The Therapeutic Potential of Phloretin: Implications for Diabetic Nephropathy and Oxidative Stress Management

Griffin Rick

Abstract:

Diabetes mellitus is a chronic metabolic disorder affecting a significant proportion of the global population. According to recent statistics, an estimated 463 million individuals worldwide are living with diabetes. Among the various complications associated with diabetes, diabetic nephropathy, a progressive kidney disease, poses a substantial burden on affected individuals. Approximately 40% of people with diabetes develop diabetic nephropathy, making it a prevalent complication of diabetes.[1]

Diabetic kidney disease, also known as diabetic nephropathy, is a complication associated with diabetes where the overall ability of the kidneys to filter blood is impaired. Patients may experience mild symptoms such as proteinuria (excessive protein in urine) and hypertension (high blood pressure) in the early stages.[2] However, as the condition progresses, more severe complications can arise, including chronic kidney disease (CKD), end-stage renal disease (ESRD), and the need for dialysis or kidney transplantation. These complications can significantly impact a person's quality of life, leading to fatigue, fluid retention, electrolyte imbalances, anemia, and an increased risk of cardiovascular problems. [3, 4]

Oxidative stress plays a significant role in diabetic kidney disease (DKD). One notable contributing factor to DKD is oxidative stress, which refers to an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them.[5] Hyperglycemia, a hallmark of diabetes, mediates oxidative stress in the kidney by triggering the overproduction of ROS during cellular metabolism, leading to cellular damage and inflammation within the renal tissue.[6, 7]

Phloretin, a dihydrochalcone, has been shown to act as a glucose transporter 2 (GLUT2) inhibitor in early cancer studies that prevent glucose absorption and metabolism in cancerous tissues. Beyond the scope of cancer cells, phloretin may serve other physiological roles, which, in the kidneys, could act in response to severe oxidative stress both proactively via mitigating glucose-induced ROS production and retroactively via acting as an antioxidant and promoting the expression of antioxidant enzymes.[8] Moreover, phloretin possesses certain chelation properties that may further prevent and treat complications related to heavy metal toxicity-induced diabetes mellitus, a relevant contributor to the development of diabetic nephropathy via oxidative stress. [9]

This paper focuses on the positive potential of phloretin therapy on overall kidney function while speculating on the negative implications for phloretin that involve short-term damage to the liver and pancreas. To this extent, this paper concludes by introducing the concepts of 1) a metformin-based phloretin treatment plan and 2) maximizing the localization of phloretin to the kidneys to minimize off-target effects.

Introduction:

Diabetic kidney disease, more commonly known as diabetic nephropathy, is a serious and relatively common complication associated with diabetes that significantly impairs kidney

function and the kidney's ability to remove harmful waste products from the bloodstream sufficiently.[2, 3]

One of the key factors contributing to the progression of diabetes and its complications is oxidative stress, which refers to an imbalance between the formation of reactive oxygen species (ROS) and the body's antioxidant defense system.[10] In diabetic nephropathy, prolonged exposure to high blood glucose concentrations can lead to increased ROS production, which is generally attributed to accelerations in cellular metabolism, namely glycolysis, whereby glucose is broken down to produce energy.[6]

In the context of chronic hyperglycemia relating to the kidneys, overstimulation of glycolysis and the citric acid cycle (TCA cycle) can lead to overproduction of NADH, a major electron carrier that is generated via the two aforementioned pathways.[11, 12] The respective increased flux of NADH flooding into the electron transport chain (ETC) within the mitochondria can overwhelm the system and lead to electron leakage, prompting the formation of reactive oxygen species. [11, 13]

Furthermore, heavy metal toxicity-induced diabetes mellitus is another relevant factor in developing diabetic nephropathy.[14, 15] Exposure to heavy metals like lead, cadmium, or arsenic can induce oxidative stress by promoting certain oxidative pathways, which can alter glucose metabolism and induce oxidative stress, leading to diabetes. [16]

Understanding the relationships between oxidative stress, diabetic nephropathy, diabetes, and central carbon metabolism is critical in discovering therapeutic opportunities for some of the underlying conditions surrounding the development of diabetic nephropathy. Targeting these processes through therapeutic interventions, like the use of phloretin, are worth exploring to mitigate oxidative stress and improve kidney function in diabetic patients.

1. ROS Formation:

1.1 Glycolysis and NADH Formation

Glycolysis is a ten-step process that results in the formation of two pyruvate molecules.

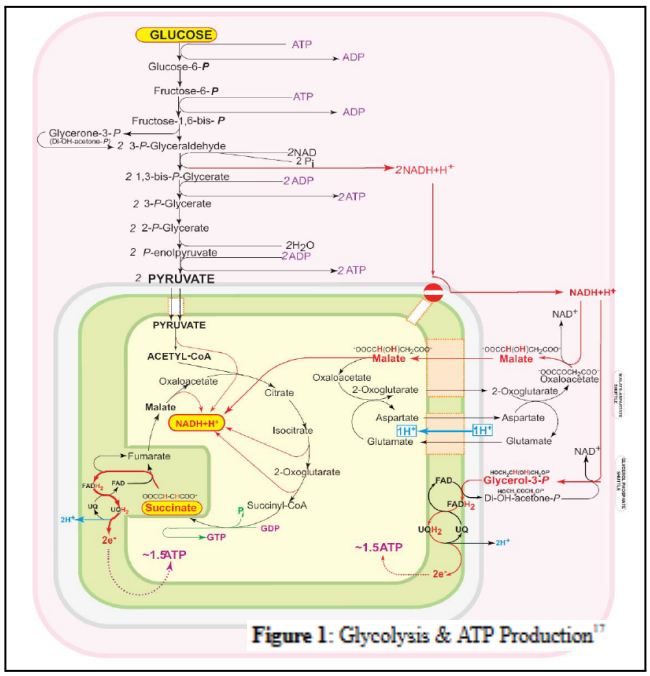

Figure 1: Glycolysis & ATP Production[17]

$$\text{Glucose} + NAD^+ + 2\ ATP + 2\ Pi \rightarrow 2\ \text{Pyruvate} + 2\ NADH + 2\ ATP$$

Pyruvate undergoes decarboxylation, forming Acetyl-CoA, which can enter the citric acid cycle. NADH, the electron-carrying molecule which can enter Complex 1 of the ETC, plays a crucial role in cellular processes. Specifically, the sixth step of glycolysis involves two glyceraldehyde 3-phosphate molecules, which react with NAD+ and Mg++ and are catalyzed by glyceraldehyde phosphate dehydrogenase to form 1,3-phosphoglycerate and NADH.

