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In silico Study to Optimize the Dosage of Oleuropein with Metformin in Diabetes Management

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Abstract

Type 2 diabetes mellitus (T2DM) is a potentially fatal metabolic disorder worldwide, in this COVID-19 era. Long-term allopathic treatment has a variety of side effects, prompting the search for alternative therapies. Oleuropein, the primary bioactive ingredient of Olive Leaf Extract (OLE), has shown noteworthy actions to control T2DM. The present study provides a dynamic study of % improvement in GLUT4 concentration with different doses of metformin (150mg-500mg) in combination with 500mg using a dynamic *in silico* model developed in Cell Designer 4.4.2, a system biology tool. The results indicated that 300mg of metformin and 500mg of oleuropein is the optimum combination to treat diabetes, ensuring a 2% improvement in GLUT4 concentration and an effective reduction in the dose of the allopathic drug metformin when taken in combination with OLE for the improvement in T2DM. The optimal dose of metformin with oleuropein was obtained successfully using our proposed *in silico* dynamic model. The research could be used to create an *in vivo* model to examine the effectiveness of herbal medicine for T2DM.

Keywords: *In silico*; Diabetes; Olive Leaf Extract (Oleuropein); Quantification; Metformin
T2DM.

1. Introduction

The COVID-19 outbreak has been declared a public health emergency of worldwide concern by the World Health Organization (WHO). The COVID-19 virus appears to make older people and people with pre-existing medical disorders (such as diabetes, heart disease, and renal disease) more susceptible to becoming seriously ill. Due to variations in blood glucose levels and, presumably, the presence of diabetes complications, it can be more difficult to treat a viral infection in people with diabetes. The immune system is weakened, making it more difficult to fight the infection and resulting in a lengthier recovery time. Also, the virus may grow in a high-blood-glucose environment. In diabetic patients, post-COVID complications are also more severe, so, the control of sugar is a critical necessity of the day. People with diabetes should be included among the priority groups for vaccination programs because of their higher risk of negative health outcomes as a result of COVID-19, as per a recent International Diabetes

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Federation (IDF) study. A total of 347 million people are at risk of getting type 2 diabetes by 2045 [1-6]. The global prevalence of diabetes has grown tremendously, with 463 million people suffering from the condition in 2019, with type 2 diabetes accounting for 95 %. The therapeutic intervention has decreased disease consequences, necessitating the early detection and prevention of diabetes in this COVID-19 era [7-12]. People with type 2 diabetes have a scarcity of Glucose Transporter 4 (GLUT4) vesicles on the plasma membrane, which is the result of the individual's signalling cascade developing resistance. Increased hunger, thirst, frequent urination, weight loss, weariness, blurred vision, infections, and sores are all common symptoms of type 2 diabetes. Diabetes can also lead to a variety of life-threatening health issues [1], [13-21]. Because morbidity and mortality are the most prevalent complications of type 2 diabetes, medications must be taken properly combined with a healthy lifestyle. It's debatable if type 2 diabetes patients can ensure precise blood glucose control. Allopathic drugs, on the other hand, have been demonstrated to be effective in the treatment of type 2 diabetes. However, for type 2 diabetes patients, a healthy lifestyle, eating habits, and drugs like metformin are the most effective treatments. However, these medications have several side effects that put a strain on patients [13], [17–23].

Metformin is the most commonly prescribed drug for the treatment of type 2 diabetes. It regulates blood glucose levels by activating the Adenosine Monophosphate Kinase enzyme (AMPK), which then enhances the translocation of GLUT4 to the plasma membrane [24]–[28]. Although metformin hasn't been linked to any serious side effects, it has been linked to lactic acidosis, dizziness, muscle discomfort, and nausea. Aside from side effects, pharmacological treatments aren't always effective, and late-stage diabetes complications are unavoidable [22], [24], [27-34]. Other complementary therapies, such as dietary supplements, exercise, medicinal herbs, acupuncture, hydrotherapy, and yoga therapies, are effective when used in conjunction with pharmaceuticals. As a result, combining chemical agents with alternative therapies such as medicinal herbs can assist in alleviating the negative effects of chemical agents to some extent [29], [35-38]. The leaves of the olive tree (Olea europaea L.) have long been utilised in traditional medicine around the world for the treatment of type 2 diabetes in several experimental and clinical investigations. They include various potentially bioactive chemicals like oleuropein that may have antioxidant, antidiabetic, and anti-inflammatory activities and have been used in the human diet as extracts, herbal teas, and powder [23], [37-43]. Clinical evidence suggests that oleuropein can significantly enhance the phosphorylation of key components of the insulin signalling pathway, such as AMPK and the glucose transporter (GLUT4). In prior *in vivo* and *in vitro* experiments, researchers have investigated the glucose uptake mechanism in skeletal muscle in response to oleuropein [23], [37], [38], [44-47]. The following section discusses the substantive research carried out in the area of the effect of medicinal herbs and metformin on people with type 2 diabetes. Olive Leaf Extract is regarded as one of the most effective complementary therapies for the prevention and control of type 2 diabetes. The literature contains well-established *in vitro* studies on the effects of oleuropein on type 2 diabetic patients [48-56]. However, *in silico* analysis of the same thing is still an open area. Furthermore, the synergistic effect of oleuropein and metformin on individuals with type 2 diabetes will be extremely beneficial in diabetes treatment. Sindi recently conducted a study to determine the usefulness of oleuropein in humans with diabetes. Several human clinical investigations were evaluated, and the findings revealed that oleuropein could lower insulin resistance and hence ameliorate type 2 diabetes [23]. Hala et.al. experimented to determine the effectiveness of the hypoglycemic effect after the consumption of Olive Leaf Extract. Oral administration of OLE showed significant improvement in the glucose level of the rats [44]. Abdel et.al. carried out an experimental study on diabetic rats. The anti-diabetic efficacy of glyburide is enhanced by OLE at 500 mg/kg in diabetic rats, according to this study. Hepatic

glucose sensitivity and metabolism are significantly influenced by the usage of such a combination. As a result, glyburide dosage may require additional attention if administered in conjunction with OLE formulations to provide patients with improved disease control [42]. When metformin is used with oleuropein for our studies, this strategy may be beneficial. Using diabetic rats, Giacometti et. al. discovered that phenolic compounds from the olive leaf play a favorable function in the control of glucose homeostasis in the skeletal muscle via GLUT4 translocation [57]. The effect of oleuropein on GLUT4 enrichment levels in human cells at an optimum dose of 10 g/mL in human hepatocellular carcinoma cells was investigated by Kocyigit et. al. in an interesting *in vitro* study [58]. To get the finest outcomes, though, it's crucial to figure out the best and safest dose of OLE. As a result, clinical and experimental animal studies are needed to identify a solution. As a result, clinical and experimental animal studies are required to determine the most effective and non-toxic treatment doses.

