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Study of Antidiabetic Activities of Carvacrol and Thymol with Structure-Based Method

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Abstract.

Background: The role of natural ingredients such as carvacrol and thymol in walnut leaves (Juglans regia L) could be a breakthrough for treating hyperglycemia and obesity.

Objective: To determine the potential carvacrol and thymol isolates of walnut oil (Juglans regia) in silico, which act on enzyme 11 dehydrogenase 1, fructose receptor 1,6 bisphosphatase, and PPRγ in the treatment of type DM 2.

Methods: Investigate carvacrol, thymol, and their amino acid interactions in silico using Biovia and Autodocks Tool software. Molecular docking between the ligand and the receptor was performed using Autodock 4 version 1.56 using a generic evolutionary algorithm (rigid docking method).

Future research: Computational modeling is the initial research that can be carried out before conducting in vivo and in vitro, further studies to investigate the potential of carvacrol and thymol in overcoming insulin resistance.

Results: The results showed the potential energy produced by the four compounds for each protein in kcal/mol (11βHSD1, F1.6BP1, PPRγ), which are oleuropein (-7.94, -6.83, -8.99), hydroxytyrosol (-3.80, - 5.52, -5.15).

***Conclusion*:** The study results found that these four isolates could be potential anti-diabetic agents.

Keywords: *type 2 diabetes mellitus; obesity; oleuropein; hydroxytyrosol.*

1 Introduction

Type 2 diabetes mellitus occurs due to several factors that trigger insulin resistance. These factors can include a lifestyle that increases the pattern of excess fat consumption marked by a BM> 30 or obesity (Kleinert et al., 2018). Insulin

resistance is triggered by inflammation due to chronic fat storage in adipose tissue [1], high carbohydrate and fibre intake, and low vegetable fat [2]

Adipose tissue is the site of fat synthesis and blood glucose regulation. The action of insulin on fat cells stimulates glucose uptake and modulates lipid metabolism by increasing the accumulation and decreasing the breakdown of triglycerides, especially in white adipose tissue [3]. Studies show that rats have increased fat accumulation in adipose tissue, especially in epidermal fat depots, due to increased lipogenesis and fatty acid absorption [4]. In cells regulated by the peroxisome proliferator active receptor-gamma transcription factor (PPARγ) [5]. Research states that decreasing PPRγ will cause insulin resistance and lipodystrophy [6]. In high-fat WAT, the production of antistress hormones increases, one of which is glucocorticoid hormones in the form of cortisol in humans [7] and corticosterone in rodents [8]. Reducing dietary fat and lifestyle modification with metformin can restore cortisol abnormalities and increase insulin sensitivity [9]. Research shows that stopping enzyme 11 beta dehydrogenase type 1 from acting as a catalyst in the production of glucocorticoid hormones will cause an increase in calorie intake, a positive energy balance, and an increase in circulating fatty acids (FA), which makes insulin regulation less effective [10].

Disruption of insulin circulation will cause impaired glucose transport by GLUT4 into cells. Finally, the body sends a signal to the liver to open up non-glucose energy reserves to start the process of gluconeogenesis. During gluconeogenesis, PEP carboxykinase in the cytoplasm converts oxaloacetate to phosphoenolpyruvate (PEP), mostly in the cytoplasm. In this process, the enzyme fructose 1,6-bisphosphate is involved in the gluconeogenic process by turning non-glucose components into glucose to meet the needs of body cells. It makes fructose 6-phosphate, which is then turned into glucose-6-phosphate (G6P) by phosphoglucoisomerase. Finally, glucose-6-phosphatase (G6Pase) dephosphorylates G6P to turn it into glucose [11]. In the design of metformin as a fructose 1,6 bisphosphate inhibitor, it can increase the binding affinity with residues on its amino acids so that it has the potential to inhibit gluconeogenesis [12]. Inhibition of the enzyme fructose 1,6 bisphosphate can cause a decrease in blood glucose [13]. Gluconeogenesis is the reversal of the process of glycolysis. In the process, GLUT2 has a role in transporting glucose into the blood through the small intestine to maintain glucose homeostasis and induce insulin secretion through glucose sensing in pancreatic cells. By controlling the resolution of the disaccharide maltose, which delays the digestion and absorption of carbohydrates, the alpha-glucosidase enzyme in the small intestine can effectively lower glucose levels. Insulin resistance can be improved by administering sodium-glucose transport two inhibitors, which improve insulin

target tissue, reduce visceral adipose fat mass, and increase glucose uptake in skeletal muscle [14].