1.2 Electron Transport Chain and ROS Formation

The electron transport chain (ETC) is an important set of reactions that occur along the mitochondrial membrane. It is instrumental in forming two components, electrons and hydrogen ions, to fuel its main goal: ATP production. The ETC is, however, an extremely complex and fickle process that requires delicate transfer of electrons between many different enzymes, all of which are susceptible to changes in function due to external factors. One major outcome of inefficient electron transfer is the formation of reactive oxygen species (ROS).

The first step of ETC is commenced by Complex 1, which comprises FMN, Fe-S, N-2, and Ubiquinone-Q, each of which directly supports the electron transport chain by aiding in the transfer of electrons (See Figure 2). To initiate the process, FMN oxidizes NADH to NAD+, and the two electrons removed during this process are then moved from FMN to Fe-S, N-2, then eventually transferred to the ubiquinone-q substrata, which can accept the electrons alongside two free hydrogen ions to form Ubiquinol.

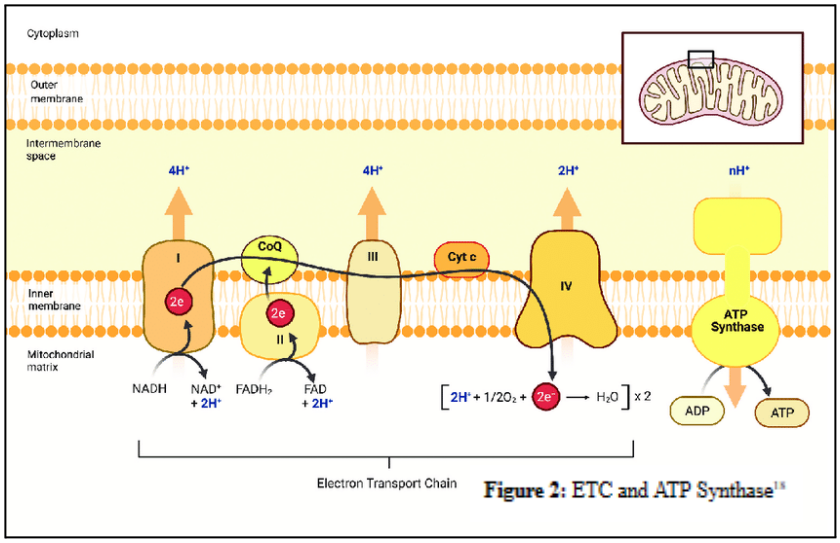

Figure 2: ETC and ATP Synthase[18]

Complex 2 involves the movement of electrons into the intermembrane space by $FADH_2$. In Complex 2, the enzyme succinate dehydrogenase is a membrane-bound enzyme that catalyzes the transport of two electrons from the $FADH_2$ molecule$_2$, which is oxidized to FAD+, and the electrons removed during this process move to Ubiquinone-Q to form Ubiquinol by taking hydrogen ions from the mitochondrial matrix.

Complex 3, the Q-cytochrome C oxidoreductase or the cytochrome bc1 complex, comprises two main subunits, cytochrome b and cytochrome c1. In the reaction, electrons from QH_2, as the result of complex 2, are transferred to cytochrome c. When two cytochrome c molecules are reduced, one molecule of QH2 is oxidized, meaning two hydrogen ions are removed from the matrix.

Ultimately, the pumping of hydrogen ions into the intermembrane space creates a concentration gradient that favours the flow of protons back into the mitochondrial matrix.

Complex 4, also known as the cytochrome oxidase complex, oxidizes cytochrome c.Electrons travel through the enzyme and eventually reduce O_2 to H_2O. During the electron transfer and reduction step, complex 4 pumps protons across the inner mitochondrial membrane from within the matrix to the intermembrane space. This also contributes to the generation of the aforementioned proton gradient that ultimately powers ATP synthase, also known as complex 5. As a result of the reaction, four electrons and four protons are used to form two water molecules (H_2O), and this ensures the disposal of electrons from the ETC thereby aiding in the prevention of electron leakage and subsequent ROS formation.

Contribution of two electrons from glycolysis-derived NADH to complex 1 is coupled with proton pumping from the mitochondrial matrix to the intermembrane space. In the case of

diabetes, kidney-associated cells experience persistently heavy glycolytic flux, leading to a surplus of NADH that saturates electron transport in the mitochondria.[19] The overflow is further perpetuated by insufficient proton pumping, and it ultimately results in substantial electron leakage and subsequent formation of superoxide (O_2^-), the most common type of ROS.[20, 21]

The ETC is considered the primary physiological event associated with ROS formation in diabetic patients, and elevated glycolytic flux in kidney tissue as a result of chronic hyperglycemia overruns the body's ability to prevent oxidative stress. [22, 23]

2. Effects of ROS on Renal Structure and Function

2.1 ROS-Induced Nephron Deterioration

The introduction of excess ROS through the glomerular apparatus can contribute significantly to structural impairments, such as damage to endothelial cells, the glomerular basement membrane, and the podocytes.[24]

The podocytes are incredibly delicate structures that, when functionally operative, allow the nephrons to screen waste for proteins that would otherwise be released through urination. The mechanism that affects the function of the podocytes is the balance of intracellular redox signaling. Excessive ROS can perturb this balance, leading to altered cellular metabolism that significantly impairs the podocyte's ability to repair its damaged tissues.[24, 25]

