Diabetic kidney disease mainly is seen as an end-stage renal disease (115).

Increased glucose level was observed in diabetic rats ($p<0.05$) (Table 5). The reason for this was attributed to the inability of the cells to use glucose due to insulin deficiency resulting from β-cell damage by STZ (110-112). It was reported that glucose levels could remain high even 8 weeks after STZ injection (116). An insignificant effect of LTEA treatments on glucose levels was observed in diabetic and nondiabetic rats ($p>0.05$).

Kidney disease develops in a significant proportion of diabetic patients. Because the complications that develop with metabolic changes caused by diabetes cause glomerular dysfunction, tubulointerstitial inflammation, and ultimately fibrosis, thereby damaging the kidneys (117).

There was no significant difference among the groups in terms of serum albumin levels ($p>0.05$) (Table 1). Whereas, the serum albumin level may reflect the eGFR values due to its filtration from the glomerular filtrate (41, 39). It was reported that serum albumin decreases but urinary albumin increases with STZ (116, 118). In normoalbuminemic conditions, renal function may also decrease but this condition may be seen in the decreased functions mainly in the early stages of diabetic kidney diseases (115). So far, we have not been able to find any study on the effect of LTEA on albumin levels in diabetics. Nevertheless, in one study, no statistically significant change in serum albumin level was observed in ducks supplemented with 300, 600, 900, and 1500 mg/kg LTEA for 28 days (119). On the other hand, a study reported the binding property of LTEA to albumin protein (120).

In our study, we found increased serum BUN, creatinine, and cystatin C levels in diabetic rats, but, of them, creatinine was not significant ($p>0.05$). It was stated that STZ induces diabetic nephropathy and causes to increase in serum BUN and creatinine levels and to decrease in creatinine clearance (121, 122). In addition, there are many studies that found high serum creatinine and urea levels in rats treated with NA+STZ (112, 113, 123-125). It has been reported that serum creatinine levels may decrease in diabetes due to decreased muscle mass, and low creatinine level poses a risk for T2DM (47, 126-129). Serum creatinine levels mainly reflect muscle mass as it is a primary metabolite of creatine, which is found in skeletal muscle (47, 130). Hyperglycemia causes muscle atrophy and reduces muscle mass (127). Bellomo et al. (128) reported that for creatinine to increase, half of the nephrons must be destroyed. These studies suggest that serum creatinine level may not be a good indicator of kidney damage in diabetes. On the other hand, cystatin C is accepted as a valid biomarker to estimate GFR and for kidney, dysfunctions than creatinine because of the fact that it is less affected by factors such as age and muscle mass (11, 50, 52). When the glomerular filtration rate decreases, serum cystatin C increases more rapidly than creatinine (38). Indeed, it has been reported that cystatin C levels are increased in diabetics (11, 50,

124, 131). However, Chen et al. (132) reported that serum cystatin C levels were low in T1DM patients with ketoacidosis and they attributed this result to acute events.

We also studied the cystatin C levels in the kidney tissues, and we saw the increased levels in diabetic rats (Figure 10) but not statistically significant ($p=0.180$). In support of our findings, Bozkurt and Pektaş (133) did not find any significant change in kidney cystatin C levels in STZ-induced rats. Similarly, according to Omoboyowa et al. (134), STZ upregulated cystatin C genes, although not statistically significant.

Oral administration of LTEA did not affect BUN and creatinine levels in diabetic and nondiabetic rats (Table 6). Altınkaynak et al. (18) found that LTEA was not effective compared to the sham group, but was effective in reducing BUN and creatinine levels in DOX-induced nephrotoxic rats. Similarly, Nagai et al. (94) expressed that LTEA improved creatinine clearance in DOX-induced rats. Malkoç et al. (17) determined that i.p. LTEA treatment decreased BUN and creatinine levels in rats with sepsis induced by cecal ligation and puncture (CLP). When we evaluated the LTEA effect on cystatin C values, we observed that it decreased serum levels in diabetic rats (Table 6). However, as with kidney tissue data (Figure 10), this was not statistically significant ($p>0.05$).

RAAS has an important role in diabetic nephropathy. Its activation causes an increase that leads the inflammation and fibrosis in the kidney (135). ACE2 is a central enzyme in the RAAS system, which adjusts electrolytes balance, and blood pressure (136). It regulates RAAS activity negatively in renal tissues. This protein hydrolyzes Ang II to Ang (1-7) and thereby protects kidney tissues from oxidative stress inflammation, apoptosis, and fibrosis (84, 85, 135). This protein is mainly expressed in tubular epithelial cells but also in glomerular epithelial cells and in renal vasculature in kidneys (137).

In our study, we found an insignificant increase ($p>0.05$, probably due to data range) in ACE2 levels in diabetic rats' kidney tissues ($p=0.310$) and serum (0.177) (Figure 11 and Table 6, respectively). There are some supporting studies. Patel et al. (138) stated that ACE2 expression and activity increased in the early stages of DM. Kajiwara et al. (139) reported that ACE2 levels increased insignificantly in renal proximal tubular epithelial cells of kidney tissues in STZ-induced diabetic mice. They determined that increased levels of the adhesion molecules resulting from diabetic inflammation cause an accumulation of ACE2 in the kidneys. On the other hand, kidney ACE2 protein levels and enzymatic activity increased in STZ-induced mice (136). Lin et al. (85) found an increase in tubular ACE2 expression in

STZ-rats. They suggested this increase as a compensatory effect, which down-regulates Ang II activity in the kidney in early diabetic conditions. On the other hand, many studies showed a decrease in ACE2 levels in DM (135, 140). It was reported that the up-regulated ACE2 expression is at the tubular level but down-regulated at the glomerular level in diabetics (84, 137). It was noted that kidney ACE2 levels were especially decreased in longstanding DM (84).

L-Theanine treatment did not significantly affect ACE2 levels in rats with and without DM ($p>0.05$) (Table 6 and Figure 10). As far as we searched, we could not come across any study that evaluates LTEA and ACE2 together. However, in a cell culture study, it was observed the suppressed effects of LTEA on Ang II-induced vascular smooth muscle cell (VSMC) migration and proliferation, by inhibiting Janus kinase/signal transducer and activators of transcription 3 (JAK/STAT3) pathway (141). The regulations of ACE2 activity and expression are important for the treatment of diabetic nephropathy (135).

Kidneys regulate fluid and electrolyte balance and control blood pressure via RAAS (62). Because of the osmotic property of glucose, in hyperglycemic conditions, water efflux from cells raises and so serum osmolality increases. Hence, electrolyte imbalance may be frequently seen in diabetics depending on impaired renal functions (61, 142, 143). In other words, hyperglycemia disturbs electrolyte balance due to its dilution effect (143). Therefore, we evaluate the serum electrolytes in our study groups (144).

One of the major electrolytes in extracellular fluids, sodium regulates acid-base and maintains osmotic balance (142). Hyperglycemia can change plasma sodium concentration via a few mechanisms. Osmotically active glucose leads to water efflux from cells, thereby diluting serum and leading to low sodium levels (143). Moreover, hyporeninemic hypoaldosteronism in DM also causes hyponatremia (143). In hyperinsulinemic conditions, because of the disturbance of the water channels (aquaporins) in gastrointestinal systems, the increased absorption of water may cause to decrease in sodium levels (143). In hyperinsulinemic conditions, a high influx of water and glucose into the cell may increase sodium levels.