Soliman et. al. also conducted an interesting study that demonstrated the significance of OLE in the prevention of type 2 diabetes-induced testicular toxicity in rats [39]. As a result, the advantages of OLE are limitless. Liguri et.al. carried out a study to examine the possible use of olive leaf polyphenols as a healthy supplement for the treatment of T2DM and summarized the scientific data for the same [51]. Alkhateeb et. al. examined glucose regulation in rat skeletal muscle in the presence of oleuropein in an *ex vivo* experiment. These findings show that oleuropein-induced enhanced glucose uptake may be mediated by AMPK activation and subsequent GLUT4 translocation in skeletal muscles [59]. Cecilia et. al. investigated the AMPK modulatory action of OLE phenolic compounds using an *in silico* methodology, in which the binding of oleuropein to AMPK is evaluated, and the results verified the modulation of AMPK by oleuropein [47]. Hadrich et. al. conducted a study in C2C12 muscle cells that demonstrated the effect of oleuropein on insulin sensitivity via AMPK activation and also established the underlying processes [46]. Wainstein et. al. conducted randomized clinical research to see if 500mg of oleuropein could improve glucose homeostasis in rats with type 2 diabetes, and the results were promising [45]. De Bock et. al. also completed a randomized clinical trial to assess the effect of OLE on glucose homeostasis in people with T2DM [60].

Also, Gupta et.al. investigated the interaction between anti-diabetic drugs and herbs and found out the clinical implications and mechanisms involved in the same. Based on the findings described, it is obvious that when herbal remedies are used with antidiabetic pharmaceutical medications, their pharmacokinetic and/or pharmacodynamic properties may be altered. Given the huge number of pathophysiological/pharmacological targets linked with the disease and the multicomponent qualities of herbs, these interactions are complicated [61]. One of the main objectives of the study is to determine the safety of consuming a lot of OLE. Guex et.al. explored this and examined the toxicity levels of Wister rats after giving them varying amounts of oleuropein throughout time. It did not cause toxicity after single and repeated dosages, according to the findings. More research is needed, however, to fully comprehend the consequences for human safety [62]. Clewell et. al. also performed a full safety investigation of OLE on Wister rats, with positive results: no side effects were found [63].
In a promising manner, the literature supports the benefits of Olive Leaf Extract over anti-diabetic medicine. Oleuropein has a well-established role in the treatment of type 2 diabetes. In type 2 diabetes patients, an *in silico* analysis of the effects of oleuropein when combined with a regular metformin dose on GLUT4 translocation is crucial. To investigate the combined effect of metformin and oleuropein on GLUT4 translocation in type 2 diabetes, a mathematical model for *in silico* investigations has yet to be established. No research quantifies the effect of an anti-diabetic medicine like metformin and a medicinal herb like oleuropein used together in humans in *in cc* or *in vivo* investigations. In addition, the optimum dose of metformin to take with

oleuropein has yet to be determined. The current study uses a computer simulation to examine the effects of oleuropein and metformin combined on GLUT4 translocation in skeletal muscles in people with type 2 diabetes. Based on the findings, an optimum dose of the medicine metformin has been identified, which should be sufficient to improve GLUT4 translocation and hence type 2 diabetes when combined with a standard dose of oleuropein. The model is created using Cell Designer software, which includes a solver for analysis [64]. The effect of oleuropein and the standard pharmacological dosage of metformin are combined in a novel *in silico* model. The work described here quantifies the effect of oleuropein in combination with the best medicine, metformin, on the surface GLUT4 concentration.

2. Oleuropein and Metformin interaction with insulin signalling pathways

In patients with type 2 diabetes, AMPK is the most common target for enhancing GLUT4 translocation in skeletal muscle in addition to insulin signalling pathways. The objective of this research is to find out how different alternative therapies and currently used medicines affect GLUT4 translocation. Understanding the fundamental processes of signalling pathways is critical to comprehending this role. The proposed method by which oleuropein and metformin drugs produce AMPK phosphorylation, which promotes GLUT4 translocation, is presented in the sections below. Earlier, the role of insulin in GLUT4 translocation had already been discussed in depth [65][66]. The signalling pathways are made up of a complicated structure with many inputs, outputs, and interactions. Although a complete understanding of this complicated structure is still a work in progress, the essential mechanisms that govern GLUT4 translocation are well understood. Oleuropein and drug-based AMPK activation pathways are principally responsible for GLUT4 translocation. In the most thorough known model of insulin signalling pathways for type 2 diabetes, *Sedaghat et. al.*, the present model integrates the influence of oleuropein and metformin on AMPK pathways for type 2 diabetes [46], [65], [66].

The basic processes of insulin, oleuropein, and metformin interaction with GLUT4 translocation are depicted in Figure 1. (Oleuropein and Metformin interaction with GLUT4) [65]. The insulin signalling pathway shows GLUT4 translocation via AMPK activation by metformin and oleuropein. The underlying mechanism for the cascade of insulin binding to GLUT4 translocation was already explained in the *Sedaghat et. al.* model [66] and also in [65]. Both oleuropein and metformin drugs independently activate AMPK. AMPK activation in turn catalyzes the phosphorylation of PKC ζ , which enhances the GLUT4 translocation to the plasma membrane [25], [46], [47], [67]. An *in silico* mathematical model to quantify the same thing has not yet been developed. The AMPK dynamics are incorporated into the Sedaghat model of insulin signalling pathways in response to oleuropein and conventional metformin doses in this present *in silico* dynamic model. The model is then used to determine the optimum dose of metformin that should be sufficient when combined with oleuropein to enhance GLUT4 translocation. When metformin is combined with oleuropein, the dose of metformin is reduced, which reduces the risk of long-term complications from the anti-diabetic medicines.

