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ORIGINAL PAPER

JMS Journal of Medical Science

Molecular docking and admet properties of *Anacardium occidentale* methanolic nut extract against inflammatory, oxidative and apoptotic markers of diabetes

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ABSTRACT

Introduction. The contemporary antidiabetic drugs have side effects and adverse reactions. This demand to search for less toxic and effective treatments for diabetes from medicinal plants using computational methods. The present research investigated the molecular docking of *Anacardium occidentale* nut methanolic extract compounds with selected proteins related to diabetes and the compounds' AMDET properties.

Material and methods. The compounds were identified using Gas chromatography-mass spectrometry analysis. The compounds'2-dimensional structure was retrieved from the PubChem compound database. Three-dimensional crystallographic structure of selected proteins; B-cell-lymphoma-2 (Bcl-2), caspase-3, glucocorticoids, interleukin-1β, myeloperoxidase and tumor necrosis factor-alpha (TNF-α) was downloaded from Protein Data Bank. Molecular docking was performed using Autodoc kvina and the active site of binding interactions was detected with the Computed Atlas of Surface Topography of proteins (CAST-P). The compounds' drug-likeness, physicochemical and ADMET were evaluated using molininspiration and admetSAR online tools.

Results. Ten compounds were identified from the *Anacardium occidentale* nut methanolic extract. All the compounds exhibited drug-likeness properties with violation of one Lipinski's rule. Two compounds, oleic acid and 3-(p-methoxyphenyl)-propionic acid exhibited the best binding energy with the active receptors site of Bcl-2, caspase-3, TNF-α and glucocorticoid. Also, tridecanoic acid exhibited good binding energy with the active site of glucocorticoid receptors. Only 3-(p-methoxyphenyl)-propionic acid exhibited moderate binding energy with the active receptors site of interleukin-1β and myeloperoxidase. All the compounds displayed excellent ADMET properties.
Conclusions. Antidiabetic drugs with the least side effects could be explored from these compounds.

Introduction

Diabetes mellitus is currently recognized as one of global epidemic diseases, affecting approximately 382 million people worldwide. The International Diabetes Federation (IDF) estimation of individuals who die from diabetes yearly is roughly 1.3 million and the global number of diabetes patients is expected to reach 629 million by 2045 [1].

Diabetes is a metabolic disorder characterized by chronic hyperglycemia accompanied by alteration in carbohydrates, fats, and proteins resulting from scanty insulin secretion or insulin action. Alterations in the metabolism of carbohydrates, fats, and proteins are associated with micro-vascular and macro-vascular diabetes complications [2].

Oxidative stress and inflammation critically contribute to the pathogenesis of diabetes mellitus-related complications. Oxidative stress and inflammation mediated by diabetes mellitus are considered to trigger apoptotic process leading to cellular injury and multiple organ damage [3].

Currently, the available antidiabetic drugs are not permanently curing diabetes and are associated with adverse effects. Therefore, much interest has been shifted towards the use and alternative medicine or derivative from food products with rich antidiabetic phytoconstituents. The presence of bioactive compounds and plant-derived products such as alkaloids, flavonoids, glycosides, gum, carbohydrates, and triterpenes with some short-peptides in medicinal plants play a major role in their therapeutic efficacy [4]. Specifically, plants nuts with rich phenolic compounds are known for their numerous biological activities including antioxidant, anti-inflammatory and antidiabetic properties [5]

Anacardium occidentale L. globally known as cashew belongs to the family *Anacardiaceae*. Several parts of the *Anacardium occidentale* tree including the leaf, stem bark and nut are often used ethno-pharmacological and investigated experimentally for their antidiabetic therapeutic efficacies [6]. Research showed that the presence of phenolic compounds, carotenoids, flavonoids, anthocyanins, tannins, and other minerals components in *Anarcadium occidentale* may be responsible for its antidiabetic properties [7]. *Anacardium occidentale* nut is well rich in unsaturated fatty acids such as oleic (ω-9) and linoleic (ω-6) acids, flavonoids, anthocyanins and tannins, fiber, folate and tocopherols [8–12]. Previously, the nuts have been reported in metabolic syndrome risk modulation [13].

However, an extensive scientific investigation is necessary on valuable traditional medicinal plants to investigate their antidiabetic efficiency using modern experimental equipment and methods. Computational molecular modeling has been identified as an important sector in the natural drug product development process. *In-silico* drug design tools improve the detection of novel drugs from natural products. Computational modeling provides much detail on the molecular recognition processes underlying the interaction between disease-related target macromolecules with naturally occurring drug-like substances [14].

The physicochemical and drug-likeness properties of bioactive compounds in *Anacardium occidentale* nut for their antidiabetic therapeutic efficacies have not been elucidated. The molecular docking, drug-likeness and ADMET of *Anacardium occidentale* nut methanolic extract compounds were investigated for further identification of major compound with potent antidiabetic therapy.

Material and methods

Anacardium occidentale Nut Collection

The nuts were freshly harvested from the *Anacardium occidentale* plant at the Agricultural

Research Farm located at Ladoke Akintola University of Technology in Ogbomosho, Oyo State, Nigeria. The nut underwent identification, and validation, and was given the voucher specimen number LH0533 by Dr. A. T. J. Ogunkunle, a faculty member of the Biology Department at Ladoke Akintola University of Technology in Ogbomoso, Oyo State, Nigeria.