We did not observe any significant difference in sodium levels among the groups. Contrary to our study, Chinaka et al. (145) found reduced sodium levels in HFD-STZ-induced DM rats. On the other hand, Akpan et al. (146) found a significant increase in sodium levels in STZ-induced rats. In addition, Datchinamoorthi et al. (144) found high

sodium levels in patients with T2DM. Unlikely, Bohara et al. (142) found a significant decrease in sodium levels in T2DM patients. However, Santosh et al. (61) reported an insignificant difference in sodium levels in diabetics.

In the present study, LTEA supplementation did not affect sodium levels (Table 6). It was reported that glutamate transport is affected by sodium gradient (147, 148). LTEA can compete with glutamate to bind the glutamate transporter due to its structural similarity (149). However, unchanged blood ACE2 levels, by LTEA treatment, support our insignificant effect on sodium levels. High sodium activates RAAS thereby decreasing ACE2 levels (84). Changing RAAS in DM may also change sodium levels in serum (61). Because, Ang II increases NaCl reabsorption (150). Not supporting our finding, Malkoç et al. (151) reported that LTEA increased sodium levels in septic rats.

The role of potassium in insulin secretion is linked to the ATP-sensitive potassium (K_{ATP}) channel in islet cells. Hypokalemia and hyperglycemia are associated with the impairment of the potassium-sensitive insulin release in response to elevated glucose (152). Hypokalemia may impair insulin secretion and may cause hyperglycemia (143). On the other hand, insulin deficiency may cause hyperkalemia in T2DM (61). Hyperglycemia attenuates the movement of water from the cells and tubular K^+ secretion may diminish (153). A study expressed that a high potassium diet may lead to the upregulation of ACE2 protein expression in kidneys (153). From the other point of view, the other study reported that ACE2 levels in kidneys might be attenuated by a high potassium diet (154).

We did not find any significant difference in terms of potassium levels among groups (Table 6). Supportingly, Akpan et al. (146) did not find any significant change in potassium levels in STZ-induced rats. Nevertheless, Chinaka et al. (145) found reduced potassium levels in HFD-STZ-induced DM rats. On the contrary, some studies determined high potassium levels in diabetics (61, 142, 144).

Although insignificant statistically ($p=0.093$), the slightly higher potassium level in the LTEA group compared to the sham group may be due to glutamate. Because, glutamate transporting is affected by extracellular potassium levels (148).

Chloride is the major extracellular anion to maintain the acid-base balance. Chloride plays a role in the regulation of RAAS (65). It was reported that chloride binds ACE2 in two different binding sites (136). We did not detect any difference in terms of serum chloride levels among the study groups. However, Chinaka et al. (145) found reduced chloride levels

in HFD-STZ-induced DM rats. Datchinamoorthi et al. (144) found high chloride levels in T2DM patients. Bohara et al. (142) reported an elevated level of chloride in T2DM patients.

Calcium functions in glucose homeostasis because of its role in insulin secretion from pancreatic β-cells, and in insulin resistance (74). With this aspect, hyperglycemia is associated with hypocalcemia (70). In diabetic nephropathy, hypocalcemia may be seen (143). Hyperphosphatemia develops as a result of decreased phosphate excretion due to acute renal failure in patients with DM. In this case, phosphate can bind calcium, leading to hypocalcemia (74). Hypomagnesemia can be the cause of hypocalcemia depending on the impairment of parathyroid hormone (PTH) action in bone and renal cells (74, 143). In hyperparathyroidic diabetic patients, hypercalcemia may be observed. In insulin deficiency, hypercalcemia may result from bone demineralization (143).

We did not perceive any significant change in terms of calcium levels among groups (Table 6). There are some studies on DM that support our results (121, 155-157). In addition, Malkoç et al. (151) noted that LTEA treatment did not affect serum calcium levels in septic rats.

Approximately 70% of circulating magnesium is filtered by the glomerulus and 90% is reabsorbed. Therefore, the kidney is crucial and elementary for the maintenance of circulating Mg^{2+} levels (158). Magnesium has many functions including the maintenance of energy homeostasis, the movement of glucose into the cell, the metabolism and stability of organic molecules, the synthesis of protein and nucleic acids, the regulation of hormones, and the functioning of nervous and immune systems (70, 71, 159, 160). Moreover, it needs for GSH synthesis and upregulates some antioxidant proteins like glutathione peroxidase. It is involved also in the cellular activity of insulin (70) and has a role in the autophosphorylation of insulin receptors (160).

In our study, diabetic conditions did not affect magnesium levels significantly (Table 6). However, many studies reported hypomagnesemia in DM (159, 161-164). In hypomagnesemia, cellular defense against oxidative stress decreases, and the risk factors for cardiovascular diseases and diabetes increase (70).

Magnesium is a glutamate receptor (NMDA) antagonist but GABA agonist, and regulates sleep quality similarly to LTEA (165). Magnesium inhibits glutamate NMDA receptor voltage-dependently (166). We could not find any significant effect of LTEA on magnesium levels.

Although iron is indispensable for life, its level must be controlled because of its potential for the production of ROS (76, 167). Therefore, an increase in iron level can also cause kidney damage by increasing oxidative stress, inflammation, and ferroptosis (76). Excess iron modulates the gene expression in β-cells and leads to pancreatic cell dysfunction.

We found insignificant increased iron levels in DM. Similarly, Zhang et al. (168) reported increased serum iron levels in STZ-induced rats and in patients with T2DM. Jelena et al. (169) observed an increase in free iron in rat serum 4 weeks after STZ induction. Özçelik et al. (170) determined high iron levels but low magnesium levels in the kidney tissues of STZ-rats. On the other hand, renal diseases can affect systemic iron homeostasis. These disorders may decrease serum iron by increasing urinary iron excretion (76). Wollide et al. (171) reported that serum Mg^{2+}, Fe^{3+}, and ferritin levels decreased but Ca^{2+} did not significant in patients with T2DM.

LTEA treatment decreased iron levels only in diabetic rats. No study was found on the effect of LTEA on iron levels. However, there are many studies showing that tea consumption causes iron deficiency (172-176). Along with other phytochelators in tea, LTEA may contribute to this type of chelation. Because, LTEA is structurally similar to glutamate amino acid (18). It is hydrolyzed to glutamic and ethylamine by phosphate-independent glutaminase in the kidney (89, 177). Phosphate-independent glutaminase activity (γ-glutamyltranspeptidase) activity increases with metabolic acidosis as observed in DM (178, 179). Glutamate or glutamic acid has the ability to chelate iron (167, 180). Metal ligands and chelators, and molecules that regulate the absorption or excretion of iron, have an important place in the treatment of diseases (167). Iron chelators can be used to form iron complexes and transport excess iron (in other words, toxic iron) from cells (181). Considering these pieces of information, it can be said that LTEA may decrease the serum iron level by increasing the excretion of iron via glutamate (increased glutaminase activity in the kidney in diabetes) chelation indirectly, or via LTEA chelation (with its glutamate-like structure) directly.

Plasma (insignificant) and kidney (significant) GSSG levels increased in the DM group (Table 6, Figure 12). On the other hand, the changes in TGSH levels in plasma and kidney tissues were not statistically significant. Oxidized or disulfide form of glutathione (GSSG) reflects oxidative stress. Decreased antioxidant and increased oxidative stress in diabetes contributes to the progression of DM. Increased GSSG levels can also cause the

glutathionylation of proteins (modify proteins) (182). Elevated GSSG and decreased GSH levels were reported in DM by many researchers (182-185).

L-Theanine treatment showed an increase in both TGSH and GSSG levels in normal kidney tissue (Figure 12). However, when diabetic rats were treated with LTEA, a decrease in plasma levels of both parameters was determined (Table 6). This difference in plasma values may be due to the effects of LTEA on non-renal tissues. There is also some literature supporting the findings of our study (17, 18).