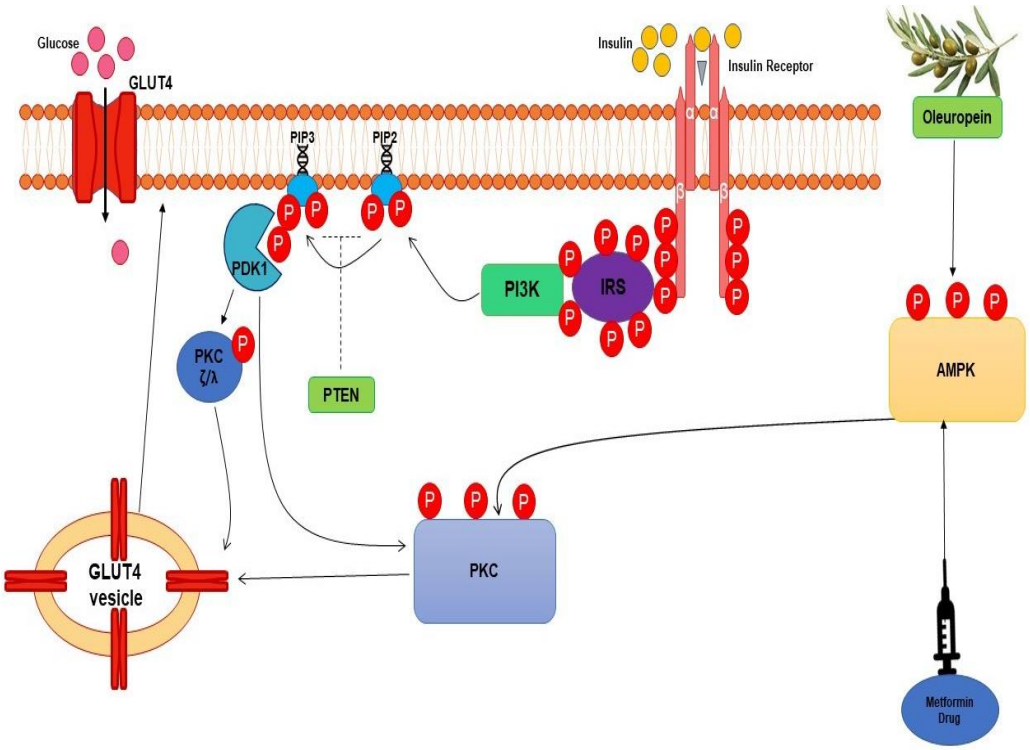

Figure 1 : Oleuropein and Metformin interaction with GLUT4

3. Methodology

Several techniques could be used to investigate the effect of oleuropein on surface GLUT4 concentration when administered with different metformin doses. As a result of the survey, it was observed that the majority of studies involving the effects of oleuropein and the drug metformin on type 2 diabetes patients were conducted using a systematic meta-analysis on the number of patients chosen for the study based on their body mass index, age, health, and a variety of other factors. *In vitro* and *in vivo* study related data was typically acquired from well-known research databases such as MEDLINE via PubMed, EBSCO, CINAHL, and the Cochrane Central Register of Controlled Trials. As a result, statistical analysis was performed on the data, taking into account various parameters as needed. Statistical approaches have always been a time-consuming process that is susceptible to demographic and statistical flaws. Only the qualitative study was carried out through clinical controlled trials and the quantification part of the interaction of medicinal herbs like oleuropein and metformin on GLUT4 translocation was missing. In the presented work on mathematical model development and *in silico* analysis, a system biology method was chosen. Much more precise information about insulin sensitivity with oleuropein and optimal metformin medication dosage was obtained in contrast to *in vitro* and *in vivo* studies. Cell Designer 4.4.2, a system biology tool, was utilized as a simulation platform to model the dynamics of AMPK interaction with GLUT4 translocation in response to metformin and oleuropein doses. The realisation of the parameters involved, as well as their interactions, was required for the development of a mathematical model. These parameters were connected by a mathematical expression, resulting in an Ordinary Differential Equation model (ODE). In addition, we used and expanded our recently published model [65] to include the influence of oleuropein on GLUT4 translocation in type 2 diabetes along with metformin [64], [68].

In order to create and simulate our model in Cell Designer, the volume of the compartment must first be chosen. Then, for the insulin signal transduction pathway components, the initial amounts for the reaction and species, as well as the kinetic rules with which they interact, as

well as the oleuropein and metformin doses and rate constant details, should be provided. The default values are calculated if none are specified. The residues can be added to the species as needed for phosphorylation, depending on whether they are active or inactive. Each species is included in the model with their reaction rates based on the interaction of the signalling pathways. As a result, a complete simulation model is now available. The simulation tab allows you to get results for each species as a function of time. The software can even export the model to other applications like JDesigner, where the differential equations underlying each signalling pathway component can be viewed. The model can also be exported to MATLAB's Simbiology (Matrix Laboratory) [64], [68]. The details of our *in silico* model are explored further below.

3.1 Model construction

Figure 2 (*In silico* model for Olive Leaf Extract (Oleuropein) and Metformin interaction with GLUT4) [65], [66] shows the *in silico* model developed in Cell Designer to investigate the effects of oleuropein and the drug metformin on GLUT4 translocation. As a foundation, we used our recently published *in silico* model c, which included the effect of metformin, physical exercise, and dynamics of insulin signalling pathways on GLUT4 translocation for type 2 diabetes [65], [66]. Now, for the present work, the effect of the medicinal herb Olive Leaf Extract-oleuropein, on surface GLUT4 translocation in type 2 diabetes was modelled. The type 2 diabetes condition was modelled by changing the value of protein tyrosine phosphatase (PTP) from 1 to 1.5. As a result, in the insulin resistance state, there was a noticeable decrease in surface GLUT4 concentration. Now, to find the optimum dose of metformin when taken with oleuropein, we have used the standard prescribed drug dosage of 500mg for both. The impact of oleuropein was modelled using the signalling pathway followed by the herb extract to interact with the insulin signal transduction components and finally enhance the GLUT4 translocation. In response to an oleuropein dose, the AMP-ADP-ATP bound AMPK gets activated, which then enhances the activation of oleuropein bound AMPK. This activates AMPK and enhances the phosphorylation of PKC ζ. In response to this, GLUT4 translocation takes place to the plasma membrane [69]–[71].

However, to analyze the interaction of 500mg oleuropein when taken with the standard drug metformin in type 2 diabetes on GLUT4 translocation, the dynamic *in silico* model was extended by incorporating the AMPK activation pathway where oleuropein binds to AMPK and activates the same. Modifications to the ordinary differential equation were made as needed. In type 2 diabetes signalling pathways, activated AMPK through metformin and oleuropein then enhances the GLUT4 translocation. The modified differential equation other than that from the *Sedaghat et.al.* model is given by **PKC ζ = k_{12} * x_{18} * (AMPK-P-ADP + AMPK-P-Metformin+ AMPK-P-Oleuropein)**, where '**P**' stands for phosphorylation, while k_{12} is the rate constant for PKC ζ activation and x_{18} is the percentage of inactivated PKC ζ as per the *Sedaghat et.al.* model. The objective of this study was to select the optimum dose of metformin to consume with 500 mg of oleuropein. This could help reduce the amount of allopathic medication used and prevent long-term consequences. Table 1 illustrates the simulation parameters that were taken into account for the model developed by combining the drug metformin and the herb oleuropein. The dissociation constant K_D for oleuropein binding to AMPK was obtained from the literature and used to determine the kinetic parameters reported in Table 1 [47]. The rest of the parameters and equations were taken from the literature and our recently published work [65], [66], [69].

Table 1: Simulation specific parameter values

Binding Reaction	Kinetic Parameters
Oleuropein-AMPK (Forward)	k_{12f} = 1 /(mM×s)
Oleuropein -AMPK (Reverse)	k_{12r} = 0.8575×10^{-2} /s

The findings of modeling the *in silico* model with different doses of metformin starting at 500mg when taken with 500mg of oleuropein on surface GLUT4 concentration are discussed in the following section.