The role of natural ingredients such as carvacrol and thymol in walnut leaves (Juglans regia L) could be a breakthrough for treating hyperglycemia and obesity. Carvacrol and thymol are the result of the hydrolysis of essential walnut oil (33.21% & 16%) [15] and can reduce blood sugar levels (Li et al., 2020). Thus, computational modelling becomes an alternative to see the further potential of carvacrol and thymol compounds against several related proteins in overcoming insulin resistance.

2 Materials and Methods

2.1 Materials

2.1.1 Protein Preparation

The protein used is essential in type 2 diabetes with obesity, especially in adipose cells and glucose metabolism. The three-dimensional X-ray crystallographic structure was taken from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (http://www.pdb.org) and saved in PDB format. Protein with PDB ID: 11βHSD1 (1XU9), F1.6BP1 (5Q09), PPRγ (5Y2O), and Alpha glycosidase (5NN5). All proteins are separated from the native ligand, and the water contained in it is removed, and then one potential chain is selected according to the similarity of the original ligand and the experimental ligand using Autodock tools and Biovia.

2.1.2 Ligand Preparation

Experimental and comparison ligands were retrieved from NCBI from the PubChem pooled database (https://pubchem.ncbi.nlm.nih.gov/) with PubChem CID: carvacrol (10364), thymol (6989), metformin (4091), canagliflozin (24812758), miglitol (441314), and acarbose with the three-dimensional structure were simulated and optimized with Avogadro application and saved in PDB format.

2.2 Method

Molecular docking between the ligand and the receptor was performed using Autodock 4 version 1.56 using a generic evolutionary algorithm (rigid docking method). Simulated docking of each ligand to the four target proteins was carried out. The probe includes bond energies, bond affinities, hydrogen bonds (H-bonds) and is visualized using Biovia and the interactions are ranked by energy. Ligand scores were compared with the original ligands.

3 Results and Discussion

3.1 Result

3.1.1 Validity Test Results

One of the most widely used methods for validating docking protocols is re-docking co-crystallized ligands to target proteins. In this study, co-crystallized ligands were extracted from four receptor proteins and reattached to the active site of each receptor. After docking, the best pose of the ligand was aligned with the cocrystal ligand, and the ligand's root means square deviation (RMSD) was calculated. This simulation helps determine the reliability and reproducibility of the docking protocol. All of the conformations of tethered ligands are within RMSD 2.0 when superimposed with their respective co-crystallized ligands. This means that the docking protocol was done correctly.

Docking of native ligands is carried out to find the 3D conformation of the native ligand to the receptor by taking into account the coordinates of the centre of mass of the structure and the grid box size of the binding site pocket in angstroms, or the number of points. Previous research showed that the target protein macromolecules 11BHSD1, F-1,6-BPase, PPRGamma, and alpha-glucosidase came from chain A, just like the natural and experimental ligands. The ligand and protein were successfully prepared, and protein was obtained without ligands and their (native) ligands. Using the Biovia program, the incomplete side chain of the target protein was used to make protein ligands that fit the 3D shape of the molecule. Biovia can separate protein chains, removing water that interferes with the simulation. Each docking algorithm carried out the native ligand validation of the target protein.