In our DM group, kidney dilatation in the Bowman's space, glomerular shrinkage, vacuolization and degeneration in the tubular epithelial cells, intertubular vasocongestion, and mild hyaline deposition were observed (Pictures 1c). Hyperglycemia damages glomerular and tubular endothelial cells. Bowman capsule thickening and tubular cell vacuolization are the characteristics of diabetic kidney diseases (186). Supporting our findings, in a study, increased diameters of glomerulus tubules and a decreased number of glomeruli were observed in STZ-DM rats (187). LTEA treatment alleviated vasocongestion, vacuolization, and degeneration in diabetic rats and almost normalize morphological architecture in glomerular structure (Picture 1d). Supportingly, there are a few studies showing that LTEA contributed to the normalization of pathological kidney morphology (17, 18).

In conclusion, LTEA treatment alleviates DM-related pathological conditions in the kidney tissues of rats. In addition to histological changes, the main effects of LTEA were seen in serum cystatin C and iron levels. The effect on the iron level in diabetics may be an issue that should be addressed for further studies, not only in terms of kidneys but also in terms of other tissues. In this respect, it would be appropriate to conduct research on its effects on humans.

6. REFERENCES

1. Sapra A, Bhandari P (2022). Diabetes Mellitus. In *StatPearls*. StatPearls Publishing.
2. Hassanein M, Shafi T (2022). Assessment of glycemia in chronic kidney disease. BMC Med 20(1):117.
3. Thomas MC (2021). Targeting the pathobiology of diabetic kidney disease. Adv Chronic Kidney Dis 28(4): 282-289.
4. Yousef Yengej FA, Jansen J, Rookmaaker MB, Verhaar MC, Clevers H (2020). Kidney organoids and tubuloids. Cells 9(6): 1326.
5. Yao B, Giela MC, Hong Y (2021). Detection of kidney disease biomarkers based on fluorescence technology. Mater Chem Front 5: 2124-2142.
6. Wen Y, Parikh CR (2021). Current concepts and advances in biomarkers of acute kidney injury. Crit Rev Clin Lab Sci 58(5): 354-368.
7. Griffin BR, Faubel S, Edelstein CL (2019). Biomarkers of drug-induced kidney toxicity. Ther Drug Monit 41(2): 213-226.
8. Kashani K, Rosner MH, Ostermann M (2020). Creatinine: From physiology to clinical application. Eur J Intern Med 72: 9-14.
9. Yao Z, Li G, Li G (2021). Correlation between serum urea nitrogen, cystatin C, homocysteine, and chronic heart failure. Am J Transl Res 13(4): 3254-3261.
10. Chen Z, Zhang J, Feng J, Zhou G, Jin X, Pan J (2021). Higher serum level of cystatin C: an additional risk factor of CAD. Medicine 100(2): e24269.
11. Jhatta C, Girdhar J, Gupta S, Verma I (2020). To compare the level of cystatin C in type 2 diabetes mellitus with obesity. Indian J Endocrinol Metab 24(4): 312-318.
12. Almengló C, Couselo-Seijas M, Agra RM, Varela-Román A, García-Acuña JM, González-Peteiro M, González-Juanatey JR, Eiras S, Álvarez E (2021). Soluble angiotensin-converting enzyme levels in heart failure or acute coronary syndrome: Revisiting its modulation and prognosis value. J Mol Med 16: 1–13.
13. Maksimowski N, Williams VR, Scholey JW (2020). Kidney ACE2 expression: Implications for chronic kidney disease. PLoS One 15(10): e0241534.
14. Lumbers ER, Delforce SJ, Pringle KG, Smith GR (2020). The lung, the heart, the novel coronavirus, and the renin-angiotensin system; the need for clinical trials. Front Med 7: 248.

15. Reshma S, Sushith S, Prathima MB, Janice D, Madan GR, Gowda P, Kumar KP, Rai M, Kalal BS (2020). Serum electrolytes levels in patients with type 2 diabetes mellitus: A cross-sectional study. Diabetes Mellitus 23(3): 223-228.
16. Vuong QV, Bowyer MC, Roach PD (2011). L-theanine: properties, synthesis and isolation from tea. J Sci Food Agric 91(11): 1931-1939.
17. Malkoç M, Patan H, Yaman SÖ, Türedi S, Kerimoğlu G, Kural BV, Örem A (2020). L-theanine alleviates liver and kidney dysfunction in septic rats induced by cecal ligation and puncture. Life Sci 249: 117502.
18. Altınkaynak Y, Kural B, Akcan BA, Bodur A, Özer S, Yuluğ E, Munğan S, Kaya C, Örem A (2018). Protective effects of L-theanine against doxorubicin-induced nephrotoxicity in rats. Biomed Pharmacother 108: 1524-1534.
19. Li MY, Liu HY, Wu DT, Kenaan A, Geng F, Li HB, Gunaratne A, Li H, Gan RY (2022). L-Theanine: A unique functional amino acid in tea (*camellia sinensis* L.) with multiple health benefits and food applications. Front Nutr 9: 853846.
20. King KM, Rubin G (2003). A history of diabetes: from antiquity to discovering insulin. Br J Nurs 12(18): 1091-5.
21. Petersmann A, Müller-Wieland D, Müller UA, Landgraf R, Nauck M, Freckmann G, Heinemann L, Schleicher E (2019). Definition, classification and diagnosis of diabetes mellitus. Exp Clin Endocrinol Diabetes 127(S01): S1–S7.
22. Chang-Chen KJ, Mullur R, Bernal-Mizrachi E (2008). Beta-cell failure as a complication of diabetes. Rev Endocr Metab Disord 9(4): 329-43.
23. Liu R, Li L, Shao C, Cai H, Wang Z (2022). The impact of diabetes on vascular disease: progress from the perspective of epidemics and treatments. J Diabetes Res 2022: 1531289.
24. Domingo-Lopez DA, Lattanzi G, H J Schreiber L, Wallace EJ, Wylie R, O'Sullivan J, Dolan EB, Duffy GP (2022). Medical devices, smart drug delivery, wearables and technology for the treatment of diabetes mellitus. Adv Drug Deliv Rev 8: 114280.
25. Reddy M, Gopalkrishna P (2022). Type 1 diabetes and periodontal disease: A literature review. Can J Dent Hyg 56(1): 22-30.
26. Wake AD (2022). Protective effects of physical activity against health risks associated with type 1 diabetes: "Health benefits outweigh the risks". World J Diabetes 13(3): 161-184.

27. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C (2020). Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci 21(17): 6275.
28. Nisar O, Pervez H, Mandalia B, Waqas M, Sra HK (2020). Type 3 diabetes mellitus: A link between Alzheimer's disease and type 2 diabetes mellitus. Cureus 12(11): e11703.
29. Michailidis M, Moraitou D, Tata DA, Kalinderi K, Papamitsou T, Papaliagkas V (2022). Alzheimer's disease as type 3 diabetes: Common pathophysiological mechanisms between Alzheimer's disease and type 2 diabetes. Int J Mol Sci 23(5): 2687.
30. Valero P, Salas R, Pardo F, Cornejo M, Fuentes G, Vega S, Grismaldo A, Hillebrands JL, van der Beek EM, van Goor H, Sobrevia L (2022). Glycaemia dynamics in gestational diabetes mellitus. Biochim Biophys Acta Gen Subj 27: 130134.
31. Bathla T, Abolbaghaei A, Reyes AB, Burger D (2022). Extracellular vesicles in gestational diabetes mellitus: A scoping review. Diab Vasc Dis Res. 19(1): 14791641221093901.
32. Abu Samra N, Jelinek HF, Alsafar H, Asghar F, Seoud M, Hussein SM, Mubarak HM, Anwar S, Memon M, Afify N, Manzoor R, Al-Homedi Z, Osman W (2022). Genomics and epigenomics of gestational diabetes mellitus: understanding the molecular pathways of the disease pathogenesis. Int J Mol Sci 23(7): 3514.
33. Hansen KL, Nielsen MB, Ewertsen C (2015). Ultrasonography of the kidney: A pictorial review. Diagnostics 6(1): 2.
34. Cortinovis M, Perico N, Ruggenenti P, Remuzzi A, Remuzzi G (2022). Glomerular hyperfiltration. Nat Rev Nephrol 18(7): 435–451.
35. Ishimoto Y, Tanaka T, Yoshida Y, Inagi R (2018). Physiological and pathophysiological role of reactive oxygen species and reactive nitrogen species in the kidney. Clin Exp Pharmacol Physiol 45(11): 1097-1105.
36. de Boer A, Hoogduin JM, Blankestijn PJ, Li X, Luijten PR, Metzger GJ, Raaijmakers AJ, Umutlu L, Visser F, Leiner T (2016). 7 T renal MRI: Challenges and promises. MAGMA 29(3): 417-33.
37. Alan SL, Yu MB, Chir B (2020). Brenner and Rector's: The kidney. 11th ed. Elsevier 27: 872-904.

38. Yang H, Lin C, Zhuang C, Chen J, Jia Y, Shi H, Zhuang C (2022). Serum cystatin C as a predictor of acute kidney injury in neonates: A meta-analysis. J Pediatr 98(3): 230-240.
39. Lang J, Katz R, Ix JH, Gutierrez OM, Peralta CA, Parikh CR, Satterfield S, Petrovic S, Devarajan P, Bennett M, Fried LF, Cummings SR, Sarnak MJ, Shlipak MG (2018). Association of serum albumin levels with kidney function decline and incident chronic kidney disease in elders. Nephrol Dial Transplant 33(6): 986-992.
40. Moman RN, Gupta N, Varacallo M (2022). Physiology, albumin. In *StatPearls*. StatPearls Publishing.
41. Molitoris BA, Sandoval RM, Yadav SPS, Wagner MC (2022). Albumin uptake and processing by the proximal tubule: physiologic, pathologic and therapeutic implications. Physiol Rev 102(4): 1625–1667.
42. Clarke H, Kim DH, Meza CA, Ormsbee MJ, Hickner RC (2020). The evolving applications of creatine supplementation: Could creatine improve vascular health? Nutrients 12(9): 2834.
43. Rae CD, Bröer S (2015). Creatine as a booster for human brain function. How might it work? Neurochem Int 89: 249-59.
44. National center for biotechnology information (2022). Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Creatinine. [Accessed 3 November 2022].
45. Teaford HR, Barreto JN, Vollmer KJ, Rule AD, Barreto EF (2020). Cystatin C: A primer for pharmacists. Pharmacy 8(1): 35.
46. Cusumano AM, Tzanno-Martins C, Rosa-Diez GJ (2021). The glomerular filtration rate: from the diagnosis of kidney function to a public health tool. Front med 8: 769335.
47. Harita N, Hayashi T, Sato KK, Nakamura Y, Yoneda T, Endo G, Kambe H (2009). Lower serum creatinine is a new risk factor of type 2 diabetes: The Kansai healthcare study. Diabetes Care 32(3): 424-426.
48. Fernando S, Polkinghorne KR (2020). Cystatin C: Not just a marker of kidney function. J Bras Nefrol 42(1): 6-7.
49. Reutens AT, Bonnet F, Lantieri O, Roussel R, Balkau B (2013). Epidemiological study on the insulin resistance syndrome study Group. The association between cystatin C

and incident type 2 diabetes is related to central adiposity. Nephrol Dial Transplant 28(7): 1820-1829.

50. Ma CC, Duan CC, Huang RC, Tang HQ (2019). Association of circulating cystatin C levels with type 2 diabetes mellitus: A systematic review and meta-analysis. Arch Med Sci 16(3): 648-656.
51. Rizk JG, Streja E, Wenziger C, Shlipak MG, Norris KC, Crowley ST, Kalantar-Zadeh K (2021). Serum creatinine-to-cystatin-C ratio as a potential muscle mass surrogate and racial differences in mortality. J Ren Nutr doi: 10.1053/j.jrn.2021.11.005.
52. Omaygenç MO, Özcan ÖU, Çakal B, Karaca O (2020). Cystatin C and uncontrolled hypertension. Anatol J Cardiol 24(5): 309-315.
53. Garcia-Carretero R, Vigil-Medina L, Mora-Jimenez I, Soguero-Ruiz C, Goya-Esteban R, Ramos-Lopez J, Barquero-Perez O (2018). Cardiovascular risk assessment in prediabetic patients in a hypertensive population: The role of cystatin C. Diabetes Metab Syndr 12(5): 625-629.
54. Chen SH, Liang YJ (2022). The role of lifestyle intervention, in addition to drugs, for diabetic kidney disease with sarcopenic obesity. Life 12(3): 380.
55. Ramchand J, Burrell LM (2020). Circulating ACE2: a novel biomarker of cardiovascular risk. Lancet 396(10256): 937-939.
56. Fernández-Ruiz I (2020). ACE2 level as a marker of CVD. Nat Rev Cardiol 17(12): 759.
57. Herman-Edelstein M, Guetta T, Barnea A, Waldman M, Ben-Dor N, Barac YD, Kornowski R, Arad M, Hochhauser E, Aravot D (2021). Expression of the SARS-CoV-2 receptorACE2 in human heart is associated with uncontrolled diabetes, obesity, and activation of the renin angiotensin system. Cardiovasc Diabetol 20(1): 90.
58. Beyerstedt S, Casaro EB, Rangel ÉB (2021). COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. Eur J Clin Microbiol Infect Dis 40(5): 905-919.
59. Cooper SL, Boyle E, Jefferson SR, Heslop CRA, Mohan P, Mohanraj GGJ, Sidow HA, Tan RCP, Hill SJ, Woolard J (2021). Role of the renin-angiotensin-aldosterone and kinin-kallikrein systems in the cardiovascular complications of COVID-19 and long COVID. Int J Mol Sci 22(15): 8255.
60. Liamis G, Liberopoulos E, Barkas F, Elisaf M (2014). Diabetes mellitus and electrolyte disorders. World J Clin Case 2(10): 488-96.