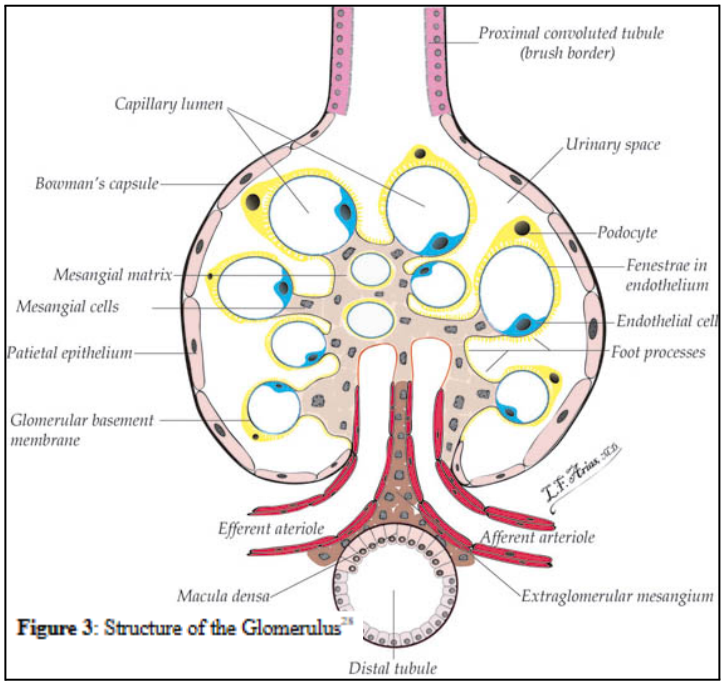

Figure 3: Structure of the Glomerulus[28]

In addition, ROS is also known to affect the cytoskeletal organization of podocytes.[26] Specifically, the actin cytoskeleton is essential for maintaining the kidneys' structural integrity and proper function of the kidneys. Disruption of the actin cytoskeleton can result in podocyte foot process effacement, leading to the flattening or loss of finger-like protrusions on the podocyte membrane.[27] This disruption can impact the efficacy of the filtration barrier, causing selective permeability and allowing proteins to leak into the urine, a condition known as proteinuria. Additionally, prolonged exposure to ROS has also been shown to promote pro-fibrotic changes within the podocytes, leading to the accumulation of extracellular matrix proteins that can result in the development of glomerulosclerosis, which is characterized by intense stiffening of the glomerular filtration apparatus and the hardening of the glomerular basement membrane (GBM), which further impairs kidney function (see figure 3).[27, 29]

One of the critical features of the GBM is its selective permeability, whereby the renin-angiotensin system (RAAS) may alter the permeability of the GBM and endothelial cells to increase or decrease the rate by which the glomerular apparatus can filter waste.[30] In the context of oxidative stress, modifications to the GBM components such as collagen IV and

heparan sulfate proteoglycans (HSPGs), both of which affect tissue organization and interact with growth factors, cytokines, enzymes, and extracellular matrix components, can lead to increased permeability and subsequent leakage of proteins into the urine. [31, 32]

ROS can also damage the structure and function of the endothelium. Increased ROS causes poly (ADP-ribose) polymerase to become active within endothelial cells, which is associated with decreased activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).[33] Reduced GAPDH activity is associated with NF-kB activation and increased expression of certain isoforms of protein kinase c (PKC), both of which decrease eNOS expression by impairing or uncoupling NOS enzymes.[34] These enzymes typically serve to dilate vascular smooth muscle cells through the production of nitric oxide (NO). In the absence of NO, vasoconstriction is likely to occur as there is a substantial lack of counteraction against potent vasoconstrictors like angiotensin II (Ang II) and endothelin-1 (ET-1).[34, 35, 36,] Ultimately, cardiovascular deterioration can occur as a result of the increased pressure exerted on these constricted capillaries.

The enhancement of alternative metabolic pathways by hyperglycemia can lead to increased diacylglycerol and a subsequent increase in the expression of particular isoforms of PKC that are typically associated with the dilation of vascular smooth muscle cells.[35, 36] In the context of the kidneys, elevated PKC may be associated with the dilation of the afferent arterioles that flow into the glomerular apparatus, ultimately leading to an increase in intraglomerular pressure.[37] As eNOS expression decreases and vasoconstrictors are released following ROS induction in the bowman's capsule, Ang II and ET-1 constrict the efferent arterioles and limit the rate at which fluid can commute from the glomerulus after initial filtration. [36]

The combination of these renal-vascular modifications forces an increased amount of blood plasma through the glomerulus and through the remainder of the nephron. Oftentimes the hyperflow of blood plasma causes increased urination by elevating the volume of fluid that is filtered through the kidneys. This hyperfiltration can contribute to certain issues related to dehydration, electrolyte imbalance, and the loss of blood and proteins.[3, 4, 38]

Overall, the damage caused by ROS in the kidneys can be severe in high enough concentrations and can significantly damage the structure and function of the kidneys. The removal of ROS or prevention of ROS formation in renal tissues is a potential way of preventing or treating diabetic nephropathy. [39]

3. Introduction to Glucose Transporter 2 (GLUT2)

Glucose transporter 2 (GLUT2) is a specialized transporter protein responsible for moving extracellular glucose to the cytoplasm, where it is the starting substrate for glycolysis. GLUT2 is most commonly found in the liver, kidneys, and pancreas, all of which do not require large amounts of glucose to maintain metabolic homeostasis. For these reasons, GLUT2 has a relatively low affinity for glucose. It requires a high venous glucose concentration to initiate glucose transport into GLUT2 cells/tissues, which can be useful in the signaling of certain sensitive pathways like insulin secretion and glycogen storage.

In the pancreas, GLUT2 is the primary glucose transporter in pancreatic β-cells, which are responsible for triggering insulin release in response to elevated blood glucose levels. Insulin secretion initiates glucose transport in GLUT4 tissues such as adipose tissue and skeletal muscle, which require insulin to facilitate glucose transport into the cytoplasm.

In the liver, the role of GLUT2 is to harvest blood glucose when in higher concentrations and store the glucose in the form of glycogen, which can then be reconverted to glucose when blood levels of this metabolite are low. The byproduct of glucose utilization by muscle is lactate, which can be re-harvested from the blood by the liver. The captured lactate can be recycled into glucose via gluconeogenesis to be used for glycogen storage or stabilization of blood glucose levels. It is also worth noting that the reason GLUT2 is found in the liver is likely because of its relatively low affinity for blood glucose uptake, making it a prime candidate for limiting the amount of glucose taken from the blood to be used in glycogen conversion/storage, making it a retroactive energy supplier to the body that serves as a critical aid in maintaining a consistent blood glucose level.