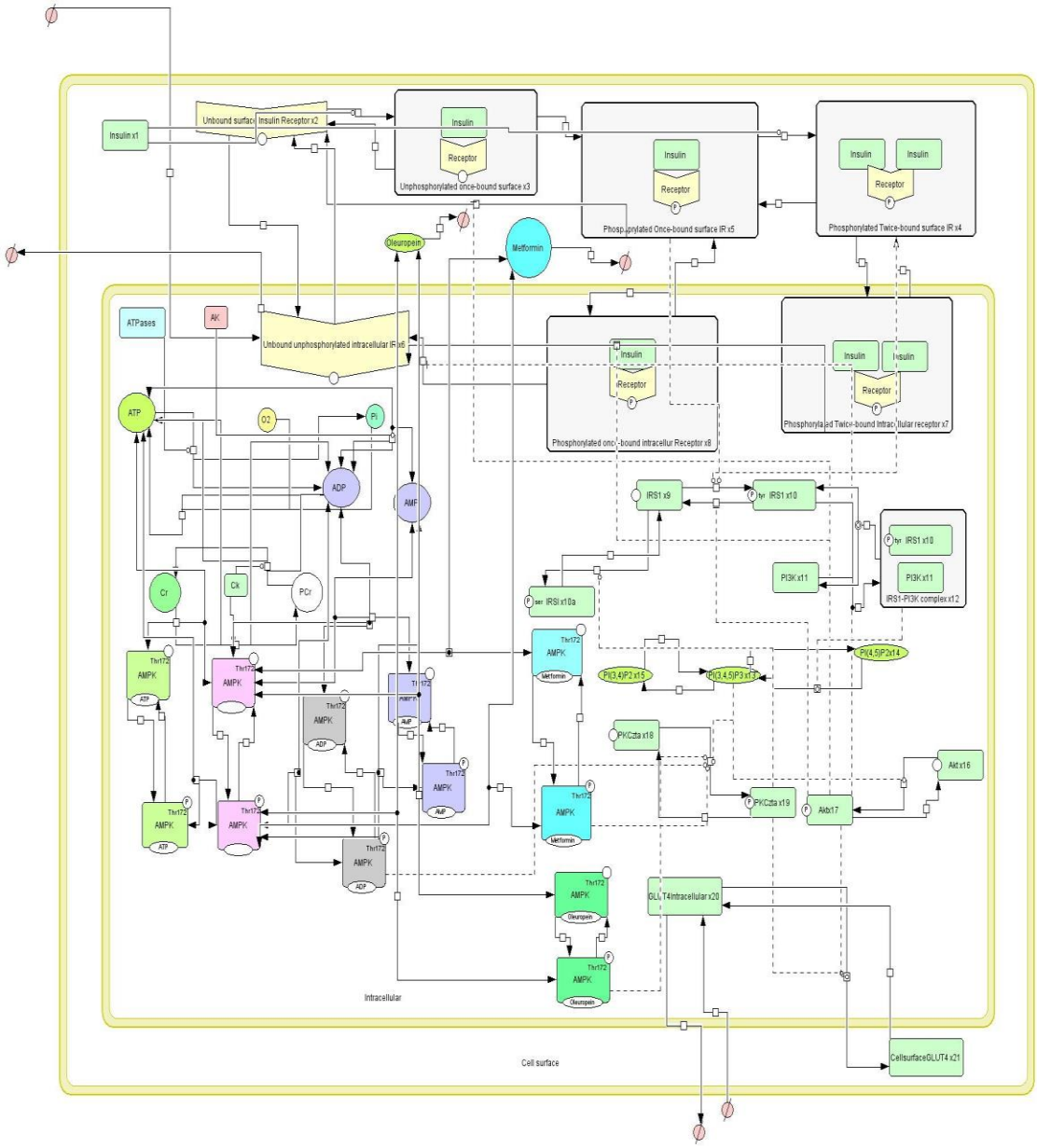

Figure 2: *In silico* model for Olive Leaf Extract (Oleuropein) and Metformin interaction with GLUT4

4. Results and Discussion

Several studies have shown that olive leaf extract-oleuropein has the potential to treat type 2 diabetes due to its anti-diabetic properties. Metformin, a regularly prescribed medicine for type 2 diabetes, requires long-term use to control glucose levels. Although it does not completely cure diabetes, it does assist in reducing the difficulties associated with high blood sugar levels. However, like allopathic medicine, it does have adverse effects. As per the current pandemic situation of COVID-19, an urgency has been raised to treat diabetes with fewer long-term complications [2], [3], [4]–[6], [10], [11], [23], [33], [37], [39], [41], [43], [46], [60].

In the proposed *in silico* model, we have incorporated the effect of Olive Leaf Extract-Oleuropein and the standard drug metformin on the surface GLUT4 concentration in type 2 diabetes. The objective here was to identify the optimal dose of metformin that would suffice when combined with 500mg of Oleuropein. As a result, unlike the natural herb, which does not have the same level of side effects, we may be able to reduce the metformin dose. This *in silico* dynamic model was facilitated by the data obtained from the mathematical model developed by *Sedaghat et.al.* [66] of insulin signalling pathways and our recently published work in [65]. Although maximum care was taken about the parameters of the model, constraints in real implementations might alter the observations. Also, the selection of specific parameters for analysis might alter the predictive capability of the model.

In addition, the influence of metformin and oleuropein on AMPK activation was modelled, with the value of the dissociation constant playing an important role. The accuracy of the concentration of various parameters used for simulation purposes determines the outcome of our *in silico* model. As a result, our *in silico* model will aid in the cost-effective execution of several studies without the use of actual cells. By properly selecting the parameters, this model might prove to be an efficient tool for analyzing the effect of various medications and natural herbs on surface GLUT4 concentration via AMPK activation in the future. Furthermore, because this is an *in silico* model, the research does not require statistical data such as population or patient counts. However, it must be taken into account while implementing the model *in vivo*. It's vital to remember that while using the *in vivo* model, the ambient and internal variables of the skeletal muscle cells must be controlled [65], [69], [70], [72]. The results achieved by modeling the *in silico* model mentioned here are detailed in the next part, keeping all of these criteria in mind.