61. Santhosh V, Gomathi DM, Khadeja-Bi A, Suganya S, Gurulakshmi G, Manjula DN (2021). Study of serum electrolytes in type 2 diabetes mellitus individuals in rural tertiary care hospital in Kancheepuram district. Biomed Pharmacol J 14(2).
62. Bell R, Mandalia R (2022). Diuretics and the kidney. BJA Education, 22(6): 216–223.
63. Shrimanker I, Bhattarai S (2021). Electrolytes. In *StatPearls*. StatPearls Publishing.
64. Ekmekcioglu C, Elmadfa I, Meyer AL, Moeslinger T (2016). The role of dietary potassium in hypertension and diabetes. J Physiol Biochem 72(1): 93-106.
65. Kataoka H (2021). Chloride in heart failure syndrome: its pathophysiologic role and therapeutic implication. Cardiol Ther 10(2): 407–428.
66. Rein JL, Coca SG (2019). "I don't get no respect": The role of chloride in acute kidney injury. Am J Physiol Renal Physiol 316(3): F587-F605.
67. Khatri M, Zitovsky J, Lee D, Nayyar K, Fazzari M, Grant C (2020). The association between serum chloride levels and chronic kidney disease progression: a cohort study. BMC Nephrol 21(1): 165.
68. Peride I, Tiglis M, Neagu TP, Niculae A, Checherita IA (2022). Magnesium-a more important role in CKD-MBD than we thought. Diagnostics 12(4): 880.
69. Tseng MH, Konrad M, Ding JJ, Lin SH (2021). Clinical and genetic approach to renal hypomagnesemia. Biomed J 45(1): 74–87.
70. Dubey P, Thakur V, Chattopadhyay M (2020). Role of minerals and trace elements in diabetes and insulin resistance. Nutrients 12(6): 1864.
71. Mathew AA, Panonnummal R. (2021). 'Magnesium'-the master cation-as a drug-possibilities and evidences. Biometals 34(5): 955–986.
72. Sakaguchi Y (2022). The emerging role of magnesium in CKD. Clin Exp Nephrol 26(5): 379-384.
73. Hanna RM, Ahdoot RS, Kalantar-Zadeh K, Ghobry L, Kurtz I (2022). Calcium transport in the kidney and disease processes. Front Endocrinol 12: 762130.
74. Ahn C, Kang JH, Jeung EB (2017). Calcium homeostasis in diabetes mellitus. J Vet Sci 18(3): 261-266.
75. Wongdee K, Krishnamra N, Charoenphandhu N (2017). Derangement of calcium metabolism in diabetes mellitus: negative outcome from the synergy between impaired bone turnover and intestinal calcium absorption. J Physiol Sci 67(1): 71-81.
76. van Swelm RPL, Wetzels JFM, Swinkels DW (2020). The multifaceted role of iron in renal health and disease. Nat Rev Nephrol 16(2): 77-98.

77. Georgieff MK (2020). Iron deficiency in pregnancy. Am J Obstet Gynecol 223(4): 516-524.
78. Zapora-Kurel A, Kuźma Ł, Zakrzewska M, Żórawski M, Dobrzycki S, Twardowska-Kawalec M, Małyszko J (2021). Novel iron parameters in patients with type 2 diabetes mellitus in relation to kidney function. J Clin Med 10(16): 3732.
79. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB (2009). The role of iron in type 2 diabetes in humans. Biochim Biophys Acta. 1790(7): 671-81.
80. Hayat K, Iqbal H, Malik U, Bilal U, Mushtaq S (2015). Tea and its consumption: Benefits and risks. Crit Rev Food Sci Nutr 55(7): 939-54.
81. Saeed M, Khan MS, Kamboh AA, Alagawany M, Khafaga AF, Noreldin AE, Qumar M, Safdar M, Hussain M, Abd El-Hack ME, Chao S (2020). L-Theanine: An astounding sui generis amino acid in poultry nutrition. Poult Sci 99(11): 5625-5636.
82. Chen Z, Wang Z, Yuan H, He N (2021). From tea leaves to factories: A review of research progress in L-Theanine biosynthesis and production. J Agric Food Chem. 69(4): 1187-1196.
83. Scheid L, Ellinger S, Alteheld B, Herholz H, Ellinger J, Henn T, Helfrich HP, Stehle P (2012). Kinetics of L-Theanine uptake and metabolism in healthy participants are comparable after ingestion of L-Theanine via capsules and green tea. J Nutr 142(12): 2091-2096.
84. Liu LP, Zhang XL, Li J (2021). New perspectives on angiotensin-converting enzyme 2 and its related diseases. World J Diabetes 12(6): 839-854.
85. Lin M, Gao P, Zhao T, He L, Li M, Li Y, Shui H, Wu X (2016). Calcitriol regulates angiotensin-converting enzyme and angiotensin converting-enzyme 2 in diabetic kidney disease. Mol Biol Rep 43(5): 397-406.
86. Kakuda T (2011). Neuroprotective effects of theanine and its preventive effects on cognitive dysfunction. Pharmacol Res 64(2): 162-168.
87. Deb S, Dutta A, Phukan BC, Manivasagam T, Justin Thenmozhi A, Bhattacharya P, PaulR, Borah A (2019). Neuroprotective attributes of L-Theanine, a bioactive amino acid of tea, and its potential role in Parkinson's disease therapeutics. Neurochem Int 129: 104478.
88. Hidese S, Ogawa S, Ota M, Ishida I, Yasukawa Z, Ozeki M, Kunugi H (2019). Effects of L-Theanine administration on stress-related symptoms and cognitive functions in healthy adults: a randomized controlled trial. Nutrients 11(10): 2362.

89. Xin Y, Ben P, Wang Q, Zhu Y, Yin Z, Luo L (2018). Theanine, an antitumor promoter, induces apoptosis of tumor cells via the mitochondrial pathway. Mol Med Rep 18(5): 4535-4542.
90. Bai H, Zhang Z, Li Y, Song X, Ma T, Liu C, Liu L, Yuan R, Wang X, Gao L (2020). L-theanine reduced the development of knee osteoarthritis in rats via its anti-inflammation and anti-matrix degradation actions: *in vivo* and *in vitro* study. Nutrients 12(7): 1988.
91. Peng WQ, Xiao G, Li BY, Guo YY, Guo L, Tang QQ (2021). L-Theanine activates the browning of white adipose tissue through the AMPK/α-ketoglutarate/Prdm16 axis and ameliorates diet-induced obesity in mice. Diabetes 70(7): 1458-1472.
92. Saha A, Chatterjee S, Chatterjee A, Roy S, Roy N, Mukhopadhyay S, Bandyopadhyay S. (2018) Insulinotropic and cytoprotective effect of L-Theanine: An *in vitro* dose dependent study. Pharmacognosy Magazine 14(55): 36-39.
93. Lin L , Zeng L , Liu A , Peng Y , Yuan D , Zhang S , Li Y , Chen J , Xiao W , Gong Z (2020). L-Theanine regulates glucose, lipid, and protein metabolism via insulin and AMP-activated protein kinase signaling pathways. Food Funct 11(2): 1798-1809.
94. Nagai K, Fukuno S, Otani K, Nagamine Y, Omotani S, Hatsuda Y, Myotoku M, Konishi H (2018). Prevention of doxorubicin-ınduced renal toxicity by theanine in rats. Pharmacology 101(3-4): 219-224.
95. Furman BL (2021). Streptozotocin-induced diabetic models in mice and rats. Curr Protoc 1(4): e78.
96. Kör S. Investigation of the effects of L-Theanine on pancreas and liver tissue damages in diabetic rats. KTU GSHS PhD Thesis. 2021.
97. Tekmeh HH. Investigation of the effect of L-Theanine on the hippocampus tissue and serumbdnf and adipocytokine levels in diabetic rats. KTU GSHS PhD Thesis. 2021.
98. Altun G, Cakiroglu Y, Pulathan Z, Yulug E, Mentese A (2020). Renoprotective potential of exogen erythropoietin on experimental ruptured abdominal aortic aneurysm model: An animal study. Iran J Basic Med Sci 23(2): 271-276.
99. Altındağ F, Rağbetli MÇ, Özdek U, Koyun N, Ismael Alhalboosi JK, Elasan S (2021). Combined treatment of sinapic acid and ellagic acid attenuates hyperglycemia in streptozotocin-induced diabetic rats. Food Chem Toxicol 156: 112443.
100. Gopinath H, Shivashankar M (2021). A study on toxicity and anti-hyperglycemic effects of *Abhrak Bhasma* in rats. J Ayurveda Integr Med 12(3): 443-451.