The validation was done to determine the conformational similarity between the native crystallographic and the native ligands resulting from docking optimization. The docking process needs to determine the coordinates of the centre where the ligand and protein interact, usually the centre of mass of the native ligand. The validation results show sufficient validity using Autodock tools and native ligands and the conformation of the docked ligands using Biovia. In simulation 11 BHSD1 (GDP IXU9) with CPS5 ligand showed conformational conformation at run 20 (figure 1), F1,6BPase (GDP 5Q09) with ligand 96A401 at run 7, PPRγ (PDB 5Y2O) with ligand 8N6501 at run 3 and Glucosidase (GDP 5NN5) NOJ1016 at run 2.

3.1.2 Bond energy result

The conformational validation results can be ligand-tethered for testing. The assay ligands carvacrol and thymol conformed to all three proteins (11BHSD1, F-1,6-BPase, PPRGamma) compared to metformin's ligand, canagliflozin. The ligand conformation test with alpha-glucosidase receptors used Acarbose and miglitol ligands as comparators. The simulation results were obtained by running 100 of each ligand with the four receptors in the order of the lowest binding energy level (**table 1**), which shows the strength of the affinity of the ligands for the receptors.

Table 1 Lowest Gibbs free energy (ΔG)

Protein	ΔG (Kcal/mol) Experimental Ligand					
	Carvacrol	**Thymol**	**Metformin**	**Canagliflozin**	**Acarbose**	**Miglitol**
11BHSD1 (1XU9)	-4,61 [d]	-4,92[c]	-5,05 [b]	-9,64[a]	-	
F-1,6-BPase (5Q09)	-5,15 [c]	-5,35 [b]	-2,86 [d]	-5,86 [a]	-	
PPARγ (5Y20)	-5,28 [c]	-5,99 [b]	-3,10 [d]	-7,67 [a]	-	
α Glucosidase (5NN5)	-5,27 [b]	-5,42 [a]	-	-	+2,66 [c]	

Annotation: [a] first lowest energy, [b] second lowest energy, [c] third lowest energy, [d] fourth lowest energy

The result of docking between the ligand and the receptor, when it produces low energy, will have a high binding affinity (**Table 2**). This simulation illustrates the drug's ability to bind to the receptor better. The smaller the binding affinity value, the higher the affinity between the receptor and the ligand, and vice versa if the more significant the binding affinity value, the lower the affinity between the receptor.

Table 2 Hydrogen bonding interactions of ligand complexes and 11BHSD1, F1,6BPase, PPRγ, α-glucosidase

Ligand Target	ΔG (Kcal/mol)	Inhibition Constant (Ki) (nM)	Hydrogen binding residues
11BHSD1			
Carvacrol	-4,61	418,95	ALA172
Thymol	-4,92	249,26	SER169
Canagliflozin	-9,64	85,81	TYR177,THR264, ASP259, LEU217
Metformin	-5,05	197,47	LEU217, GLN234, SER 260
F-1,6-BPase			
Carvacrol	-5,15	167,67	TYR113
Thymol	-5,35	119,44	THR31
Canagliflozin	-7,67	2,40	GLY26, TYR113, THR27, THR27, ALA24
Metformin	-2,86	8,07	GLU20
PPARγ			
Carvacrol	-5,28	135,15	HIS449
Thymol	-5,99	40,79	SER289
Canagliflozin	-5,86	50,25	TYR327, TYR327, SER289, ARG288, LYS230
Metformin	-3,10	5,32	PHE282, PHE282, TYR473
α Glucosidase			
Carvacrol	-5,27	137,44	-
Thymol	-5,42	106,02	ASP404
Acarbose	+2,66	-	ASP616, ASP616, ASP616, ARG600, ASP282
Miglitol	-7,81	1.89 uM	ARG600, ASP616, ASP518, HIS674, HIS 674, ASP404, ASP404,

Walnuts (Juglans regia) contain the main components of Carvacrol and thymol, and from several research reports, they can reduce hyperglycemia. *Carvacrol* is a phenol that is a natural monoterpene derivative of cymene and has a molecular weight of 150.22 Da. In comparison, thymol has a molecular weight of 150 Da and has met the requirements set out by the Lipinski rule. The comparisons used in this study were canagliflozin and metformin as the main antidiabetic of choice for obesity. Canagliflozin, a reversible inhibitor of sodium-glucose co-transporter 2, a second-choice antidiabetic drug in obesity, shows commensurate potential as an antidiabetic candidate for obesity.