For individuals with type 2 diabetes, the *in silico* model extensively simulates the measurement of the effect of metformin at various dosages, like 100mg to 500mg and Olive Leaf Extract (oleuropein) at 500mg. There were no formal recommendations for how much olive leaf extract to take. Participants in the human investigations in [60] took 500–1,000mg of standard Olive Leaf Extract per day on average. The Food and Drug Administration (FDA) recommends a normal dose of metformin of 500mg to a maximum of 2000 mg per day. The purpose was to quantify the effect of oleuropein and metformin on GLUT4 translocation as well as to find the optimum dose of metformin to prevent side effects. As a result, we used the highest available dose of 500mg oleuropein and continued to reduce the dose of metformin from 500mg by 10% to examine the effect of the herb and metformin interaction. In comparison to the *Sedaghat et. al.* model, 10^{-7} molar insulin was also used as an input to the model for 15 minutes. As a result, for each simulation outcome, the reduction in the surface GLUT4 concentration occurs after 15 minutes. The effect of oleuropein and metformin on surface GLUT4 concentration was quantified as shown in Table 2. A percentage improvement in surface GLUT4 concentration concerning type 2 diabetes was obtained as a result of different metformin drug dosages and oleuropein. When only metformin at the standard drug dosage of

500mg was consumed, only a 0.394% improvement in type 2 diabetes was observed, but when 500mg of oleuropein was given as a supplement to metformin, a 2.067% improvement in GLUT4 concentration was observed. The percentage improvement shows the enhancement in GLUT4 translocation. Thus, to find the optimum dose of metformin, we kept the oleuropein dose of 500mg constant throughout the simulation, while the metformin dose was reduced by 10% in each phase. The results were satisfactory, as it was discovered that a 300mg dose of metformin combined with 500mg oleuropein produced a suitable quantity of GLUT4 translocation enhancement. As a result, it was determined that 300mg metformin combined with 500mg oleuropein should be sufficient to improve type 2 diabetes as per the observation from our *in silico* model.

Table 2: Quantification of the effect of oleuropein and metformin on surface GLUT4 concentration

Drug/Herb Dosage		%Improvement in GLUT4 concentration on the cell surface
Oleuropein	**Metformin**	
-	500mg	0.394
500mg	-	1.953
500mg	500mg	2.067
500mg	450mg	2.049
500mg	400mg	2.030
500mg	350mg	2.018
500mg	**300mg**	**2.000**
500mg	250mg	1.981
500mg	200mg	1.961
500mg	150mg	1.942
500mg	100mg	1.930

Figure 3 (Oleuropein and Metformin induced GLUT4 translocation) shows the separate as well as a combined effect of 500 mg oleuropein and different doses of metformin drug on surface GLUT4 concentration. Oleuropein and metformin-induced surface GLUT4 translocation was observed clearly in the presence of type 2 diabetes. Figure 3 (a) shows the result when 500mg metformin and 500mg oleuropein were taken separately. As a result, the enhancement in the surface GLUT4 concentration was 0.394% and 1.953% for metformin and oleuropein, respectively. Figure 3 (b) shows the GLUT4 translocation obtained when, along with 500mg oleuropein, a standard dose of metformin was also given in type 2 diabetes as a supplement. It was observed that the combined effect provided a 2.067% improvement in surface GLUT4 concentration. It has been discovered that when metformin 500mg was taken alone versus when combined with oleuropein for type 2 diabetes patients, the incorporation of oleuropein produced better results in terms of insulin resistance improvement.

Further, we kept the oleuropein dosage of 500mg constant and we reduced the dose of metformin by 10%. Thus, Figure-3 (c), Figure 3 (d), Figure 3 (e), Figure 3 (f), Figure 3 (g), Figure 3 (h), Figure 3 (i) and Figure 3 (j) show the results obtained when the oleuropein dose was 500mg and the metformin dose was 450mg, 400mg, 350mg, 300mg, 250mg, 200mg, 150mg and 100mg respectively. The percentage improvement in surface GLUT4 concentration was 2.049%, 2.03%, 2.018%, 2%, 1.981%, 1.961%, 1.942% and 1.93% respectively. It was observed that at increased metformin concentration, the results obtained were even better than those obtained when the patient was on a dose of 100mg metformin with 500mg oleuropein.

However, to avoid allopathy's side effects, we found that a lower dose of 300 mg metformin in addition to 500 mg oleuropein is sufficient. To minimize long-term consequences, we also propose increasing the dose of Olive Leaf Extract (oleuropein) rather than using excessive quantities of allopathic medications. As a result, it demonstrates the significance of the natural medicinal herb oleuropein in improving surface GLUT4 concentration. This combination yields considerable improvements in both the time required for GLUT4 translocation and the concentration of GLUT4 on the surface. As a result, the *in silico* model is effective in showing the influence of the natural herb oleuropein in the reduction of insulin resistance, as dependence on high pharmacological dosage results in late stage problems in type 2 diabetes patients.

As a result, the unique *in silico* model described here is a useful tool for quantifying oleuropein's effect on surface GLUT4 translocation. This has also resulted in a better knowledge of the signalling dynamics and underlying mechanisms. The model's ability to quantify oleuropein's effect and optimize metformin dose is a driving force behind experimental attempts to reduce insulin resistance, which contributes to metabolic illnesses including type 2 diabetes.

5. Conclusion

Oleuropein, a bioactive compound found in Olive Leaf Extract, has the potential to treat type 2 diabetes. It stimulates GLUT4 translocation to the plasma membrane via increasing the phosphorylation of AMPK. However, because metformin is the most commonly given standard treatment for type 2 diabetes, it was critical to determine the optimal and safe dose in order to achieve the best benefits while avoiding side effects. So, the natural herb oleuropein from Olive Leaf Extract was used to observe its effect on surface GLUT4 concentration in type 2 diabetes when taken with metformin. Also, to understand the underlying mechanisms involved in the effect of oleuropein and metformin on the surface GLUT4 concentration, an *in silico* modeling and simulation approach was chosen. The model was quantified using different doses of metformin, such as (100mg-500mg) in combination with 500mg of oleuropein. As a result, the objective of the study was achieved with a 2% improvement in GLUT4 concentration with 500mg oleuropein in combination with 300mg metformin as the optimum dose. Thus, we developed a novel *in silico* model that depicts the influence of the allopathic medicine metformin and the natural herb Olive Leaf Extract (oleuropein) on surface GLUT4 concentration in type 2 diabetes. Our *in silico* model could be used as a starting point for simulating the effects of other medications as well as various natural herbs. Finally, the connection between herbal and pharmaceutical treatments must be addressed by ongoing research into the potential hazards. Such a finding is critical for future clinical guidelines aimed at improving healthcare outcomes.