3.1 The Role of GLUT 2 in the Kidneys:

In the kidneys, GLUT 2 is utilized primarily in the proximal tubules, which are responsible for filtering blood and turning its waste into urine. The main function of GLUT2 in the kidneys is to facilitate the movement of glucose across the luminal membrane of the proximal tubule cells, allowing glucose to be transported from the tubular fluid into the cells.[40] Once inside these cells, glucose can either be utilized in glucose metabolism or be transported across the basolateral membrane into the interstitial fluid and then back into the bloodstream in a process known as reabsorption or reuptake. This process of glucose reabsorption is crucial in maintaining glucose homeostasis in the body.[38]

Normally, the kidneys filter a large amount of glucose from the blood into the glomerular filtrate in the initial phase of filtration, which occurs in the glomerulus/bowman's capsule.[41, 42] Still, under normal physiological conditions, almost all of this filtered glucose is reabsorbed by the proximal tubules via GLUT2, preventing excess loss of glucose in the urine. [40,]

Due to GLUT2 having a low affinity for glucose, the kidneys are able to reabsorb glucose in response to varying glucose concentrations in the blood, ensuring efficient glucose reabsorption.[40] The primary physiological issue with GLUT2 reabsorption in the kidneys is that in diabetes, proximal reabsorption of glucose is extremely high because of glucosuria, or increased glucose concentration in the filtrate, which indicates to the GLUT2 cells that glucose should be reabsorbed into the blood, thus preventing excess glucose from being filtered out. [40, 43, 44]

Furthermore, sodium-glucose cotransporter (SGLT2) is responsible for maintaining venous electrolyte balance via reabsorption of both glucose and salts in the proximal tubules.[45] Therefore, a change in urine glucose concentrations common in glycosuria may perpetuate blood electrolyte imbalance, leading to a variety of negative health effects like certain neurological abnormalities such as cardiac arrhythmia.[46, 47] Hence, the regulation of this interface is crucial in maintaining overall homeostasis.

4. Phloretin Therapy – GLUT 2 Inhibitors:

One potential therapeutic option for mitigating chronic hyperglycemia is inhibiting proximal reabsorption of glucose in the kidneys, which would facilitate the passage of glucose through the nephron body towards the bladder for eventual secretion.

Similarly, phloretin possesses certain chelation properties that may further prevent and treat complications related to heavy metal toxicity-induced diabetes mellitus. This characteristic

of phloretin may be significant in defending against heavy metal-ROS formation pathways like the Fenton and Haber-Weiss0 pathways.

4.1 GLUT2 in Competitive Inhibition

Phloretin works to inhibit GLUT2 through a process known as competitive inhibition.[48] Phloretin competes with glucose to bind the active site of the GLUT2 transport protein, which prevents glucose from being transported across the cell, and has been shown to inhibit GLUT2 in cancer-based clinical trials.[8, 49, 50, 51] Because phloretin only remains in the active site for a brief period of time, it can bind and unbind from the active site in predictable intervals and as necessary.[52, 53,] Additionally, the predictability of this interaction is beneficial because varying concentrations and quantities of phloretin can be used for different strengths of interference, allowing for diversification of treatment plans for different patients at varying stages of diabetic nephropathy.

The specific molecular mechanism surrounding the interaction of phloretin with GLUT2 is largely unknown. However, we can draw likely conclusions based on the molecular composition of the phloretin molecule, namely a combination of hydrophobic and hydrogen bond interactions.

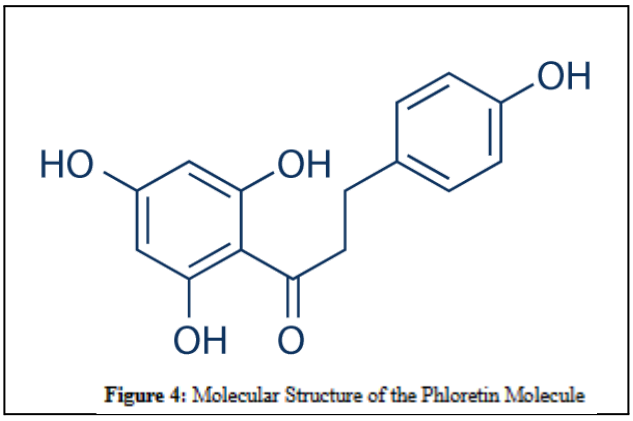

Figure 4: Molecular Structure of the Phloretin Molecule

Phloretin is composed of aromatic rings and hydrophobic regions (See Figure 4). The hydrophobic regions of the phloretin molecule can interact with the hydrophobic amino acid residues present in the transmembrane region of the GLUT2 protein that includes the active site. This interaction largely involves Van der Waals forces. It enables the formation of non-polar contacts between phloretin and the protein, which are not particularly strong in terms of bond strength.

In addition to the hydrophobic interactions, phloretin also contains hydroxyl groups (-OH) that can act as hydrogen bond donors or acceptors: so these hydroxyl groups may form hydrogen bonds with specific amino acid residues in the active site of GLUT2, leading to stronger bonds between the active site and the phloretin molecule than the previously mentioned contacts involving Van der Waals forces

It is also important to note that the actual bond affinity of phloretin to GLUT2 is unknown, so it is unknown whether glucose or phloretin is more likely to bind to GLUT2. Nevertheless, phloretin, if proven to hold a stronger affinity towards GLUT2, may have implications for fast-acting effects in cases of acute renal failure.

4.2 GLUT2 in the Antioxidant Defense System

Phloretin exhibits antioxidant activity primarily through its ability to scavenge free radicals and inhibit oxidative stress.[54] Phloretin possesses phenolic groups that can donate hydrogen atoms or electrons to free radicals, neutralizing their reactivity.

Phloretin is also a highly capable chelation molecule, which can bind to metal ions like iron or copper. Metal ions, in high concentrations, can catalyze the generation of free radicals through Fenton and Haber-Weiss reactions, and by chelating these ions, phloretin may help

prevent heavy metals' participation in these reactions by essentially wrapping the metal ion in the phloretin molecules, reducing the harmful free radical production.

The Fenton reaction involves the catalytic role of iron II in generating hydroxyl radicals.

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH$$

Hydroxyl radicals are highly reactive and can cause oxidative damage to cellular components such as lipids, proteins, and nucleic acids.[55]

The significance of the chelation properties of phloretin lies in the correlation between heavy metal toxicity and diabetes. Elevated venous heavy metal concentration has been shown to contribute to the severity of diabetes and some of its complications.[56, 57] Specifically, exposure to lead, cadmium, arsenic, and mercury has been associated with an increased risk of developing diabetes or exacerbating its symptoms, specifically in developing insulin resistance and inducing pancreatic β-cell death.[58]

Heavy metals are also fairly common in the environment. They can enter the body through various sources such as contaminated water, food, air pollution, occupational exposure, and many consumer products. Prolonged exposure to any heavy metal source significantly increases the risk of developing diabetes or worsening its complications.[56, 58] It is also worth noting that by removing harmful heavy metals from the bloodstream via chelation, phloretin may prevent the formation of advanced glycation end-products by sequestering metal ions.