A molecular docking study was conducted to understand the molecular interactions between Carvacrol, thymol, and the enzyme 11β-HSD1 as receptors (**Figure1**). Carvacrol interacted with 11 -HSD1 in its pocket with the lowest binding energy score of -4.61 Kcal/mol and an inhibition constant of 418.95 nM. Carvacrol (A) undergoes molecular interactions in the CO group with amino acid residues in the pocket-forming hydrogen bonds with ALA172. At the same time, the aromatic ring makes hydrophobic contact by forming phi alkyl bonds with residues ILE218, ALA223, and LEU 215. In comparison, thymol (B) poses binding on bag 11*β*-HSD1 with a -4.92 Kcal/mol bond energy lower than Carvacrol. The molecular interaction of thymol on the OH group forms a hydrogen bond with the amino acid residue SER218. In thymol, several hydrophobic contacts were formed between the amino acid residue ILE218 with the aromatic ring, and the C group formed a phi alkyl bond interaction and the C group and residues LEU178 and ALA223. In Carvacrol and thymol, hydrophobic contact is more so that it requires high energy to interact. Molecular interactions in the comparison ligands metformin and canagliflozin resulted in bond energies of -5.05 Kcal/mol and -9.64 Kcal/mol. Canagliflozin in the first position has the lowest energy required, followed by metformin in the second position so that it has a higher affinity than the test ligand.

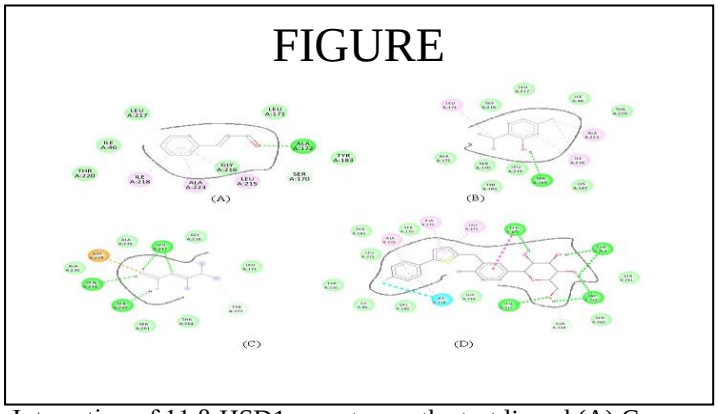

Figure 1 Interaction of 11 β-HSD1 receptor on the test ligand (A) Carvacrol (B) Thymol (C) Metformin, and (D) Canagliflozin.

There are seven hydrogen bonds in Canagliflifozin (D) which bind to residues TYR177, THR264, ASP259, LEU217 and one hydrophobic contact LEU217 (**Figure 2**). The residue is an active site as antidiabetic obesity on 11 β-HSD1 inhibition. The ligand metformin (C) interaction comparison with four hydrogen bonds on the amino acid residue is LEU217 with two hydrogen bonds and GLN234, SER260 in each hydrogen bond.

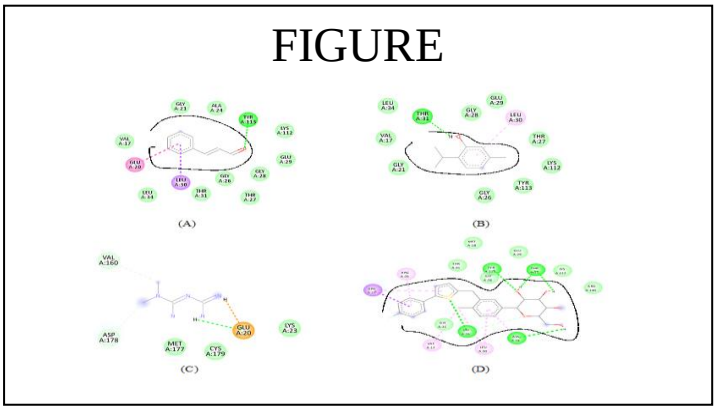

Figure 2 F1,6BPase receptor interaction with test ligand (A) Carvacrol, (B) Thymol, (C) Metformin, and (D) Canagliflozin