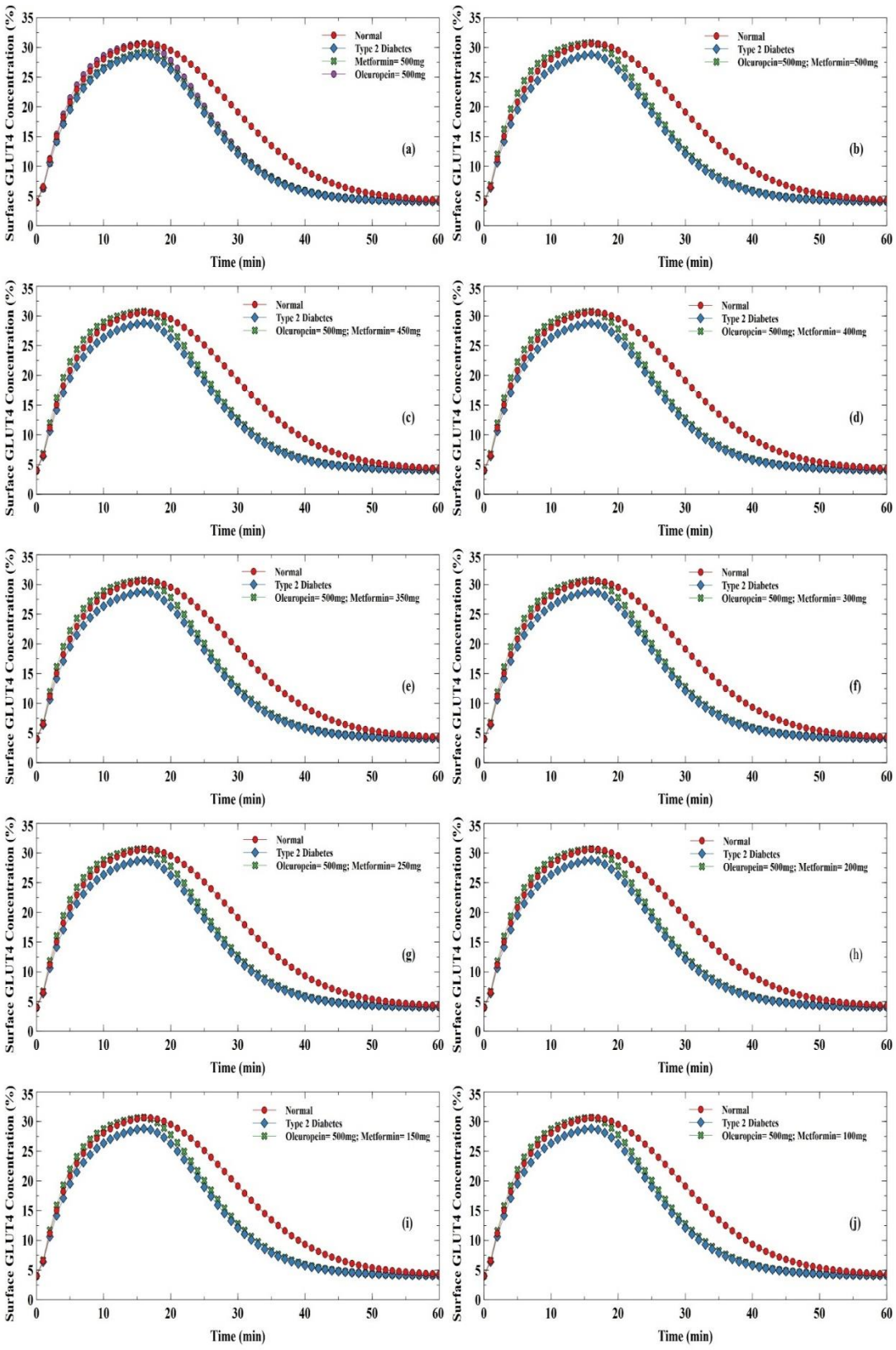

Figure 3: Oleuropein and Metformin induced GLUT4 translocation

6. Acknowledgment

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7. Statement of compliance

This article does not contain any studies involving animals performed by any of the authors.

8. Disclosure and conflict of interest

The authors declare that they have no conflicts of interest.

References

[1] 9th edn Internation Diabetes Federation. IDF Diabetes Atlas, *International Diabetes Federation*. 2019.

[2] I. K. Jeong, K. H. Yoon, and M. K. Lee, "Diabetes and COVID-19: Global and regional perspectives," *Diabetes Res. Clin. Pract.*, vol. 166, 2020.

[3] Z. J. Gazzaz, "Diabetes and COVID - 19," *Open Life Scicences*, pp. 297–302, 2021.

[4] A. K. Singh, R. Gupta, A. Ghosh, and A. Misra, "Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations," *Diabetes Metab. Syndr. Clin. Res. Rev.*, vol. 14, no. 4, 2020.

[5] A. Abdi, M. Jalilian, P. A. Sarbarzeh, and Z. Vlaisavljevic, "Diabetes and COVID-19: A systematic review on the current evidences," *Diabetes Research and Clinical Practice*, vol. 166. 2020.

[6] Y. Hwang *et al.*, "Diabetes and COVID-19: IDF perspective in the Western Pacific region," *Diabetes Res. Clin. Pract.*, vol. 166, 2020.

[7] H. I. Mcdonald, D. Nitsch, E. R. C. Millett, A. Sinclair, and S. L. Thomas, "New estimates of the burden of acute community-acquired infections among older people with diabetes mellitus: A retrospective cohort study using linked electronic health records," *Diabet. Med.*, vol. 31, no. 5, 2014.

[8] J. Pearson-Stuttard, S. Blundell, T. Harris, D. G. Cook, and J. Critchley, "Diabetes and infection: Assessing the association with glycaemic control in population-based studies," *The Lancet Diabetes and Endocrinology*, vol. 4, no. 2. 2016.

[9] J. C. N. Chan *et al.*, "The Lancet Commission on diabetes: using data to transform diabetes care and patient lives," *The Lancet*, vol. 396, no. 10267. 2020.

[10] R. Muniyappa and S. Gubbi, "COVID-19 pandemic, coronaviruses, and diabetes mellitus," *American Journal of Physiology - Endocrinology and Metabolism*, vol. 318, no. 5. pp. E736–E741, 2020.

[11] Y. J. Chee, S. K. Tan, and E. Yeoh, "Dissecting the interaction between COVID-19 and diabetes mellitus," *Journal of Diabetes Investigation*, vol. 11, no. 5. pp. 1104–1114, 2020.

[12] A. L. Wicaksana, N. S. Hertanti, A. Ferdiana, and R. B. Pramono, "Diabetes management and specific considerations for patients with diabetes during coronavirus diseases pandemic: A scoping review," *Diabetes Metab. Syndr. Clin. Res. Rev.*, vol. 14, no. 5, pp. 1109–1120, 2020.

[13] K. Blaslov, F. S. Naranđa, I. Kruljac, and I. P. Renar, "Treatment approach to type 2 diabetes: Past, present and future," *World J. Diabetes*, vol. 9, no. 12, pp. 209–219, 2018.

[14] D. E. Kelley *et al.*, "The effect of non-insulin-dependent diabetes mellitus and obesity on glucose transport and phosphorylation in skeletal muscle," *J. Clin. Invest.*, vol. 97, no. 12, pp. 2705–2713, 1996.

[15] R. A. DeFronzo, "Pathogenesis of type 2 diabetes mellitus," *Medical Clinics of North America*, vol. 88, no. 4. pp. 787–835, 2004.

[16] S. E. Kahn, M. E. Cooper, and S. Del Prato, "Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future," *The Lancet*, vol. 383, no. 9922. pp. 1068–1083, 2014.

[17] R. A. DeFronzo *et al.*, "Type 2 diabetes mellitus," *Nat. Rev. Dis. Prim.*, vol. 1, 2015.