Anacardium occidentale Nuts Methanolic Extraction

The *Anacardium occidentale* nuts finely powered weighing 500 g was placed in a Soxhlet apparatus for extraction using 95% methanol as the solvent for a duration of 48 hours, the temperature was carefully maintained below the boiling point of the solvent. The extracts were filtered using a white muslin cloth and subsequently subjected to a double filtration process using white Whatman filter paper. The filtrate was then concentrated in a rotary evaporator at a controlled temperature of 35°C and reduced pressure until the extract had fully dried, yielding a concentrated methanolic extract. The obtained extract was then stored in a refrigerator at a constant temperature of 4°C for utilization at a later time.

Identification of *Anacardium occidentale* Nut Compounds

The bioactive compounds of the *Anacardium occidentale* nut methanolic extract were identified by gas chromatography-mass spectrometry (GC-MS) technique. GC-MS analysis of the methanolic extract was performed using on Ralte et al. [15] adopted method.

Target Proteins

Five target proteins, B-cell lymphoma-2 (Bcl-2) for anti-apoptotic, caspase-3 for apoptotic, interleukin-1β (IL-1β) for anti-inflammatory, tumor necrosis factor-alpha (TNF-α) for inflammatory, myeloperoxidase for insulin resistance and glucocorticoids receptor for insulin antagonist related to diabetes mellitus progression were selected for their interactions with the bioactive compounds from *Anacardium occidentale* nuts.

Preparation of Target Proteins

The 3-dimensional (3D) X-ray crystallographic structuzres of target proteins; B-cell lymphoma-2(Bcl-2) (PDB ID: 2YV6), caspase-3 (PDB ID: 1QX3), glucocorticoids receptor (PDB ID: 1GDC), interleukin-1β (IL-1β) (PDB ID: 9ILB), myeloperoxidase (MPO) (PDB ID: 3F9P) and tumor necrosis factor-alpha (TNF-α) (PDB ID: 1TNF) were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (The proteins were prepared by(a) removal of hetero-atoms (water, ions), (b) addition of polar hydrogens, and (c) assignment of Kollman charges using "Clean Protein" and "Prepare Protein" Discovery Studio software (version 19.1) and the proteins were converted from PDB format into pdbqt file format via Auto-dock Tools 4.2 software.

Ligand Molecules Preparation

After the GC-MS analysis, The ligand molecules were downloaded from the pubchem database (https://pubchem.ncbi.nlm.nih.gov) in a structure database file (SDF) and converted to pdb format using Pymol [16], then to pdbqt format via Autodock tool (version 4.2) for molecular docking.

The ligand molecules were docked with all target proteins using Auto-dock 4.0 software by setting up 4 energy range and exhaustiveness value of eight 8 as default to obtain 10 different poses of ligand molecules [17]. The 2D binding interactions was detected via LigPlot+ v.2.2 (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). After the docking process, ligands with the lowest binding energy were selected to visualize the ligand-protein interaction in Pymol.

Physicochemical and ADMET Determination

Drug-likeness physicochemical and ADMET properties of *Anacardium occidentale* nut methanolic extract compounds were determined using the molinspiration web tool and ADMETsar online server [18].

Results

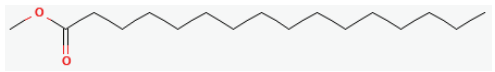
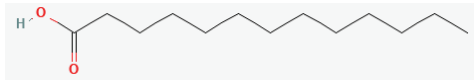
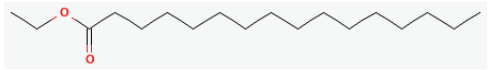
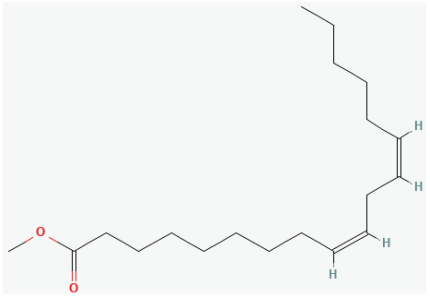
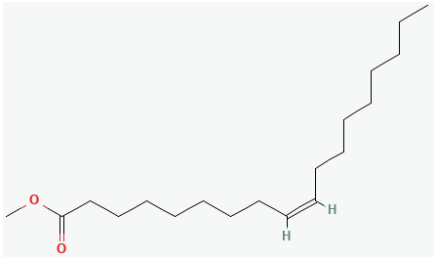
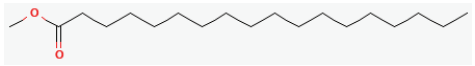
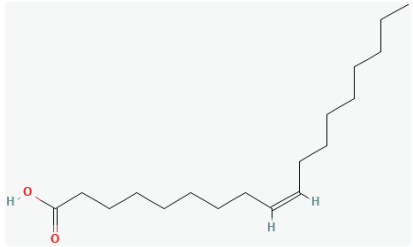
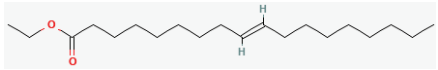
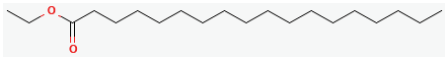
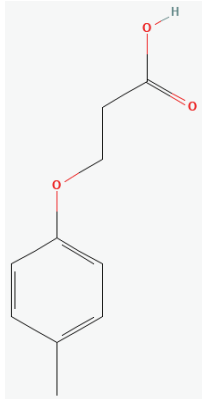
Anacardium occidentale Nut Compounds

A total of 10 compounds were identified from *Anacardium occidentale* nut methanolic extract via the GC-MS analysis. The details of the compounds are given in **Table 1**.