The interaction between the F1,6BPase receptor and all test ligands resulted in low energy, namely carvacrol -5.15, thymol -5.35, metformin -2.86, and canaglifazone -7.67 (**Figure 3**). The binding affinity of carvacrol and thymol is higher than that of metformin, even though metformin acts on the liver. Canagliflozin itself has the lowest and best bond energy compared to the other three ligands with hydrogen bonds, namely five bonds, and the active site is at residues GLY26, TYR113, THR27, THR27, ALA24. The hydrogen bonds of carvacrol on the TYR113 residue with the CO group and the aromatic ring form two pi-bond interactions. Thymol forms one hydrogen bond in the OH group with the THR31 residue, and the aromatic ring forms a one-phi bond. Metformin, with the highest bond energy compared to the three test ligands, had hydrogen bonds and pi bonds at the GLU20 residue.

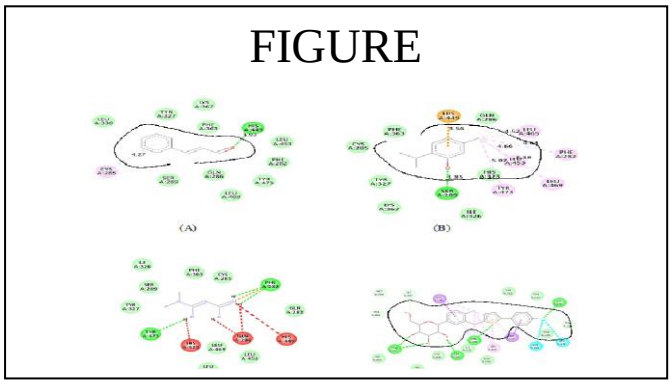

Figure 3 Interaction of PPRGamma receptors on test ligands (A) Carvacrol (B)Thymol (C) Metformin, and (D) Canagliflozin

The interaction of PPRgamma with carvacrol, thymol and canagliflozin ligands has low binding energy and high affinity compared to metformin. Respectively (-5.28, -5.99, -5.86 and -3.10) with one hydrogen bond to carvacrol at the HIS449 residue with a CO group and a phi bond on the aromatic ring. In comparison, Thymol itself has one hydrogen bond (SER289) and six phi bonds. Canagliflozin itself has hydrogen bond interactions of 5 ARG 288 bonds with the S group, two hydrogen bonds in TYR327 with the CO group and SER289 with the OH group. In canagliflozin, there are seven pi bonds and two halogen bonds. Metformin itself has three hydrogen bonds, namely TYR473 and PHE282.

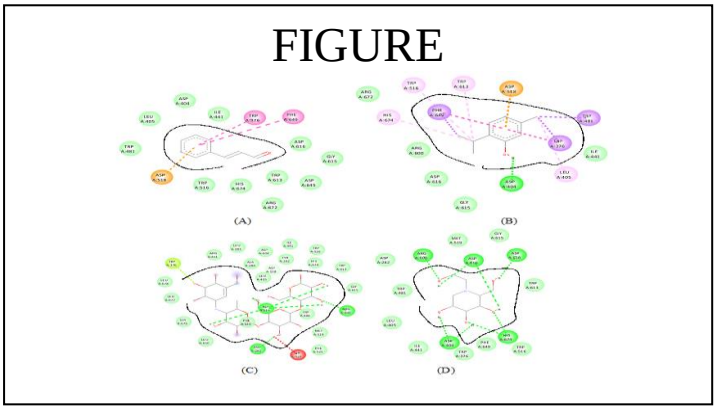

Figure 4 Interaction of α -glucosidase receptors on test ligands (A) Carvacrol (B) Thymol (C) Acarbose (D) Miglitol

Modeling alpha-glucose inhibition with acarbose as the primary choice, contrary to Lipinski's rule where the molecular weight must be approximately 500 daltons. Acarbose cannot enter the alpha-glucosidase enzyme's cavity, so the bond energy required to bind is very high. In contrast, in vitro experiments and clinical practice applications have proven that acarbose can inhibit and lower glucose levels.