101. Taher M, Tg Zakaria TM, Susanti D, Zakaria ZA (2016). Hypoglycaemic activity of ethanolic extract of *Garcinia mangostana Linn.* in normoglycaemic and streptozotocin-induced diabetic rats. BMC Complement Altern Med 16: 135.
102. Chen H, Brahmbhatt S, Gupta A, Sharma AC (2005). Duration of streptozotocin-induced diabetes differentially affects p38-mitogen-activated protein kinase (MAPK) phosphorylation in renal and vascular dysfunction. Cardiovasc Diabetol 4: 3.
103. Zeng L, Lin L, Chen L, Xiao W, Gong Z (2021). L-Theanine ameliorates d-galactose-induced brain damage in rats via inhibiting AGE formation and regulating sirtuin1 and BDNF signaling pathways. Oxid Med Cell Longev 2021: 8850112.
104. Saeed M, Yatao X, Hassan FU, Arain MA, Abd El-Hack ME, Noreldin AE, Sun C (2018). Influence of graded levels of L-Theanine dietary supplementation on growth performance, carcass traits, meat quality, organs histomorphometry, blood chemistry and immune response of broiler chickens. Int J Mol Sci 19(2): 462
105. Sumathi T, Shobana C, Thangarajeswari M, Usha R (2015). Protective effect of L-Theanine against aluminium induced neurotoxicity in cerebral cortex, hippocampus and cerebellum of rat brain - histopathological, and biochemical approach. Drug Chem Toxicol 38(1): 22-31.
106. Thangarajan S, Deivasigamani A, Natarajan SS, Krishnan P, Mohanan SK (2014). Neuroprotective activity of L-Theanine on 3-nitropropionic acid-induced neurotoxicity in rat striatum. Int J Neurosci 124(9): 673-684.
107. Unno T, Suzuki Y, Kakuda T, Hayakawa T, Tsuge H (1999). Metabolism of theanine, gamma-glutamylethylamide, in rats. J Agric Food Chem 47(4): 1593–1596.
108. Mani V, Arfeen M, Sajid S, Almogbel Y (2022). Aqueous Ajwa dates seeds extract improves memory impairment in type-2 diabetes mellitus rats by reducing blood glucose levels and enhancing brain cholinergic transmission. Saudi J Biol Sci 29(4): 2738-2748.
109. Kumar S, Prashant M, Ram B, Dwivedi KN (2021). Role of katankateryadi kwatha in insulin secretion and restoration of biochemical changes in streptozotocin-nicotinamide induced diabetes mellitus type 2 in rats. Int J Appl Bio Pharm 12 (2021): 362-379.
110. Abdel Aziz SM, Ahmed OM, Abd El-Twab SM, Al-Muzafar HM, Amin KA, Abdel-Gabbar M (2020). Antihyperglycemic effects and mode of actions of *musa paradisiaca*

leaf and fruit peel hydroethanolic extracts in nicotinamide/streptozotocin-induced diabetic rats. Evid Based Complement Alternat Med. 2020: 9276343.

111. Zhu BT (2022). Pathogenic mechanism of autoimmune diabetes mellitus in humans: Potential role of streptozotocin-induced selective autoimmunity against human islet β-cells. Cells 11(3): 492.
112. Ali AM, Gabbar MA, Abdel-Twab SM, Fahmy EM, Ebaid H, Alhazza IM, Ahmed OM (2020). Antidiabetic potency, antioxidant effects, and mode of actions of *Citrus reticulata* fruit peel hydroethanolic extract, hesperidin, and quercetin in 84 nicotinamide/streptozotocin-induced wistar diabetic rats. Oxid Med Cell Longev 2020: 1730492.
113. Sasongko H, Nurrochmad A, Rohman A, Nugroho AE (2022). Characteristic of streptozotocin-nicotinamide-induced inflammation in a rat model of diabetes-associated renal injury. Open Access Maced J Med Sci 3(8): 16-22.
114. Wu J, Yan LJ (2015). Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes Metab Syndr Obes 8: 181-188.
115. Piwkowska A, Zdrojewski Ł, Heleniak Z, Dębska-Ślizień A (2022). Novel markers in diabetic kidney disease—current state and perspectives. Diagnostics 12(5): 1205.
116. Mestry SN, Dhodi JB, Kumbhar SB, Juvekar AR (2016). Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract. J Tradit Complement Med 7(3): 273-280.
117. Kene K, Wondimnew T, Welde M, Mateos T, Adugna T, Gerema U, Abdisa D, Abera D (2021). Prevalence and determinants of impaired serum creatinine and urea among type 2 diabetic patients of Jimma medical center. Endocrinol Metab 3: 100096.
118. Fang J, Wei H, Sun Y, Zhang X, Liu W, Chang Q, Wang R, Gong Y (2013). Regulation of podocalyxin expression in the kidney of streptozotocin-induced diabetic rats with Chinese herbs (Yishen capsule). BMC Complement Altern Med 13: 76.
119. Zhang C, Chen KK, Zhao XH, Wang C, Geng ZY (2019). Effect of L-Theanine on the growth performance, immune function, and jejunum morphology and antioxidant status of ducks. Animal 13(6): 1145-1153.
120. Roslan AA, Mohamad SB, Tayyab S (2021). Docking evaluation of the interaction between green tea active ıngredient, L-Theanine and human serum albumin. Natl Acad Sci Lett 44: 17–19.

121. Guo CJ, Xie JJ, Hong RH, Pan HS, Zhang FG, Liang YM (2019). Puerarin alleviates streptozotocin (STZ)-induced osteoporosis in rats through suppressing inflammation and apoptosis via HDAC1/HDAC3 signaling. Biomed Pharmacother 115: 108570.
122. Bilen A, Calik I, Yayla M, Dincer B, Tavaci T, Cinar I, Bilen H, Cadirci E, Halici Z, Mercantepe F (2021). Does daily fasting shielding kidney on hyperglycemia-related inflammatory cytokine via TNF-α, NLRP3, TGF-β1 and VCAM-1 mRNA expression. Int J Biol Macromol 190: 911-918.
123. Shao H, Xiao M, Zha Z, Olatunji OJ (2022). UHPLC-ESI-QTOF-MS2 analysis of Acacia pennata extract and its effects on glycemic indices, lipid profile, pancreatic and hepatorenal alterations in nicotinamide/streptozotocin-induced diabetic rats. Food Sci Nutr 10(4): 1058-1069.
124. Al Za'abi M, Ali H, Ali BH (2021). Effect of flaxseed on systemic inflammation and oxidative stress in diabetic rats with or without chronic kidney disease. PLoS One 16(10): e0258800
125. Bayat E, Rahpeima Z, Dastghaib S, Gholizadeh F, Erfani M, Asadikaram G, Mokarram P (2020). *Stevia rebaudiana* extract attenuate metabolic disorders in diabetic rats via modulation of glucose transport and antioxidant signaling pathways and aquaporin-2 expression in two extrahepatic tissues. J Food Biochem 44(8): e13252.
126. Takeuchi M, Imano H, Muraki I, Shimizu Y, Hayama-Terada M, Kitamura A, Okada T, Kiyama M, Iso H (2018). Serum creatinine levels and risk of incident type 2 diabetes mellitus or dysglycemia in middle-aged Japanese men: A retrospective cohort study. Vet Rec 6(1): e000492.
127. Kashima S, Inoue K, Matsumoto M, Akimoto K (2017). Low serum creatinine is a type 2 diabetes risk factor in men and women: The yuport health checkup center cohort study. Diabetes Metab 43(5): 460-464.
128. Bellomo R, See EJ (2021). Novel renal biomarkers of acute kidney injury and their implications. Intern Med J 51(3):316-318.
129. Hjelmesæth J, Røislien J, Nordstrand N, Hofsø D, Hager H, Hartmann A (2010). Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study. BMC Endocr Disord 10: 6.