Physicochemical Properties Analysis

The physicochemical properties and drug-likeness of the selected *Anacardium occidentale* nut

Table 1. Identified compounds from *Anacardium occidentale* nut methanolic extract.

No	Compounds structures, molecular names and formulas	Molecular weight (g/mol)	Retention time
1	 Hexadecanoic acid-methyl ester Molecular formula $C_{17}H_{34}O$	271	37.008
2	 Tridecanoic acid Molecular formula $C_{16}H_{32}O_2$	256	37.420
3	 Hexadecanoic acid-ethyl ester Molecular formula $C_{18}H_{36}O_2$	285	37.589
4	 9,12-Octadecadienoic acid (Z,Z)-methyl ester Molecular formula $C_{19}H_{34}O$	295	38.265
5	 9-Octadecenoic acid (Z)-methyl ester Molecular formula $C_{19}H_{36}O$	296	38.302
6	 Methyl stearate Molecular formula $C_{19}H_{38}O_2$	299	38.465
7	 Oleic acid Molecular formula $C_{18}H_{32}O_2$	283	38.609
8	 (E)-9-Octadecenoic acid-ethyl ester Molecular formula $C_{20}H_{38}O_2$	310	38.697
9	 Octadecanoic acid-ethyl ester Molecular formula $C_{20}H_{40}O_2$	313	38.847
10	 3-(4-Methoxyphenyl)-propionic acid Molecular formula $C_{16}H_{12}O_3$	180	40.342

methanolic extract compounds were determined using Lipinski's rule of five (RO5) [19], which stated that, a compound is considered a drug-like molecule if no more than one of the following criteria is violated: molecular weight < 500KDa, LogP < 5, hydrogen bond donor ≤ 5, hydrogen bond acceptor ≤ 10. Nine compounds of *Anacardium occidentale* nut methanolic extract violated one Lipinski's rule as their lipophilicity expressed as logP (octanol-water partition coefficient) greater than the acceptable range and one compound 3-(p-methoxyphenyl)-propionic acid violated none of the rules (**Table 2**).

Compounds ADMET Profile Analysis

The canonical SMILES of the compounds were saved and uploaded to the admetSAR web server or admet properties analysis. The compounds are screening for ADMET properties including acute oral toxicity, blood-brain barrier, carcinogenicity, cytochrome P450 inhibitors isoforms (CYP inhibitors)1, hepato-toxicity, human ether-a-go-go-related gene inhibition (hERG), human Intestinal Absorption, human oral bioavailability and P-glycoprotein inhibitor (P-gpi) (**Table 3**).

The selected compounds of *Anarcardium occidentale* nut methanolic extract have no acute oral toxicity as the majority of the compounds displayed Class III category. However, compound II (tridecanoic acid) and compound VII (oleic acid) fall into the Class IV category.

In addition, the compounds have high human intestinal absorption, good blood-brain barrier

Table 2. Physicochemical properties of Anacardium occidentale nut methanolic extract compounds.

No	Compounds	Lipinski rule violation	Molecular weight (g/mol)	nHD	nHA	Log P
1	Hexadecanoic acid	1	270.45	0	2	7.37
2	Tridecanoic acid	1	214.33	1	1	5.54
3	Hexadecanoic acid-ethyl ester	1	284.27	0	2	7.448
4	9,12-octadecadienoic acid (Z,Z)-methyl ester	1	294.26	0	2	6.992
5	9-octadecenoic acid-methyl ester	1	296.49	0	2	7.746
6	Methyl stearate	1	298.29	0	2	8.049
7	Oleic acid	1	282.26	1	2	7.131
8	(E)-9-octadecenoic acid-ethyl ester	1	310.52	0	2	7.531
9	Octadecanoic acid-ethyl ester	1	312.3	0	2	8.286
10	3-(p-methoxyphenyl)-propionic acid	0	180.08	1	3	2.148

Table 3. ADMET properties of Anacardium occidentale nut methanolic extract compounds.

No	Acute oral toxicity	Blood-Brain Barrier	Carcinogenicity	CYP1A2 inhibitor	CYP2C19inhibitior	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Hepatotoxicity	hERG inhibitor	Human Intestinal Absorption	Human Oral Bioavailability	p-glycoprotein inhibitor
1	Class III	+	-	-	-	-	-	-	-	-	+	-	-
2	Class IV	+	-	+	-	-	-	-	-	-	+	-	-
3	Class III	+	-	-	-	-	-	-	-	-	+	-	-
4	Class III	+	-	+	-	-	-	-	-	+	+	-	-
5	Class III	+	-	+	-	-	-	-	-	+	+	-	-
6	Class III	+	-	-	-	-	-	-	-	-	+	-	-
7	Class IV	+	-	+	-	-	-	-	-	-	+	-	-
8	Class III	+	-	+	-	-	-	-	-	+	+	-	-
9	Class III	+	-	-	-	-	-	-	-	-	+	-	-
10	Class III	+	-	-	-	-	-	-	-	-	+	+	-

permeation and well human oral bioavailability and compound X exhibited poor human oral bioavailability. Furthermore, the compounds are non-carcinogenic, non-hepato-toxic and non-inhibitors of permeability glycoprotein (P-gp).

Furthermore, the majority of the compounds are non-inhibitors of CYP450 isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) and compounds II, IV, V, VII and VIII exhibited CYP1A2 inhibitors.