[18] M. Stumvoll, B. J. Goldstein, and T. W. Van Haeften, "Type 2 diabetes: Principles of pathogenesis and therapy," in *Lancet*, 2005, vol. 365, no. 9467, pp. 1333–1346.

[19] A. B. Olokoba, O. A. Obateru, and L. B. Olokoba, "Type 2 diabetes mellitus: A review of current trends," *Oman Medical Journal*, vol. 27, no. 4. pp. 269–273, 2012.

[20] R. Taylor, "Insulin resistance and type 2 diabetes," *Diabetes*, vol. 61, no. 4. pp. 778–779, 2012.
[21] S. L. Samson and A. J. Garber, "Type 2 diabetes," in *Encyclopedia of Endocrine Diseases*, 2018, pp. 116–121.
[22] J. J. Marín-Peñalver, I. Martín-Timón, C. Sevillano-Collantes, and F. J. del Cañizo-Gómez, "Update on the treatment of type 2 diabetes mellitus," *World J. Diabetes*, vol. 7, no. 17, p. 354, 2016.
[23] H. A. Sindi, "Evidence that supports the antidiabetic, antihypertensive, and antihyperlipidemic effects of olive (Olea europaea L.) leaves extract and its active constituents (oleuropein) in human.," *J. Biochem. Technol.*, vol. 11, no. 2, pp. 41–45, 2020.
[24] J. M. Kristensen *et al.*, "Metformin does not compromise energy status in human skeletal muscle at rest or during acute exercise: A randomised, crossover trial," *Physiol. Rep.*, vol. 7, no. 23, 2019.
[25] V. Luna *et al.*, "Metformin improves atypical protein kinase C activation by insulin and phosphatidylinositol-3,4,5-(P04)3 in muscle of diabetic subjects," *Diabetologia*, vol. 49, no. 2, pp. 375–382, 2006.
[26] N. Musi *et al.*, "Metformin increases AMP-activated protein-kinase activity in skeletal muscle of subjects with type 2 diabetes," *Diabetes*, vol. 51, no. 7, pp. 2074–2081, 2002.
[27] K. S. Madsen, Y. Chi, M. I. Metzendorf, B. Richter, and B. Hemmingsen, "Metformin for prevention or delay of type 2 diabetes mellitus and its associated complications in persons at increased risk for the development of type 2 diabetes mellitus," *Cochrane Database of Systematic Reviews*, vol. 2019, no. 12. 2019.
[28] Y. W. Wang *et al.*, "Metformin: A review of its potential indications," *Drug Design, Development and Therapy*, vol. 11. pp. 2421–2429, 2017.
[29] A. Pandey, P. Tripathi, R. Pandey, R. Srivatava, and S. Goswami, "Alternative therapies useful in the management of diabetes: A systematic review," *J. Pharm. Bioallied Sci.*, vol. 3, no. 4, pp. 504–512, 2011.
[30] E. S. Muise *et al.*, "Pharmacological AMPK activation induces transcriptional responses congruent to exercise in skeletal and cardiac muscle, adipose tissues and liver," *PLoS One*, vol. 14, no. 2, pp. 1–29, 2019.
[31] H. Nasri and M. Rafieian-Kopaei, "Metformin: Current knowledge," *Journal of Research in Medical Sciences*, vol. 19, no. 7. pp. 658–664, 2014.
[32] W. Mao, S. Zhang, L. Wang, and S. Shen, "Efficacy and safety of metformin in the treatment of gestational diabetes: A protocol for systematic review and meta-analysis," *Medicine (Baltimore).*, vol. 100, no. 1, p. e23954, 2021.
[33] J. Wang *et al.*, "Association of Metformin with Susceptibility to COVID-19 in People with Type 2 Diabetes," *J. Clin. Endocrinol. Metab.*, vol. 106, no. 5, pp. 1255–1268, 2021.
[34] H. Ouyang, A. Al-Mureish, and N. Wu, "Research progress of metformin in gestational diabetes mellitus: A narrative review," *Annals of Palliative Medicine*, vol. 10, no. 3. pp. 3423–3437, 2021.
[35] A. Sampath Kumar *et al.*, "Exercise and insulin resistance in type 2 diabetes mellitus: A systematic review and meta-analysis," *Ann. Phys. Rehabil. Med.*, vol. 62, no. 2, pp. 98–103, 2019.
[36] S. R. Bird and J. A. Hawley, "Update on the effects of physical activity on insulin sensitivity in humans," *BMJ Open Sport Exerc. Med.*, vol. 2, no. 1, pp. 1–26, Mar. 2017.
[37] M. Matheka, "Complementary and alternative medicine for type 2 diabetes mellitus: Role of medicinal herbs," *J. Diabetes Endocrinol.*, vol. 3, no. 4, pp. 44–56, 2012.
[38] D. Dastan *et al.*, "Protective effects of the traditional herbal formulation on oxidative stress, learning and memory in the animal model of type 2 diabetes," *Physiol. Pharmacol.*, vol. 24, no. 3, pp. 174–184, 2020.
[39] G. A. Soliman, A. S. Saeedan, R. F. Abdel-Rahman, H. A. Ogaly, R. M. Abd-Elsalam, and M. S. Abdel-Kader, "Olive leaves extract attenuates type II diabetes mellitus-induced testicular damage in rats: Molecular and biochemical study," *Saudi Pharm. J.*, vol. 27, no. 3, 2019.
[40] Á. Guinda, J. M. Castellano, J. M. Santos-Lozano, T. Delgado-Hervás, P. Gutiérrez-Adánez, and M. Rada, "Determination of major bioactive compounds from olive leaf," *LWT - Food Sci. Technol.*, vol. 64, no. 1, 2015.
[41] H. Abunab, W. L. Dator, and S. Hawamdeh, "Effect of olive leaf extract on glucose levels in diabetes-induced rats: A systematic review and meta-analysis," *J. Diabetes*, vol. 9, no. 10, 2017.
[42] M. S. Abdel-Kader, G. A. Soliman, R. F. Abdel-Rahman, A. S. Saeedan, R. M. Abd-Elsalam, and H. A. Ogaly, "Effect of olive leaves extract on the antidiabetic effect of glyburide for possible herb-