130. Song DK, Hong YS, Sung YA, Lee H (2022). Association of serum creatinine levels and risk of type 2 diabetes mellitus in Korea: A case control study. BMC Endocr Disord 22(1): 4.
131. Esmat A, Alzahrani AM, Alharthy BT, Ramadan WS, Sattar Ahmad M (2022). potential nephroprotective effect of dorsomorphin homolog 1 (dmh1) in a rat model of diabetic nephropathy. Eur Rev Med Pharmacol Sci 26(7): 2489-2500.
132. Cheng Y, Xu C, Wang S, Hou L, Guan Q, Zhou X (2020). Serum cystatin C levels are decreased in type 1 diabetes mellitus patients with diabetic ketoacidosis. Minerva Endocrinol 45(2): 106–116.
133. Bozkurt E, Pektaş G (2020). The regulatory effects of Resveratrol on the expression of renal MMP-2 and MMP-9 in the rat models of diabetes. Ant J Bot 4(2): 85-91.
134. Omoboyowa DA, Karigidi KO, Aribigbola TC. (2020). Bridelia ferruginea Benth leaves attenuates diabetes nephropathy in STZ-induced rats via targeting NGAL/KIM-1/cystatin C gene. Clin Phytosci 6: 63.
135. Nomura H, Kuruppu S, Rajapakse NW (2022). Stimulation of angiotensin converting enzyme 2: A novel treatment strategy for diabetic nephropathy. Front Physiol 12: 813012.
136. Rushworth CA, Guy JL, Turner AJ (2008). Residues affecting the chloride regulation and substrate selectivity of the angiotensin-converting enzymes (ACE and ACE2) identified by site-directed mutagenesis. The FEBS J 275(23): 6033–6042.
137. Soler MJ, Wysocki J, Batlle D (2013). ACE2 alterations in kidney disease. Nephrol Dial Transplant 28(11): 2687-2697.
138. Patel VB, Parajuli N, Oudit GY (2014). Role of angiotensin-converting enzyme 2 (ACE2) in diabetic cardiovascular complications. Clin Sci (Lond) 126(7): 471-482.
139. Kajiwara K, Sawa Y, Fujita T, Tamaoki S (2021). Immunohistochemical study for the expression of leukocyte adhesion molecules, and FGF23 and ACE2 in *P. gingivalis* LPS-induced diabetic nephropathy. BMC Nephrol 22(1): 3.
140. Ahmed YM, Abdelgawad MA, Shalaby K, Ghoneim MM, AboulMagd AM, Abdelwahab NS, Hassan HM, Othman AM (2022). Pioglitazone synthetic analogue ameliorates streptozotocin-induced diabetes mellitus through modulation of ACE 2/angiotensin 1-7 via PI3K/AKT/mTOR signaling pathway. Pharmaceuticals 15(3): 341.

141. Ben P, Hu M, Wu H, Zhang Z, Gao Y, Luo L, Yin Z (2018). L-Theanine down-regulates the JAK/STAT3 pathway to attenuate the proliferation and migration of vascular smooth muscle cells induced by angiotensin II. Biol Pharm Bull 41(11): 1678-1684.
142. Bohara J, Kunwar S, Akela G, Joshi SR, Gurung S (2021). Serum electrolytes disturbances in type 2 diabetic patients. IIJHSR 11(7): 105-110.
143. Khanduker S, Ahmed R, Khondker F, Aharama A, Afrose N, Chowdhury M (2018). Electrolyte disturbances in patients with diabetes mellitus. Bangladesh J Med Biochem 10(1): 27–35.
144. Datchinamoorthi S, Vanaja R, Rajagopalan B (2016). Evaluation of serum electrolytes in type II diabetes mellitus. Int J Pharm Sci Rev Res 40: 251-253.
145. Chinaka N, Chuku LC, Monago-Ighorodje C, Edeogu O (2018). Antioxidant supplementation and kidney function status of wistar rats following high fat diet-streptozotocin (HFT-STZ) induced type 2 diabetes. BM BJ 4(3): 24.
146. Akpan O (2013). *Ocimum Gratissimum* alleviates derangements in serum and biliary bilirubin, cholesterol and electrolytes in streptozotocin- induced diabetic rats. Int J Biochem Res 3: 171-189.
147. Thomason EJ, Suárez-Pozos E, Afshari FS, Rosenberg PA, Dupree JL, Fuss B (2022). Deletion of the sodium-dependent glutamate transporter GLT-1 in maturing oligodendrocytes attenuates myelination of callosal axons during a postnatal phase of central nervous system development. Front Cell Neurosci 16: 905299.
148. Rimmele TS, Rocher AB, Wellbourne-Wood J, Chatton JY (2017). Control of glutamate transport by extracellular potassium: Basis for a negative feedback on synaptic transmission. Cereb Cortex 27(6): 3272-3283.
149. Wang L, Brennan M, Li S, Zhao H, Lange KW, Brennan C (2022). How does the tea L-Theanine buffer stress and anxiety. Food Sci Hum Wellness 11(3): 467-475.
150. Vio CP, Gallardo P, Cespedes C, Salas D, Diaz-Elizondo J, Mendez N (2020). Dietary potassium downregulates angiotensin-I converting enzyme, renin, and angiotensin converting enzyme 2. Front Pharmacol 11: 920.
151. Malkoç M, Patan H, Özer Yaman S, Türedi S, Ersöz Ş, Vanizor Kural B, Örem A. (2022). The effect of L-Theanine on the oxidant-antioxidant balance in serum and lung tissue in experimentally induced sepsis in rats. IJBB 59: 139-147.

152. Coregliano-Ring L, Goia-Nishide K, Rangel ÉB (2022). Hypokalemia in diabetes mellitus setting. Medicina 58(3): 31.
153. Gonzalez AA, Gallardo M, Cespedes C, Vio CP (2019). Potassium intake prevents the induction of the renin-angiotensin system and increases medullary ACE2 and COX-2 in the kidneys of angiotensin II-dependent hypertensive rats. Front Pharmacol 10: 1212.
154. Slotki IN, Skorecki KL (2016). Disorders of sodium balance: The kidney, Brenner and Rector"s. 11th ed. Philadelphia: Elsevier, Philadelphia: 390–459.
155. Gholami A, Dabbaghmanesh MH, Ghasemi Y, Talezadeh P, Koohpeyma F, Montazeri-Najafabadyet N (2020). Probiotics ameliorate pioglitazone-associated bone loss in diabetic rats. Diabetol Metab Synd 12: 78.
156. Omoruyi FO, Budiaman A, Eng Y, Olumese FE, Hoesel JL, Ejilemele A, Okorodudu AO (2013). The potential benefits and adverse effects of phytic acid supplement in streptozotocin-induced diabetic rats. Adv Pharmacol Sci 2013: 172494.
157. Ward DT, Yau SK, Mee AP, Mawer EB, Miller CA, Garland HO, Riccardi D (2001). Functional, molecular, and biochemical characterization of streptozotocin-induced diabetes. J Am Soc Nephrol 12(4): 779-790.
158. van de Wal-Visscher ER, Kooman JP, van der Sande FM (2018). Magnesium in chronic kidney disease: Should we care? Blood Purif 45(1-3): 173-178.
159. Pelczyńska M, Moszak M, Bogdański P (2022). The Role of magnesium in the pathogenesis of metabolic disorders. Nutrients 14(9): 1714.
160. Morais JB, Severo JS, Santos LR (2017). Role of magnesium in oxidative stress in individuals with obesity. Biol Trace Elem Res 176(1): 20-26.
161. Manoj Chandra MC, Laxmegowda (2022). Association of serum magnesium levels among type 2 diabetes mellitus patients with diabetic retinopathy. J Assoc Physicians India 70(4): 11-12.
162. Batar PK (2022). Study of serum magnesium level in diabetes mellitus and it's correlation with micro and macro complications. J Assoc Physicians India 70(4): 11-12.
163. Barooti A, Kamran M, Kharazmi F, Eftakhar E, Malekzadeh K, Talebi A, Soltani N (2019). Effect of oral magnesium sulfate administration on blood glucose hemostasis via inhibition of gluconeogenesis and FOXO1 gene expression in liver and muscle in diabetic rats. Biomed Pharmacother 109: 1819-1825.