Also, compounds I, II, III, VI, VII, IX and X are non-inhibitor of the human ether-a-go-go related gene (hERG) and compounds IV, V and VIII exhibited hERG inhibitors.

Ligands Molecular Docking Interaction Analysis

The ten identified compounds were successfully docked with the target protein receptors and compounds with notable significant docking binding energy were selected.

Ligands Molecular Interaction with B-cell lymphoma-2 (BCL-2)

Oleic acid and 3-(p-methoxyphenyl)-propionic acid were recognized as the best-docked compounds to active receptors site of target protein B-cell lymphoma-2 (Bcl-2) (PDB ID: 2YV6) with a binding energy of -6.1 and -5.8 Kcal/mol respectively. Oleic acid established binding interaction with active receptors site of Bcl-2 via one hydrogen bond forming residue ASP 103 significant than the standard drug (metformin) and eight residues with GLY 145, ARG 107, ASP 111, PHE 153, GLU, 152, LEU 137, VAL 148 and ALA 100 via hydrophobic interactions and six forming residues with MET 115, PHE 112, VAL, 156, ALA 149, PHE, 104 and TYR 108 through alkyl-pi-alkyl interactions. The ligand 3-(p-methoxyphenyl)-propionic acid had binding interaction with active receptors site of Bcl-2 via one hydrogen bond forming residue ARG 127 and 6 residues with HIS 184, PHE 138, TYR 180, GLU 135, VAL 134 and ALA 131 through hydrophobic interactions (**Figure 1: a** & **b**).

Ligands Molecular Interaction with Caspase-3

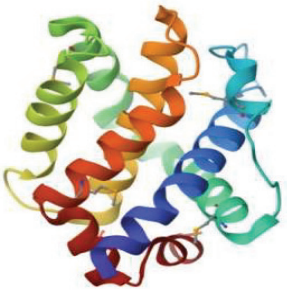
Oleic acid and 3-(p-methoxyphenyl)-propionic acid were identified as the most docked compounds with target protein caspase-3 (PDB ID: 1QX3) active receptors site and displayed lower binding energy of -3.4 and -4.0 Kcal/mol respectively in comparison with reference drug metformin (-4.3 Kcal/mol). Oleic acid had interactions with caspase-3 through one hydrogen bond forming residue TYR 195 and six residues with MET 268, ARG 164, TYR 197, GLY 125, LEU 136 and ASP 135 via hydrophobic interactions. Also, 3-(p-methoxyphenyl)-propionic acid had interactions with caspase-3 via one hydrogen bond forming residue LYS 105 and three residues via hydrophobic interactions with ARG 147, SER 150, and CYS 148 (**Figure 2: c** & **d**).

Ligands Molecular Interaction with Glucocorticoids

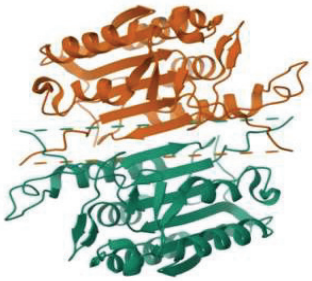
Three compounds' tridecanoic acid, oleic acid and 3-(p-methoxyphenyl)-propionic acid were discovered as excellent docked compounds with target protein glucocorticoids (PDB ID: 1GDC) active receptors site with binding energy of -4.0, -4.1 and -5.0Kcal/mol respectively. Tridecanoic acid had interactions with glucocorticoids active receptors site through one hydrogen bond forming residue SER 440 and two forming residues with TYR 455 and ARG 477 through hydrophobic interactions. Also, Oleic acid had interactions with active receptors site of glucocorticoid via one hydrogen bond forming residue ARG 477 and four residues with SER 440, TYR 455, ARG 470 and PRO 474 via hydrophobic interactions. Further, 3-(p-methoxyphenyl)-propionic acid had interactions with glucocorticoids active receptors site via two hydrogen bonds forming residues SER 440 and ARG 477 and three forming residues with TYR 455, PHE 444 and VAL 443 via hydrophobic interactions (**Figure 2: e**, **f** & **g**).

Ligands Molecular Interaction with Interleukin-1beta (IL-1β)

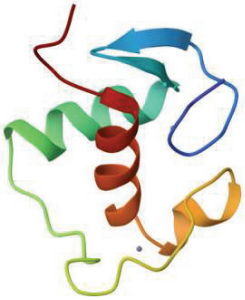
The compound's 3-(p-methoxyphenyl)-propionic acid exhibited a docking pattern of the binding energy of – 5.2 Kcal/mol with the protein interleukin-1β (PDB: 9ILB) active receptors site of all the 10 compounds. 3-(p-methoxyphenyl)-propionic acid had no hydrogen bonding forming residue interactions with IL-1β. The stabilization of interaction of 3-(p-methoxyphenyl)-propionic acid with the active receptors site was mediated via hydrophobic interactions forming residues with LYS 63, TRY 68, TRY 90, ASN 66, SER 5, GLU



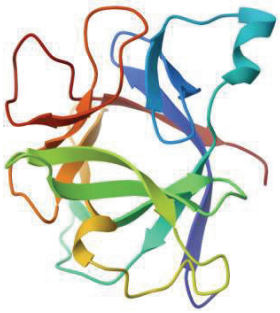
a: B-cell lymphoma-2 (Bcl-2)