The second option as an alpha-glucosidase inhibitor is miglitol, which has a molecular weight following Lipinski's rule with a very low energy level showing a high energy affinity among all ligands, namely -7.81 with seven hydrogen bonds in the OH group with the active site at the ARG600 residue, ASP616, ASP518, and two residues HIS674 and ASP404. Acarbose and miglitol have the same active site on ARG600. Carvacrol and thymol have bond energies of -5.27 and -5.42, where the dominance of pi bonds in both ligands is quite large. Even carvacrol itself does not have hydrogen bonds. There is one hydrogen bond in thymol in the OH group with the active site ASP404 as in Canagliflozin.

3.2 Discussion

The study used a molecular docking study to understand the molecular interactions between carvacrol, thymol, and the enzyme 11β-HSD1 as receptors. Carvacrol, a phenol derivative of cymene, has a molecular weight of 150.22 Da, while thymol has a molecular weight of 150 Da and meets the Lipinski rule.

The comparisons used were canagliflozin and metformin as the main antidiabetic of choice for obesity. Canagliflozin, a reversible inhibitor of sodium-glucose co-transporter 2, shows commensurate potential as an antidiabetic candidate for obesity. The binding affinity of carvacrol and thymol is higher than that of metformin, even though metformin acts on the liver.

The interaction of PPRgamma with carvacrol, thymol, and canagliflozin ligands has low binding energy and high affinity compared to metformin. Carvacrol has the lowest and best bond energy compared to other three ligands with hydrogen bonds, while thymol has one hydrogen bond and six phi bonds.

Modeling alpha-glucose inhibition with acarbose as the primary choice, contrary to Lipinski's rule, shows that acarbose cannot enter the alpha-glucosidase enzyme's cavity, so the bond energy required to bind is very high. Miglitol, with a low energy level and high energy affinity among all ligands, has a very low energy level and high energy affinity among all ligands.

4 Conclusion

Overall, the docking results show that the electron-withdrawing moiety is more favorable for interaction with the active site residue so that the potential for carvacrol and thymol can be candidates for receptor-inhibiting antidiabetic drugs that cause insulin resistance. In the docking model, carvacrol and thymol can be compared to have better binding energy than metformin as the main antidiabetic of choice but not superior to Canagliflozin as an inhibitor of sodium-glucose transport 2.

In vivo and in vitro studies need to be carried out to study the potential of carvacrol and thymol further.

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References

[1] H. M. Lawler, C. M. Underkofler, P. A. Kern, C. Erickson, B. Bredbeck, and N. Rasouli, "Adipose Tissue Hypoxia, Inflammation, and Fibrosis in Obese Insulin-Sensitive and Obese Insulin-Resistant Subjects," *J Clin Endocrinol Metab*, vol. 101, no. 4, pp. 1422–1428, Apr. 2016, doi: 10.1210/jc.2015-4125.

[2] H. Kahleova, S. Dort, R. Holubkov, and N. Barnard, "A Plant-Based High-Carbohydrate, Low-Fat Diet in Overweight Individuals in a 16-Week Randomized Clinical Trial: The Role of Carbohydrates," *Nutrients*, vol. 10, no. 9, p. 1302, Sep. 2018, doi: 10.3390/nu10091302.

[3] P. Morigny, J. Boucher, P. Arner, and D. Langin, "Lipid and glucose metabolism in white adipocytes: pathways, dysfunction and therapeutics," *Nat Rev Endocrinol*, vol. 17, no. 5, pp. 276–295, May 2021, doi: 10.1038/s41574-021-00471-8.