drug interaction," *Saudi Pharm. J.*, vol. 27, no. 8, pp. 1182–1195, 2019.
[43] G. Annunziata, "Oleuropein as a novel anti-diabetic nutraceutical. An overview," *Arch. Diabetes Obes.*, vol. 1, no. 3, 2018.
[44] H. A. H. Khattab, S. S.Moselhy, and A. A. O. Aljafri, "Olive leaves extract alleviate diabetic nephropathy in diabetic male rats: Impact on oxidative stress and protein glycation," *Int. J. Pharm. Res. Sci.*, vol. 9, no. 1, pp. 130–141, 2020.
[45] J. Wainstein *et al.*, "Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats," *J. Med. Food*, vol. 15, no. 7, pp. 605–610, 2012.
[46] F. Hadrich *et al.*, "Oleuropein activated AMPK and induced insulin sensitivity in C2C12 muscle cells," *Life Sci.*, vol. 151, 2016.
[47] C. Jiménez-Sánchez *et al.*, "AMPK modulatory activity of olive-tree leaves phenolic compounds: Bioassay-guided isolation on adipocyte model and in silico approach," *PLoS One*, vol. 12, no. 3, 2017.
[48] C. G. Guex, F. Z. Reginato, P. R. de Jesus, J. C. Brondani, G. H. H. Lopes, and L. de F. Bauermann, "Antidiabetic effects of Olea europaea L. leaves in diabetic rats induced by high-fat diet and low-dose streptozotocin," *J. Ethnopharmacol.*, vol. 235, 2019.
[49] F. Vlavcheski, M. Young, and E. Tsiani, "Antidiabetic effects of hydroxytyrosol: In vitro and in vivo evidence," *Antioxidants*, vol. 8, no. 6. 2019.
[50] M. A. Temiz and A. Temur, "The effect of olive leaf extract on digestive enzyme inhibition and insulin production in streptozotocin-induced diabetic rats," *Ankara Univ. Vet. Fak. Derg.*, vol. 66, no. 2, 2019.
[51] G. Liguri and M. Stefani, "Recent advances in basic and clinical research on the prevention and treatment of the metabolic syndrome and related disorders by the use of olive polyphenols," *Journal of Gerontology and Geriatrics*, vol. 65, no. 1. 2017.
[52] M. Gürbüz and S. Öğüt, "Antidiabetic effect of olive leaf extract on streptozotocin-induced diabetes mellitus in experimental animals," *Nutr. Hosp.*, vol. 37, no. 5, 2020.
[53] M. Benlarbi *et al.*, "Neuroprotective effects of oleuropein on retina photoreceptors cells primary culture and olive leaf extract and oleuropein inhibitory effects on aldose reductase in a diabetic model: Meriones shawi," *Arch. Physiol. Biochem.*, 2020.
[54] N. Acar-Tek and D. Ağagündüz, "Olive Leaf (Olea europaea L. folium): Potential Effects on Glycemia and Lipidemia," *Annals of Nutrition and Metabolism*, vol. 76, no. 1. 2020.
[55] L. Al-Hayaly, A. Al-Sultan, and S. M. S. Sultan, "Effect of Olive Leaves Extract on Alloxan Induced Diabetes in Male Albino Mice," 2020.
[56] A. G. Ağgül, F. Gür, and M. Gülaboğlu, "Streptozotocin-Induced Oxidative Stress in Rats: The Protective Role of Olive Leaf Extract," *Bull. Korean Chem. Soc.*, vol. 42, no. 2, 2021.
[57] J. Giacometti, D. Muhvić, T. Grubić-Kezele, M. Nikolić, T. Šoić-Vranić, and S. Bajek, "Olive leaf polyphenols (Olps) stimulate glut4 expression and translocation in the skeletal muscle of diabetic rats," *Int. J. Mol. Sci.*, vol. 21, no. 23, 2020.
[58] A. Kocyigit *et al.*, "An Optimum Dose of Olive Leaf Extract Improves Insulin Receptor Substrate-1, Tyrosine Kinase, and Glucose Transporters, While High Doses Have Genotoxic and Apoptotic Effects," *Am. J. Plant Sci.*, vol. 10, no. 11, pp. 1933–1948, 2019.
[59] H. Alkhateeb, M. Al-Duais, and E. Qnais, "Beneficial effects of oleuropein on glucose uptake and on parameters relevant to the normal homeostatic mechanisms of glucose regulation in rat skeletal muscle," *Phyther. Res.*, vol. 32, no. 4, 2018.
[60] M. de Bock *et al.*, "Olive (Olea europaea L.) Leaf Polyphenols Improve Insulin Sensitivity in Middle-Aged Overweight Men: A Randomized, Placebo-Controlled, Crossover Trial," *PLoS One*, vol. 8, no. 3, 2013.
[61] R. C. Gupta, D. Chang, S. Nammi, A. Bensoussan, K. Bilinski, and B. D. Roufogalis, "Interactions between antidiabetic drugs and herbs: An overview of mechanisms of action and clinical implications," *Diabetology and Metabolic Syndrome*, vol. 9, no. 1. 2017.
[62] C. G. Guex *et al.*, "Safety assessment of ethanolic extract of Olea europaea L. leaves after acute and subacute administration to Wistar rats," *Regul. Toxicol. Pharmacol.*, vol. 95, pp. 395–399, 2018.
[63] A. E. Clewell *et al.*, "A Comprehensive Toxicological Safety Assessment of an Extract of Olea Europaea L. Leaves (Bonolive™)," *Int. J. Toxicol.*, vol. 35, no. 2, 2016.
[64] Funahashi, A.; Matsuoka, Y.; Jouraku, A.; Morohashi, M.; Kikuchi, N.; Kitano, H, "CellDesigner

4.3 Startup guide," 2013.

[65] D. M. Joshi, J. Patel, and H. Bhatt, "In silico study to quantify the effect of exercise on surface GLUT4 translocation in diabetes management," *Netw. Model. Anal. Heal. Informatics Bioinforma.*, vol. 10, no. 1, 2021.

[66] A. R. Sedaghat, A. Sherman, and M. J. Quon, "A mathematical model of metabolic insulin signaling pathways," *Am. J. Physiol. Metab.*, vol. 283, no. 5, pp. E1084–E1101, 2002.

[67] D. D. J. Habets *et al.*, "Involvement of atypical protein kinase C in the regulation of cardiac glucose and long-chain fatty acid uptake," *Front. Physiol.*, vol. 3 SEP, no. September, pp. 1–9, 2012.

[68] B. A. Funahashi, Y. Matsuoka, A. Jouraku, M. Morohashi, N. Kikuchi, and H. Kitano, "A Versatile Modeling Tool for Biochemical Networks," *Ieee*, vol. 96, no. 8, pp. 1254–1265, 2008.

[69] I. Coccimiglio and D. C. Clarke, "ADP is the dominant controller of AMP-activated protein kinase activity in skeletal muscle during exercise," p. In revision., 2019.

[70] P. Vicini and M. J. Kushmerick, "Cellular energetics analysis by a mathematical model of energy balance: Estimation of parameters in human skeletal muscle," *Am. J. Physiol. - Cell Physiol.*, vol. 279, no. 1 48-1, pp. 213–224, 2000.

[71] M. J. Lambeth and M. J. Kushmerick, "A computational model for glycogenolysis in skeletal muscle," *Ann. Biomed. Eng.*, vol. 30, no. 6, pp. 808–827, 2002.

[72] A. Sacan, S. Ekins, and S. Kortagere, "Applications and limitations of in silico models in drug discovery," *Methods in Molecular Biology*, vol. 910. pp. 87–124, 2012.