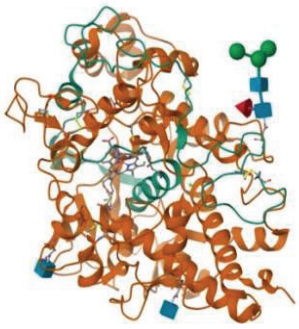
b: Caspase-3



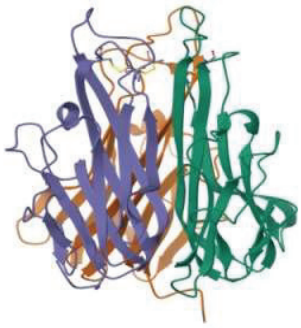
c: Glucocorticoid receptors



d: Interleukin-1β (IL-1β)



e: Myeloperoxidase



f: Tumor necrosis factor-alpha (TNF-α)

Figure 1. Depict 3-D crystallographic structure of the target proteins related to diabetes for Molecular docking.

64 and SER 43 and alkyl-pi-alkyl interaction residue with PRO 87 (**Figure: 2h**).

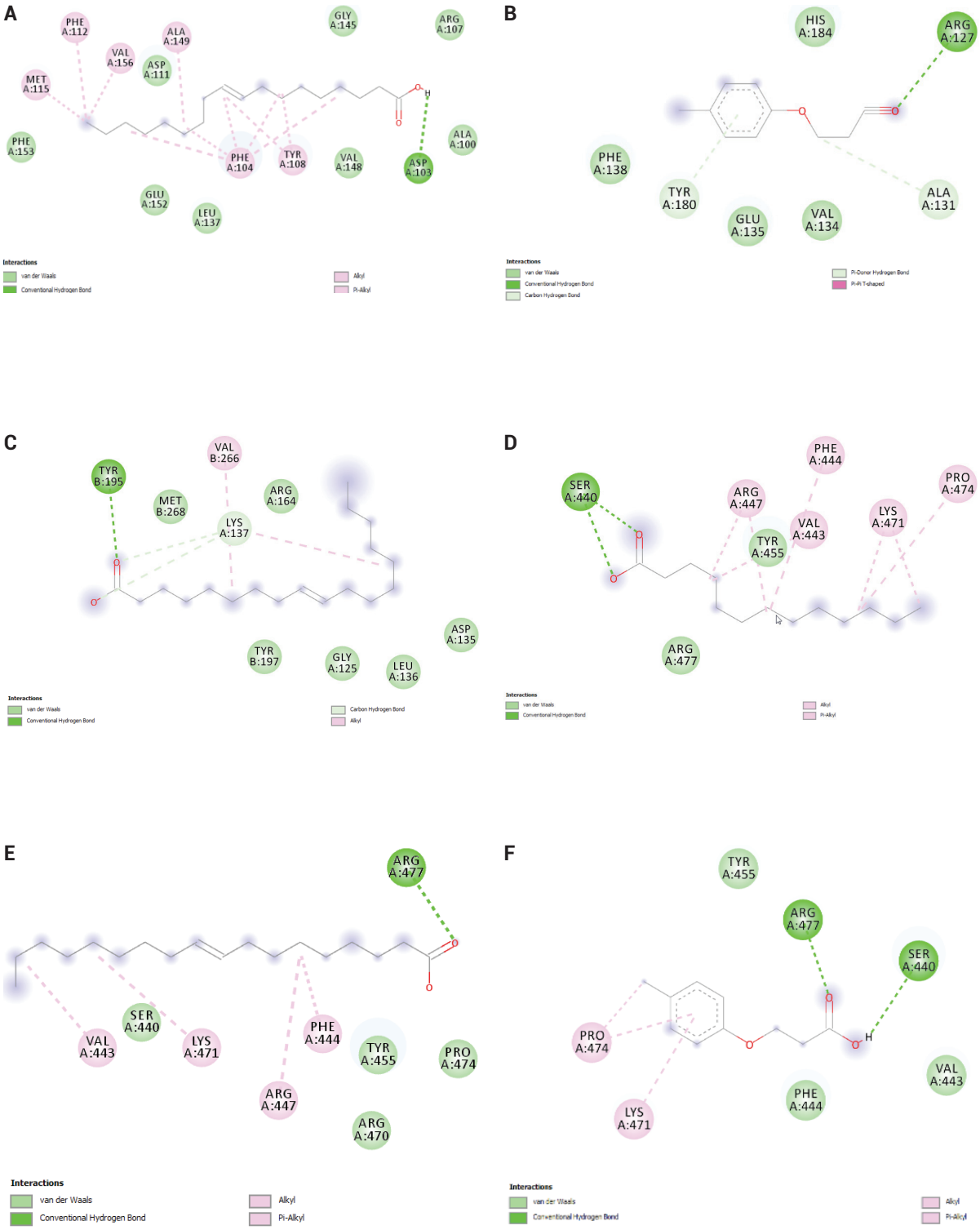
Ligands Molecular Interaction with Myeloperoxidase (MPO)

The docking analysis revealed only compound 3-(p-methoxyphenyl)-propionic acid showed binding energy of –5.3Kcal/mol with protein myeloperoxidase (PDB: 3F9P) and had interactions with through one hydrogen bond forming residue ARG 507 and six forming residues with TRY 273, TRY 313, LEU 310, THR 312, TRP 513 and PRO 311 via hydrophobic interactions and four alkyl-pi-alkyl interactions forming residues with ALA 529, TRP 514, ILE 290 and ARG 294 (**Figure: 2i**).