[4] J. Miranda, I. Eseberri, A. Lasa, and M. P. Portillo, "Lipid metabolism in adipose tissue and liver from diet-induced obese rats: a comparison between Wistar and Sprague-Dawley strains," *J Physiol Biochem*, vol. 74, no. 4, pp. 655–666, Nov. 2018, doi: 10.1007/s13105-018-0654-9.

[5] J.-E. Lee, H. Schmidt, B. Lai, and K. Ge, "Transcriptional and Epigenomic Regulation of Adipogenesis," *Mol Cell Biol*, vol. 39, no. 11, Jun. 2019, doi: 10.1128/MCB.00601-18.

[6] J. Joseph, R. D. Shamburek, E. K. Cochran, P. Gorden, and R. J. Brown, "Lipid Regulation in Lipodystrophy Versus the Obesity-Associated Metabolic Syndrome: The Dissociation of HDL-C and Triglycerides," *J Clin Endocrinol Metab*, vol. 99, no. 9, pp. E1676–E1680, Sep. 2014, doi: 10.1210/jc.2014-1878.

[7] R. H. Oakley and J. A. Cidlowski, "The biology of the glucocorticoid receptor: New signaling mechanisms in health and disease," *Journal of Allergy and Clinical Immunology*, vol. 132, no. 5, pp. 1033–1044, Nov. 2013, doi: 10.1016/j.jaci.2013.09.007.

[8] M. Bekhbat, E. R. Glasper, S. A. Rowson, S. D. Kelly, and G. N. Neigh, "Measuring corticosterone concentrations over a physiological dynamic range in female rats," *Physiol Behav*, vol. 194, pp. 73–76, Oct. 2018, doi: 10.1016/j.physbeh.2018.04.033.

[9] A. Stomby, J. Otten, M. Ryberg, R. Andrew, B. R. Walker, and T. Olsson, "Diet-induced weight loss alters hepatic glucocorticoid metabolism in type 2 diabetes mellitus," *Eur J Endocrinol*, vol. 182, no. 4, pp. 447–457, Apr. 2020, doi: 10.1530/EJE-19-0901.

[10] K. Peng *et al.*, "11β-Hydroxysteroid Dehydrogenase Type 1(11β-HSD1) mediates insulin resistance through JNK activation in adipocytes," *Sci Rep*, vol. 6, no. 1, p. 37160, Dec. 2016, doi: 10.1038/srep37160.

[11] X. Zhang, S. Yang, J. Chen, and Z. Su, "Unraveling the Regulation of Hepatic Gluconeogenesis," *Front Endocrinol (Lausanne)*, vol. 9, Jan. 2019, doi: 10.3389/fendo.2018.00802.

[12] D. J. Timson, "Fructose 1,6- bis phosphatase: getting the message across," *Biosci Rep*, vol. 39, no. 3, Mar. 2019, doi: 10.1042/BSR20190124.

[13] A. Pinto *et al.*, "International practices in the dietary management of fructose 1-6 biphosphatase deficiency," *Orphanet J Rare Dis*, vol. 13, no. 1, p. 21, Dec. 2018, doi: 10.1186/s13023-018-0760-3.

[14] N. Waseda, H. Satoh, C. Yoshida, F. Ikeda, A. Kanazawa, and H. Watada, "Effects of SGLT2 Inhibitors on Insulin Secretion and Insulin Resistance—Results from a Cross-Sectional Study," *Diabetes*, vol. 67, no. Supplement 1, pp. 1187-P, Jul. 2018, doi: 10.2337/db18-1187-P.

[15] H. Moravej *et al.*, "Chemical Composition and the Effect of Walnut Hydrosol on Glycemic Control of Patients with Type 1 Diabetes," *Int J Endocrinol Metab*, vol. 14, no. 1, Jan. 2016, doi: 10.5812/ijem.34726.