164. Gommers LM, Hoenderop JG, Bindels RJ, de Baaij JH (2016). Hypomagnesemia in type 2 diabetes: A vicious circle? Diabetes 65(1): 3-13.
165. Dasdelen MF, Er S, Kaplan B, Celik S, Beker MC, Orhan C, Tuzcu M, Sahin N, Mamedova H, Sylla S, Komorowski J, Ojalvo SP, Sahin K, Kilic E (2022). A novel theanine complex, Mg-L-Theanine improves sleep quality via regulating brain electrochemical activity. Front Nutr 9: 874254.
166. Collingridge GL, Abraham WC (2022). Glutamate receptors and synaptic plasticity: The impact of Evans and Watkins. Neuropharmacology 206: 108922.
167. Kontoghiorghes GJ, Kontoghiorghe CN (2020). Iron and chelation in biochemistry and medicine: New approaches to controlling iron metabolism and treating related diseases. Cells 9(6): 1456.
168. Zhang R, Huang X, Li Y, Yu Z, Wu Y, Zha B, Ding H, Zang S, Liu J (2021). Serum ferritin as a risk factor for type 2 diabetes mellitus, regulated by liver transferrin receptor 2. Endocr Connect 10(12): 1513-1521.
169. Jelena A, Mirjana M, Desanka B, Svetlana IM, Aleksandra U, Goran P, Ilijana G (2013). Haptoglobin and the inflammatory and oxidative status in experimental diabetic rats: Antioxidant role of haptoglobin. J Physiol Biochem 69(1): 45-58.
170. Ozcelik D, Tuncdemir M, Ozturk M, Uzun H (2011). Evaluation of trace elements and oxidative stress levels in the liver and kidney of streptozotocin-induced experimental diabetic rat model. Gen Physiol Biophys 30(4): 356-363.
171. Wolide AD, Zawdie B, Alemayehu T, Tadesse S (2016). Evaluation of serum ferritin and some metal elements in type 2 diabetes mellitus patients: Comparative cross-sectional study. Diabetes Metab Syndr Obes 9: 417-424.
172. Buerkli S, Salvioni L, Koller N, Zeder C, Teles MJ, Porto G, Habermann JH, Dubach IL, Vallelian F, Frey BM, Moretti D, Baumgartner J, Zimmermann MB (2022). The effect of a natural polyphenol supplement on iron absorption in adults with hereditary hemochromatosis. Eur J Nutr 61(6): 2967-2977.
173. Lazrak M, El Kari K, Stoffel NU, Elammari L, Al-Jawaldeh A, Loechl CU, Yahyane A, Barkat A, Zimmermann MB, Aguenaou H (2021). Tea consumption reduces iron bioavailability from NaFeEDTA in nonanemic women and women with iron deficiency anemia: Stable iron isotope studies in Morocco. J Nutr 151(9): 2714-2720.

174. Al-Momen H, Hussein HK, Al-Attar Z, Hussein MJ (2020). Green tea influence on iron overload in thalassemia intermedia patients: A randomized controlled trial. F1000Res 9: 1136.
175. Koonyosying P, Kongkarnka S, Uthaipibull C, Svasti S, Fucharoen S, Srichairatanakool S (2018). Green tea extract modulates oxidative tissue injury in beta-thalassemic mice by chelation of redox iron and inhibition of lipid peroxidation. Biomed Pharmacother 108: 1694-1702.
176. Saewong T, Ounjaijean S, Mundee Y, Pattanapanyasat K, Fucharoen S, Porter JB, Srichairatanakool S (2010). Effects of green tea on iron accumulation and oxidative stress in livers of iron-challenged thalassemic mice. Med Chem 6(2): 57-64.
177. Yamada T, Terashima T, Kawano S, Furuno R, Okubo T, Juneja LR, Yokogoshi H (2009). Theanine, gamma-glutamylethylamide, a unique amino acid in tea leaves, modulates neurotransmitter concentrations in the brain striatum interstitium in conscious rats. Amino Acids 36(1): 21-27.
178. Appagere V, Kashinath RT, Sandya HP, Indumati V, Shekanawar MS, Amareshwara M, Rajeshwari, Krishnaswamy D (2014). Glutaminase activity in various tissues and their relative organ weights in long term (90 days) alloxan diabetic rats. Medica Innovative 3(1): 133-138.
179. Curthoys NP, Kuhlenschmidt T (1975). Phosphate-independent glutaminase from rat kidney. Partial purification and identity with gamma-glutamyltranspeptidase. J Biol Chem 250(6): 2099-2105.
180. Cui M, Gu M, Lu Y, Zhang Y, Chen C, Ling HQ, Wu H (2020). Glutamate synthase 1 is involved in iron-deficiency response and long-distance transportation in *Arabidopsis*. J Integr Plant Biol 62(12): 1925-1941.
181. Kang H, Han M, Xue J, Baek Y, Chang J, Hu S, Nam H, Jo MJ, El Fakhri G, Hutchens MP, Choi HS, Kim J (2019). Renal clearable nanochelators for iron overload therapy. Nat Commun 10(1): 5134.
182. Azarova I, Klyosova E, Polonikov A (2021). The link between type 2 diabetes mellitus and the polymorphisms of glutathione-metabolizing genes suggests a new hypothesis explaining disease initiation and progression. Life 11(9): 886.
183. Gao H, Wu H (2022). Maslinic acid activates renal AMPK/SIRT1 signaling pathway and protects against diabetic nephropathy in mice. BMC Endocr Disord 22(1):25.

184. Sánchez-Duarte S, Márquez-Gamiño S, Montoya-Pérez R, Villicaña-Gómez EA, Vera-Delgado KS, Caudillo-Cisneros C, Sotelo-Barroso F, Melchor-Moreno MT, Sánchez-Duarte E (2021). Nicorandil decreases oxidative stress in slow- and fast-twitch muscle fibers of diabetic rats by improving the glutathione system functioning. J Diabetes Investig 12(7): 1152-1161.
185. Rukavina-Mikusic IA, Rey M, Martinefski M, Tripodi V, Valdez LB (2021). Temporal evolution of cardiac mitochondrial dysfunction in a type 1 diabetes model. Mitochondrial complex I impairment, and H_2O_2 and NO productions as early subcellular events. Free Radic Biol Med 162: 129-140.
186. Winiarska A, Knysak M, Nabrdalik K, Gumprecht J, Stompór T (2021). Inflammation and oxidative stress in diabetic kidney disease: The targets for SGLT2 inhibitors and GLP-1 receptor agonists. Int J Mol Sci 22(19): 10822.
187. Cyrus J, Shiva R, Mohammad Reza S (2019). *Falcaria vulgaris* extract attenuates diabetes-induced kidney injury in rats. Asian Pac J Trop Biomed 9(4): 150-157.

APPENDIX