Ligands Molecular Interaction with Tumor Necrosis Factor-Alpha (TNF-α)

The compounds' oleic acid and 3-(p-methoxyphenyl)-propionic acid exhibited a significant docked pattern of the binding energy of -4.1 and -4.1Kcal/mol respectively with target protein tumor necrosis factor- alpha (PDB ID: 1TNF) active receptors site. Oleic acid

had interaction with the active receptor site of TNF-α through two hydrogen bonds forming residues TYR 59 and ALA 14 and six residues with TYR 119, TRY 151, ILE 154, LEU 36, and VAL via hydrophobic interactions and three alkyl-pi-alkyl interactions residues with LUE 57, ILE 155 and HIS. The interaction of compound 3-(p-methoxyphenyl)-propionic acid with TNF-α active receptor site was mediated via hydrophobic interactions forming residues with GLY 68,



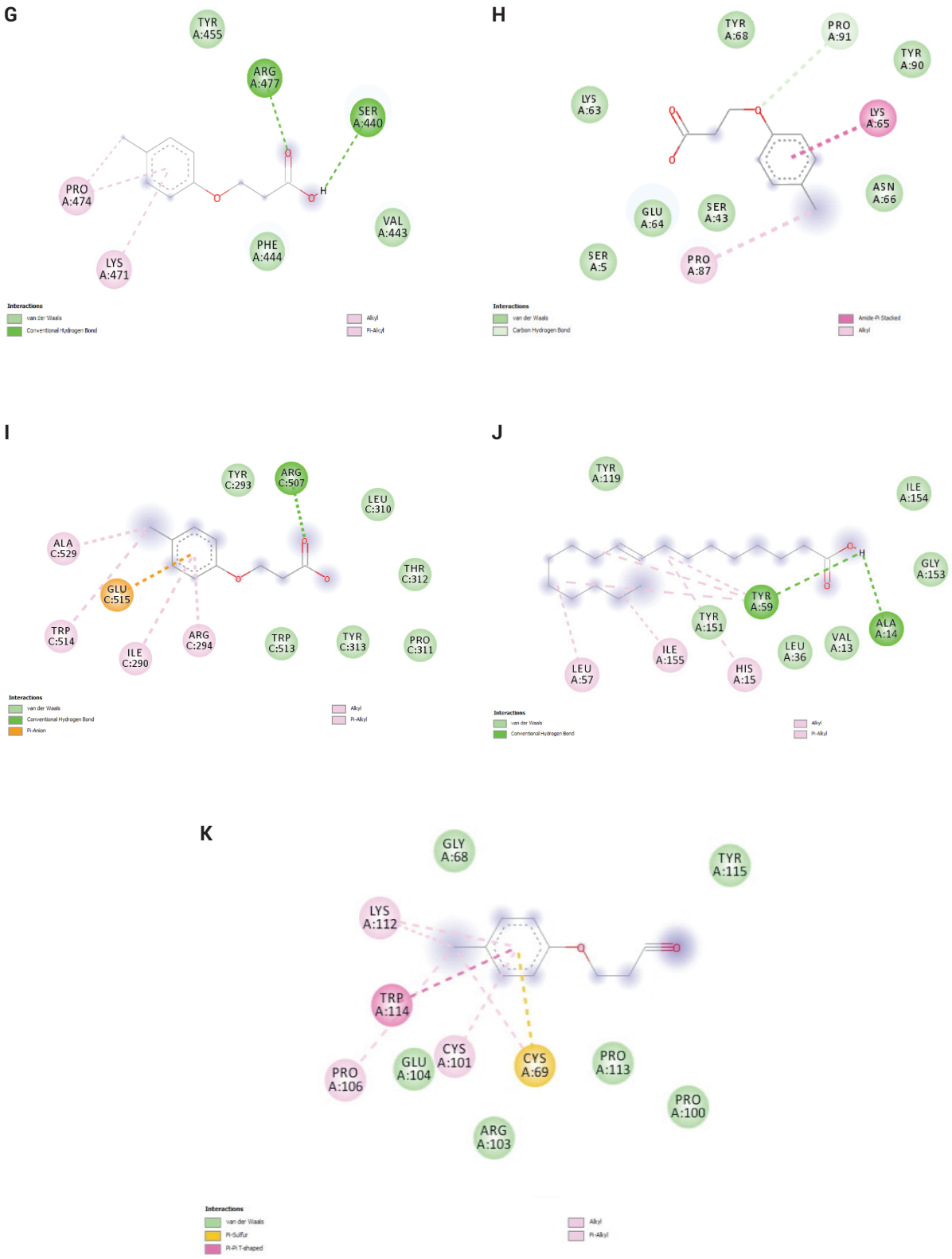


Figure 2. Depicted the 2-D structure molecular binding interactions of (**A**) oleic acid and Bcl-2, (**B**) 3-(p-methoxyphenyl)-propionic acid and Bcl-2, (**C**) oleic acid and caspase-3, (**D**) 3-(p-methoxyphenyl)-propionic acid and caspase-3, (**E**) tridecanoic acid and glucocorticoids receptor (**F**) oleic acid and glucocorticoids receptor, (**G**) 3-(p-methoxyphenyl)-propionic acid and glucocorticoid, (**H**) 3-(p-methoxyphenyl)-propionic acid and IL-1β, (**I**) 3-(p-methoxyphenyl)-propionic acid and myeloperoxidase, (**J**) oleic acid and TNF-α, (**K**) 3-(p-methoxyphenyl-)-propionic acid and TNF-α.