For instance, chronically elevated blood glucose levels can lead to the glycation of hemoglobin, known as HbA1c.[59] Importantly, iron can promote the glycation of hemoglobin specifically by utilizing the Fenton reaction within the bloodstream. The process of glycation is facilitated by the attachment of glucose to nitrogen in the formation of a Schiff base. It is formed largely as a result of hyperglycemia. The Schiff base turns into a ketone via the Amadori Adduct, which results in the crosslinking of arginine and lysine residues, subsequently forming an Amadori product that can easily bind to hemoglobin. The glycation of HbA1c renders it incapable of transporting oxygen in the bloodstream. The introduction of phloretin could both minimize hyperglycemia and limit reactive iron concentrations in the blood thereby mitigating the effects of glycation.

Phloretin has also been shown to induce the expression of certain antioxidant enzymes, including superoxide dismutase (SOD) and catalase, both of which play crucial roles in neutralizing free radicals and detoxifying reactive oxygen species.[60]

4.3 Improvements in Kidney Function

Phloretin treatment will likely lead to improvements in kidney function by decreasing ROS formation and increasing proactive mechanisms to help eliminate radicals. Additionally, the decrease in the formation of advanced glycation end products (AGEs), specifically advanced glycated enzymes, will likely lead to decreased intraglomerular hypertension. The corresponding decrease in blood pressure may result in lessening the damaging effects of ROS-induced glomerulosclerosis, heightened glomerular filtration rate, glomerular basement membrane deterioration, podocyte dysfunction, and in severe cases, endothelial dysfunction.

5. Negative Externalities:

5.1 Renal Function

Although the potential local effects of inhibiting GLUT2 in the kidneys are fairly optimistic, it is also important to understand the possible negative impacts phloretin could have on the kidneys and other organ systems.

Although overall function may improve, the kidneys may see negative effects due to phloretin-induced glucosuria. As mentioned above, sodium-glucose cotransporter (SGLT2) is responsible for maintaining venous electrolyte balance through reabsorption in the proximal tubules, and a change in urine glucose concentration in glucosuria may perpetuate venous electrolyte imbalance.[45] In addition, chronic glucosuria may result in progressive deterioration of the kidneys in events similar to glycation. It could limit reuptake potentials for other proteins/cells and lead to osmotic diuresis as the body attempts to level the glucose gradient by attempting to dilute the urine by increasing water secretion.

5.2 Hepatic Function

In the liver, GLUT2 is crucial in maintaining glucose homeostasis all over the body, and inhibiting GLUT2 may disrupt the normal uptake and utilization of glucose by the hepatocytes, which may lead to altered glucose metabolism, abnormal blood glucose levels, and further contribute to altered hepatic insulin resistance.

The liver's primary function in terms of blood glucose management is the storage of glucose in the form of glycogen, which can be broken down and released back into the bloodstream in the form of glucose when concentrations decrease such that metabolic homeostasis is jeopardized. The role of GLUT2 in the liver is to regulate this exchange, and by inhibiting GLUT2, glucose uptake by the liver may change such that glycogen synthesis, storage, and signaling are jeopardized, leading to a decreased capacity to store glycogen and regulate blood glucose concentrations.

GLUT2 inhibition may also impact liver metabolism beyond glycolysis, as the liver plays a crucial role in various metabolic processes, including lipid metabolism, protein synthesis, and blood detoxification. One such metabolic change could alter the process of gluconeogenesis, such that lactate, a major input for gluconeogenesis, shifts to a point where lactate concentrations increase in the bloodstream and can cause serious adverse health effects like metabolic acidosis, increased myocardial oxygen consumption, tissue hypoxia, or even organ failure.

5.3 Pancreatic Function

In the pancreas, GLUT2 is primarily expressed in pancreatic β-cells responsible for producing and secreting insulin into the bloodstream. GLUT2 allows for the entry of glucose into beta cells, which can trigger insulin release in response to elevated blood glucose levels to attempt to increase cellular glucose uptake by GLUT4 (insulin-dependent) tissues to decrease blood glucose levels. Inhibiting GLUT2 can disrupt normal glucose sensing and impair glucose-stimulated insulin secretion, leading to reduced β-cell insulin production and further compromising blood glucose regulation.

In addition, GLUT2 is also involved in glucose metabolism within pancreatic β-cells, and limiting normal glucose utilization and metabolism within beta cells could potentially affect energy production and other cellular processes.

GLUT2 inhibition may also lead to changes in gene expression within the pancreas, including genes related to insulin synthesis, glucose metabolism, and pancreatic cell differentiation. By disrupting the normal expression of these genes, developmental abnormalities may present as reduced cellular proliferation, differentiation, apoptosis, and impaired immune response.

6. Phloretin v. Other Antioxidants

Concerning their antioxidant properties, phloretin and other antioxidants share a few characteristics in terms of their ability to regulate oxidative stress and limit free radical damage. Compared to some of its antioxidant counterparts like vitamin C, vitamin E, resveratrol, and curcumin, phloretin does not perform as well, mainly because of the wide range of tissues that the alternate antioxidants can target. Due to its insolubility in water, phloretin cannot readily transverse the bloodstream, making it difficult to promote as an all-around solution to inhibiting oxidative damage across the entirety of the kidneys.

For example, Vitamin C (ascorbic acid) is a potent antioxidant that, like phloretin, scavenges free radicals and protects against oxidative damage. Unlike phloretin, Vitamin C's water-soluble characteristics allow it to maneuver across many different tissue types. Vitamin E (tocopherol) is a fat-soluble antioxidant that primarily protects cell membranes, specifically, from oxidative damage by neutralizing lipid peroxyl radicals, and also carries specific anti-inflammatory properties, which could be an option for limiting oxidative damages within the glomerular basement membrane and podocyte foot processes as well as being able to navigate fatty tissues easily.

However, as stated before, the beneficial aspects of phloretin extend beyond its ability to prevent oxidative damage. Examples of its beneficial effect include retroactively inducing antioxidant enzymes to help prevent oxidative stress in the long term, anti-diabetic chelation potential, the ability to inhibit glycolysis and subsequent electron leakage in GLUT2 renal tissues, and anti-AGE formation.[54, 55, 59]

It is also worth noting that the possibility of using phloretin as a broad-spectrum antioxidant is relatively slim, as the damage to the pancreas and liver would likely be too severe for the benefits in antioxidant prevention to outweigh the negative externalities. However, there are some modern solutions that could address the challenge of localization.

Modern Implications for Therapeutic Phloretin Interventions

Modern innovation in localized drug administration could have significant implications for the delivery method of phloretin to renal tissues without the invasive injection method that phloretin would require for localization. Phloretin, because of its water-insoluble nature, is not likely to transfer to other organ systems in large quantities if administered directly to the kidneys themselves, so the administration of phloretin in an organ-specific manner would likely be an effective work-around to the administration of phloretin intravenously.

Modern nanotechnology and advancements in delivery systems could mean a simple oral administration of phloretin encapsulated in nanoparticles designed to target the kidney could provide the level of localization required to aid the body in treating the oxidative damages of diabetic nephropathy.[61, 62]

Specifically, through targeting renal tubular cells directly involved in diabetic nephropathy, surface modifications on nanoparticles can be designed to bind to receptors or transporters expressed in renal tubular cells, allowing for the delivery of phloretin directly to the desired site of action within the kidneys, maximizing therapeutic efficacy and minimizing the effects on other organ systems.[62]

Metformin is commonly known and used as an oral medication for type 2 diabetes. Recent research has explored the potential protective effects of metformin to be used on the kidneys specifically. Still, the combination of metformin and phloretin might be a serious therapeutic option for diabetic nephropathy.

Metformin has demonstrated renoprotective properties in preclinical studies, and metformin reduces inflammation and fibrosis in the kidneys, which have implications for diabetic nephropathy.[63, 64] Metformin is also commonly used for its ability to lower blood glucose levels in individuals with diabetes, and by improving insulin sensitivity and reducing glucose production by the liver, metformin can help control blood sugar levels.

The ability of metformin to remove glucose from the bloodstream by reactivating insulin-dependent cells/tissues in type 2 diabetes alongside the beneficial properties of phloretin on the kidneys may lead to significant improvements in overall kidney function by allowing for urination to occur without perpetuating glucosuria, electrolyte imbalances, and dehydration, making this combination an appealing treatment for cases of acute renal failure.

Conclusion

The use of phloretin as a therapeutic option for mitigating chronic hyperglycemia and heavy metal toxicity-induced diabetes mellitus holds promise. Its ability to inhibit GLUT2 and compete with glucose for binding sites shows potential for fast-acting effects in cases of acute renal failure. Additionally, phloretin's antioxidant properties, including scavenging free radicals and chelating metal ions, offer a means to prevent oxidative stress and limit the formation of advanced glycation end-products. While negative externalities such as potential impacts on renal, hepatic, and pancreatic function should be considered, modern innovations in localized drug administration, such as nanotechnology-based delivery systems, may help address these concerns. Combining phloretin with other therapeutic agents like metformin could provide a comprehensive approach to treating diabetic nephropathy and improving overall kidney function. Overall, the potential benefits of phloretin-based antioxidant therapy in preventing and treating diabetic nephropathy are significant and warrant further investigation and development.

Bibliography

1.Zhu T, Li K, Herrero P, Georgiou P. Deep Learning for Diabetes: A Systematic Review. *IEEE Journal of Biomedical and Health Informatics*. 2021;25(7):2744-2757. doi:https://doi.org/10.1109/jbhi.2020.3040225

2.Sheira G, Noreldin N, Tamer A, Saad M. Urinary biomarker N-acetyl-β-D-glucosaminidase can predict severity of renal damage in diabetic nephropathy. *Journal of Diabetes & Metabolic Disorders*. 2015;14(1). doi:https://doi.org/10.1186/s40200-015-0133-6

3.Fu H, Liu S, Bastacky SI, Wang X, Tian XJ, Zhou D. Diabetic kidney diseases revisited: A new perspective for a new era. *Molecular Metabolism*. 2019;30:250-263. doi:https://doi.org/10.1016/j.molmet.2019.10.005

4.Doria A. Genetics of Diabetes Complications. *Current Diabetes Reports*. 2010;10(6):467-475. doi:https://doi.org/10.1007/s11892-010-0147-x

5.Turkmen K. Inflammation, oxidative stress, apoptosis, and autophagy in diabetes mellitus and diabetic kidney disease: the Four Horsemen of the Apocalypse. *International Urology and Nephrology*. 2016;49(5):837-844. doi:https://doi.org/10.1007/s11255-016-1488-4

6.Bhatti JS, Sehrawat A, Mishra J, et al. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. *Free Radical Biology and Medicine*. 2022;184:114-134. doi:https://doi.org/10.1016/j.freeradbiomed.2022.03.019

7.Rochette L, Zeller M, Cottin Y, Vergely C. Diabetes, oxidative stress and therapeutic strategies. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 2014;1840(9):2709-2729. doi:https://doi.org/10.1016/j.bbagen.2014.05.017

8.Wu C, Yuan Soon Ho, Chia Ti Tsai, et al. *In vitro and in vivo study* of phloretin-induced apoptosis in human liver cancer cells involving inhibition of type II glucose transporter. *Cancer*. 2009;124(9):2210-2219. doi:https://doi.org/10.1002/ijc.24189

9.Hu H, Bai X, Xu K, Zhang C, Chen L. Effect of phloretin on growth performance, serum biochemical parameters and antioxidant profile in heat-stressed broilers. 2021;100(8):101217-101217. doi:https://doi.org/10.1016/j.psj.2021.101217

10.Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current neuropharmacology*. 2009;7(1):65-74. doi:https://doi.org/10.2174/157015909787602823

11.Naudi A, Jove M, Ayala V, et al. Cellular Dysfunction in Diabetes as Maladaptive Response to Mitochondrial Oxidative Stress. *Experimental Diabetes Research*. 2012;2012:e696215. doi:https://doi.org/10.1155/2012/696215

12.Mokini Z, Marcovecchio ML, Chiarelli F. Molecular pathology of oxidative stress in diabetic angiopathy: Role of mitochondrial and cellular pathways. *Diabetes Research and Clinical Practice*. 2010;87(3):313-321. doi:https://doi.org/10.1016/j.diabres.2009.11.018

13.Galvan DL, Green NH, Danesh FR. The hallmarks of mitochondrial dysfunction in chronic kidney disease. *Kidney International*. 2017;92(5):1051-1057. doi:https://doi.org/10.1016/j.kint.2017.05.034

14.Mitra S, Chakraborty AJ, Tareq AM, et al. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *Journal of King Saud University - Science*. 2022;34(3):101865. doi:https://doi.org/10.1016/j.jksus.2022.101865

15.Cooper RG. Effect of tobacco smoking on renal function. 2006;124(3):261-268.