TYR 115, GLU 104, ARG 103, PRO 100 and PRO 113 and three alkyl-pi-alkyl interactions forming residues with LYS 112, PRO 106 and CYS 101 (**Figure 2: j** & **k**).

Discussion

Diabetes is emerging as the third "quiet killer" of humankind, following cancer and cardiovascular diseases owing to its escalation prevalence, morbidity and mortality [20]. Despite significant advancements in the drug discovery field, effectively managing diabetes remains challenging and poses a major problem within the medical arena [21].

Molecular docking serves a cardinal role in the development and designing of novel drugs. It precisely envisions the binding mode and affinity of natural compounds within the active binding site of the drug target [22]. Furthermore, *in-silico* techniques serve as a screening tool to acquire physicochemical, drug-likeness and ADMET information for drug designing [23]. The current study investigated drug-likeness, ADMET properties and molecular docking of compounds identified from *Anacardium occidentale* nut methanolic extract with target protein of diabetes mellitus progression using *in-silico* technique.

Hyperglycemia has been implicated in the induction of β-cell apoptosis in diabetes mellitus [24]. Anti-apoptotic B-cell lymphoma-2 (Bcl-2) is a member of the Bcl proteins family. The up-regulation of the Bcl-2 suppresses apoptosis by regulating the sensitivity of cells to apoptotic stimuli [25]. Oleic acid and 3-(p-methoxyphenyl)-propionic acid of *Anacardium occidentale* nut methanolic extract-compounds interact with active receptors site of Bcl-2 protein which are implicated in the pathogenesis of human diabetes. These novel compounds interact in the same manner as the reference drug (metformin) and efficiently fit the binding pocket of Bcl-2 protein receptors and may facilitate the up-regulation of the Bcl-2 to impede β-cell apoptosis.

Caspase-3 protein is a member of the caspase family and plays a critical role in cell apoptosis execution [26]. Inhibitors of caspase-3 peptide avert β-cell apoptosis and improve the function of islet graft [27]. Also, these two compounds oleic acid and 3-(p-methoxyphenyl)- propionic acid fit accurately into the binding pocket of the caspase-3 protein active receptors site. Therefore, could serve as an effective caspase-3 peptide inhibitors candidate to treat diabetes mellitus.

Glucocorticoids are powerful insulin action antagonists and promote hepatic gluconeogenesis thereby leading to hyperglycemia in diabetes. The determination of active glucocorticoids to their receptors at the tissue level is governed by 11beta-hydroxysteroid dehydrogenase type 1(11β-HSD1) [28]. 11β-HSD1 is an enzyme that depends on nicotinamide adenine dinucleotide phosphate (NADPH) to catalyze the inter-conversion of glucocorticoids, cortisone, and cortisol in humans. Elevated circulating levels of the active glucocorticoid cortisol can instigate insulin resistance causing hepatic gluconeogenesis eventually leading to insulin-resistant and macro-vascular diabetes complications [29]. Tridecanoic acid, oleic acid and 3-(p-methoxyphenyl) propionic acid compounds of *Anacardium occidentale* nut methanolic extract fit clearly into the binding pocket of 11β-HSD1 active receptors site and these interactions suggested that the three compounds are novel inhibitors of 11β-HSD1for diabetes therapy.

Cytokines (small proteins produced by immune cells and other cell types) are considered in diabetes pathogenesis. Active innate immune cells assemble to cause activation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) [30]. Elevated circulating levels of cytokine TNF-α (the protein responsible for inflammation) and diminished levels of anti-inflammatory interleukin-1β (IL-1β) protein have been proposed to induce β-cells apoptosis and insulin resistance leading to chronic hyperglycemia of diabetes mellitus [31,32]. 3-(p-methoxyphenyl)-propionic acid compound of all the compounds interact with target IL-1β protein receptors binding pocket. Furthermore, two compounds oleic acid and 3-(p-methoxyphenyl)-propionic acid compactly interact with the binding pocket of TNF-α protein active receptor site like metformin, showing the compounds possess inhibitory potential on pro-inflammatory cytokine TNF-α, and suggest it anti-inflammatory efficacy for diabetes.

Also, the heme protein myeloperoxidase (MPO) derived from leukocytes greatly contributes to the instigation and progression of diabetes. A mildly

elevated level of myeloperoxidase is associated with macro-vascular diabetes complications [33]. 3-(p-methoxyphenyl)-propionic acid compound tightly interacts with the binding pocket of myeloperoxidase protein active receptors site as the metformin which confirms the compound's myeloperoxidase inhibitory potent for therapy of macro-vascular complication of diabetes.

Efficacy and risk-free are the main goals for searching for a novel drug as every drug can assist to treat diseases as well as induce perilous effects [34]. *In-silico* analysis has served an enormous role to evaluate multiple ADMET (pharmacokinetics) properties of compounds in drug research, discovery and design [35]. The drug-likeness characteristics of the selected compounds were screened by Lipinski's rule of five (Ro5) criteria in the current study. All the compounds fulfill the drug-likeness criteria as they violated one rule of five.

The selected compounds also passed the drug-like evaluation as potential candidates via the ADMET analysis. The compounds are safe from acute oral toxicity as some of the compounds belong to Class III except for tridecanoic acid and oleic acid. High absorption from the gastrointestinal tract (GIT) and blood-brain barrier permeation connote that these compounds could be better absorbed from GIT excellently via oral administration than other routes of administration and can achieve bioavailability as well in neurological pathways, thus, serving as therapeutic for neurological degeneration.