16.Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology*. 2011;283(2-3):65-87. doi:https://doi.org/10.1016/j.tox.2011.03.001

17.Dasika S. Glycolysis and TCA Cycle. ResearchGate. Published January 2007. https://www.researchgate.net/figure/Glycolysis-and-TCA-cycle-13_fig5_241825789

18.Turton N. Coenzyme Q 10 and the exclusive club of diseases that show a limited response to treatment. ResearchGate. Published May 2021. https://www.researchgate.net/figure/Diagram-of-electron-transport-chain-ETC-and-ATP-synthase-illustrating-the-pumping-of-H_fig2_351722900

19.Kanasaki K, Maeshima A, Taduri G, Revuelta I. Combating Kidney Fibrosis. *BioMed Research International*. 2014;2014:1-2. doi:https://doi.org/10.1155/2014/679154

20.Jankovic A, Korac A, Buzadzic B, et al. Redox implications in adipose tissue (dys)function—A new look at old acquaintances. *Redox Biology*. 2015;6:19-32. doi:https://doi.org/10.1016/j.redox.2015.06.018

21.Oliveira HCF, Vercesi AE. Mitochondrial bioenergetics and redox dysfunctions in hypercholesterolemia and atherosclerosis. *Molecular Aspects of Medicine*. 2020;71:100840. doi:https://doi.org/10.1016/j.mam.2019.100840

22.He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. *Cellular Physiology and Biochemistry*. 2017;44(2):532-553. doi:https://doi.org/10.1159/000485089

23.Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*. Published online March 30, 2020. doi:https://doi.org/10.1038/s41580-020-0230-3

24.Rask-Madsen C, King George L. Vascular Complications of Diabetes: Mechanisms of Injury and Protective Factors. *Cell Metabolism*. 2013;17(1):20-33. doi:https://doi.org/10.1016/j.cmet.2012.11.012

25.Singh A, Kukreti R, Saso L, Kukreti S. Mechanistic Insight into Oxidative Stress-Triggered Signaling Pathways and Type 2 Diabetes. *Molecules*. 2022;27(3):950. doi:https://doi.org/10.3390/molecules27030950

26.Hsu HH, Hoffmann S, Endlich N, et al. Mechanisms of angiotensin II signaling on cytoskeleton of podocytes. 2008;86(12):1379-1394. doi:https://doi.org/10.1007/s00109-008-0399-y

27.Suleiman HY, Roth R, Jain S, Heuser JE, Shaw AS, Miner JH. Injury-induced actin cytoskeleton reorganization in podocytes revealed by super-resolution microscopy. *JCI Insight*. 2017;2(16). doi:https://doi.org/10.1172/jci.insight.94137

28.Murray I. Normal Histology: Glomeruli - Interstitium - Tubules - Vessels . Kidney Pathology. Published October 12, 2006. https://kidneypathology.com/English_version/Glomeruli_histology.html

29.Hu C, Sun L, Xiao L, et al. Insights into the Mechanisms Involved in the Expression and Regulation of Extracellular Matrix Proteins in Diabetic Nephropathy. *Current Medicinal Chemistry*. 2015;22(24):2858-2870. doi:https://doi.org/10.2174/0929867322666150625095407

30.Jourde-Chiche N, Fakhouri F, Dou L, et al. Endothelium structure and function in kidney health and disease. *Nature Reviews Nephrology*. 2019;15(2):87-108. doi:https://doi.org/10.1038/s41581-018-0098-z

31.Marchand M, Monnot C, Muller L, Germain S. Extracellular matrix scaffolding in angiogenesis and capillary homeostasis. *Seminars in Cell & Developmental Biology*. 2019;89:147-156. doi:https://doi.org/10.1016/j.semcdb.2018.08.007

32.Karamanos NK, Theocharis AD. Matrix modeling and remodeling: A biological interplay regulating tissue homeostasis and diseases. *Matrix Biology*. 2019;75-76:1-11. doi:https://doi.org/10.1016/j.matbio.2018.08.007

33.Fiorentino T, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced Oxidative Stress and its Role in Diabetes Mellitus Related Cardiovascular Diseases. *Current Pharmaceutical Design*. 2013;19(32):5695-5703. doi:https://doi.org/10.2174/1381612811319320005

34.Pandolfi A, De EA. Chronic hyperglycemia and nitric oxide bioavailability play a pivotal role in pro-atherogenic vascular modifications. *Genes & Nutrition*. 2007;2(2):195-208. doi:https://doi.org/10.1007/s12263-007-0050-5

35.C. Adam Kirton, Loutzenhiser R. Alterations in basal protein kinase C activity modulate renal afferent arteriolar myogenic reactivity. *American Journal of Physiology*. 1998;275(2):H467-H475. doi:https://doi.org/10.1152/ajpheart.1998.275.2.h467

36.Gschwend SK. *Small Artery Tone under Control of the Endothelium: On the Importance of EDHF and Myogenic Tone in Organ (Dys)Function*. University of Gronigen; 2003. https://pure.rug.nl/ws/portalfiles/portal/3024541/thesis.pdf

37.Sasson AN. Renal hyperfiltration related to diabetes mellitus and obesity in human disease. *World Journal of Diabetes*. 2012;3(1):1. doi:https://doi.org/10.4239/wjd.v3.i1.1

38.Liamis G. Diabetes mellitus and electrolyte disorders. *World Journal of Clinical Cases*. 2014;2(10):488. doi:https://doi.org/10.12998/wjcc.v2.i10.488

39.Jha JC, Gray SP, Barit D, et al. Genetic Targeting or Pharmacologic Inhibition of NADPH Oxidase Nox4 Provides Renoprotection in Long-Term Diabetic Nephropathy. *Journal of the American Society of Nephrology*. 2014;25(6):1237-1254. doi:https://doi.org/10.1681/asn.2013070810

40.Vallon V. Glucose transporters in the kidney in health and disease. *Pflügers Archiv - European Journal of Physiology*. 2020;472(9):1345-1370. doi:https://doi.org/10.1007/s00424-020-02361-w

41.Eaton DC. *Vander's Renal Physiology*. Mcgraw-Hill Medical; 2018.

42.Sasaki T, Tsuboi N, Haruhara K, et al. Bowman Capsule Volume and Related Factors in Adults With Normal Renal Function. *Kidney International Reports*. 2018;3(2):314-320. doi:https://doi.org/10.1016/j.ekir.2017.10.007

43.Gerich JE. Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: therapeutic implications. *Diabetic Medicine*. 2010;27(2):136-142. doi:https://doi.org/10.1111/j.1464-5491.2009.02894.x

44.Vallon V, Thomson SC. The tubular hypothesis of nephron filtration and diabetic kidney disease. *Nature Reviews Nephrology*. 2020;16(6):317-336. doi:https://doi.org/10.1038/s41581-020-0256-y

45.Bjornstad P, Greasley PJ, Wheeler DC, et al. The Potential Roles of Osmotic and Nonosmotic Sodium Handling in Mediating the Effects of Sodium-Glucose Cotransporter 2 Inhibitors on Heart Failure. *Journal of Cardiac Failure*. 2021;27(12):1447-1455. doi:https://doi.org/10.1016/j.cardfail.2021.07.003

46.Arnold R, Issar T, Krishnan AV, Pussell BA. Neurological complications in chronic kidney disease. *JRSM Cardiovascular Disease*. 2016;5(5):204800401667768. doi:https://doi.org/10.1177/2048004016677687

47.R. Brouns. Neurological complications in renal failure: a review. *Clinical Neurology and Neurosurgery*. 2004;107(1):1-16. doi:https://doi.org/10.1016/j.clineuro.2004.07.012

48.Hytti M, Ruuth J, Kanerva Ii, et al. Phloretin inhibits glucose transport and reduces inflammation in human retinal pigment epithelial cells. *Molecular and Cellular Biochemistry*. 2022;478(1):215-227. doi:https://doi.org/10.1007/s11010-022-04504-2

49.Choi B. Biochemical Basis of Anti-Cancer-Effects of Phloretin—A Natural Dihydrochalcone. *Molecules*. 2019;24(2):278. doi:https://doi.org/10.3390/molecules24020278

50.Ho YS. The apple polyphenol phloretin inhibits breast cancer cell migration and proliferation via inhibition of signals by type 2 glucose transporter. *Journal of Nutrition & Food Sciences*. 2018;08. doi:https://doi.org/10.4172/2155-9600-c1-054

51.Shriwas P, Roberts D, Li Y, et al. A small-molecule pan-class I glucose transporter inhibitor reduces cancer cell proliferation in vitro and tumor growth in vivo by targeting glucose-based metabolism. *Cancer & Metabolism*. 2021;9(1). doi:https://doi.org/10.1186/s40170-021-00248-7

52.Cheon D, Kim J, Shin HC, Kim Y. Target Proteins of Phloretin for Its Anti-Inflammatory and Antibacterial Activities Against Propionibacterium acnes-Induced Skin Infection. *Molecules*. 2019;24(7):1319. doi:https://doi.org/10.3390/molecules24071319

53.Behzad S, Sureda A, Barreca D, Nabavi SF, Rastrelli L, Nabavi SM. Health effects of phloretin: from chemistry to medicine. *Phytochemistry Reviews*. 2017;16(3):527-533. doi:https://doi.org/10.1007/s11101-017-9500-x

54.Anand V, Ramachandran Vinyagam, Vinothkumar Rajamanickam, Sankaran V, Venkatesan S, David E. Pharmacological Aspects and Potential Use of Phloretin: A Systemic Review. 2019;19(13):1060-1067. doi:https://doi.org/10.2174/1389557519666190311154425

55.Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*. 2007;39(1):44-84. doi:https://doi.org/10.1016/j.biocel.2006.07.001

56.Peng S, Liu L, Zhang X, et al. A nested case-control study indicating heavy metal residues in meconium associate with maternal gestational diabetes mellitus risk. *Environmental Health*. 2015;14(1). doi:https://doi.org/10.1186/s12940-015-0004-0

57.Flores CR, Puga MP, Wrobel K, Garay Sevilla ME, Wrobel K. Trace elements status in diabetes mellitus type 2: possible role of the interaction between molybdenum and copper in the progress of typical complications. *Diabetes Research and Clinical Practice*. 2011;91(3):333-341. doi:https://doi.org/10.1016/j.diabres.2010.12.014

58.Chen YW, Yang CY, Huang CF, Hung DZ, Leung YM, Liu SH. Heavy metals, islet function and diabetes development. *Islets*. 2009;1(3):169-176. doi:https://doi.org/10.4161/isl.1.3.9262

59.Lippi G, Targher G. Glycated hemoglobin (HbA1c): old dogmas, a new perspective? *Clinical Chemistry and Laboratory Medicine*. 2010;48(5). doi:https://doi.org/10.1515/cclm.2010.144

60.Yang YC, Lii CK, Lin AH, et al. Induction of glutathione synthesis and heme oxygenase 1 by the flavonoids butein and phloretin is mediated through the ERK/Nrf2 pathway and protects

against oxidative stress. 2011;51(11):2073-2081. doi:https://doi.org/10.1016/j.freeradbiomed.2011.09.007

61.Khalil I, Yehye WA, Etxeberria AE, et al. Nanoantioxidants: Recent Trends in Antioxidant Delivery Applications. *Antioxidants*. 2019;9(1):24. doi:https://doi.org/10.3390/antiox9010024

62.Li CW, Li LL, Chen S, Zhang JX, Lu WL. Antioxidant Nanotherapies for the Treatment of Inflammatory Diseases. *Frontiers in Bioengineering and Biotechnology*. 2020;8. doi:https://doi.org/10.3389/fbioe.2020.00200

63.Nasri H, Azar Baradaran, Ardalan M, Saeed Mardani, Momeni A, Mahmoud Rafieian-Kopaei. Bright renoprotective properties of metformin: beyond blood glucose regulatory effects. 2013;7(6):423-428.

64.De Broe Marc E, Kajbaf F, Lalau JD. Renoprotective Effects of Metformin. *Nephron*. 2017;138(4):261-274. doi:https://doi.org/10.1159/000481951