The LogP value predicts a compound's permeability through a lipid membrane. For a potential drug, it should be ≤5. The number of hydrogen bond acceptors and donors describes its ability to bind with other compounds, which therefore describes its solubility and permeability [36]. Among the compounds, only 3-(p-methoxyphenyl)-propionic acid has better lipophilicity with LogP < 5, implying its good permeability across the lipid membrane. All the compounds exhibited an excellent number of hydrogen-bound acceptors and donors and this suggests the compounds' high solubility and affinity to bind with other compounds.

Metabolism plays a significant role in drug bioavailability as well as drug-drug interactions. Permeability glycoprotein (P-gp) belongs to the ATP-binding cassette transporters (ABC) and is essential for assessing active efflux through biological membranes (from the wall of GIT to the lumen or from the central nervous system) [37]. CYP450 enzymes with isoforms CYP450 (CYP 3A4, CYP 2D6, CYP 1A2, CYP 2C9, and CYP 2C19) facilitate drug elimination via metabolic biotransformation [38]. Both P-gp and CYP 450 have been proposed to synergistically process small molecules to improve tissue protection [39]. Inhibition of these iso-enzymes may lead to pharmacokinetics-associated drug-drug interactions that might result in detrimental effects by diminishing the solubility and the drug metabolites. Except for compounds, IV, V and VIII that demonstrated inhibitor of CYP1A2 of CYP 450 isoform, all the top hit compounds of methanolic extract of *Anacardium occidentale* nut are non-inhibitor of P-gp and CYP 450 enzymes. These compounds of non-inhibitor of P-gp and CYP 450 will be metabolized normally and safe from inducing unwanted adverse side effects.

The aptness of small molecules to be selected as candidate compounds in drug discovery depends on the compound's toxicity levels [40]. AdmetSar predicts toxicity and carcinogenic of compounds. All the selected compounds are non-carcinogenic and non-hepato-toxic and, therefore, free from inducing DNA mutation(s) and hepatic damage upon ingestion.

The human ether-a-go-go-related gene (hERG) encodes the potassium channel known for normal heart function. Research showed that many drugs have been withdrawn from use owing to their cardio-toxicity through the blockage of hERG activity [41,42]. The selected compounds are also non-inhibitory of hERG, hence safe from cardio-toxicity induction. Moreover, compounds IV, V, and VIII are hERG inhibitors and might probably induce cardiac blockage.

Conclusions

The docking analysis revealed oleic acid, 3-(p-methoxyphenyl)-propionic acid and tridecanoic acid from *Anacardium occidentale* nut methanolic extract were excellent molecules with drug-likeness owing to their inhibitory potentials on selected proteins related to diabetes mellitus pathogenesis progression. The compounds also exhibited good ADMET properties and may lead to

the design of potent novel antidiabetic drugs with minimal side effects. In *vitro and in vivo* studies of these compounds can be further investigated.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

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Association of the interleukin-10 (IL-10) gene polymorphisms with ovarian cancer risk: a systematic review and meta-analysis

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Keywords: ovarian cancer, interleukin-10, IL-10, polymorphisms, genetic predisposition

ABSTRACT

Introduction. Ovarian cancer is a cancer with high fatality due to its symptomless nature, which leads to a late diagnosis. Therefore, there is an urgent need to discover genetic markers related to predisposition to the disease. With anti-inflammatory cytokines playing a major role in cancer predisposition, the present systematic review and meta-analysis were undertaken to evaluate the association of the interleukin-10 (IL-10) gene polymorphisms with ovarian cancer risk.

Material and methods. Online databases were searched for articles dating from June 2023 until inception for studies assessing the frequencies of IL-10 polymorphisms in ovarian cancer patients and controls. The odds ratios of the genotypes, alongside their respective 95% confidence intervals, were calculated under three different genetic models.

Results. A total of 5 records studying the IL-10-819 C>T and IL-10-1082 G>A polymorphisms were included in the quantitative analysis. The meta-analysis suggested that the IL-10-819 C>T polymorphism was significantly associated with the risk of ovarian cancer under a dominant (CT + TT vs CC) inheritance model (OR = 2.67; 95% CI = [1.17,6.12]; p = 0.02).

Conclusions. The meta-analysis suggested that the T allele of the IL-10-819 C>T is associated with an increased risk of ovarian cancer. However, no statistically significant association exists between the IL-10-1082 G>A polymorphism and ovarian cancer. Future studies are required to verify these results further.

Introduction

Ovarian cancer is the fatal type of gynaecological cancer globally and accounts for about 2.5% of all malignant neoplastic diseases among females [1]. Approximately only 50% of ovarian cancer patients survive for more than five years after diagnosis [1,2]. The reason underlying this low survival rate is the fact that ovarian cancer is often symptomless in the initial stages, lead-

ing to late diagnosis at stages where classical therapeutic strategies may fail to be successful [3]. Therefore, discovering methods that may help an earlier diagnosis of malignancy in women can improve survival rates [4]. Over the recent years, the discovery of genetic polymorphisms related to cancer has significantly assisted clinicians in identifying patients who are at high risk of developing malignancies and, therefore, achieving earlier diagnosis through means of continuous screening [5].

The interleukin genes, encoding for a group of cytokines, have been shown to be associated with carcinogenesis, and studies have indicated that some polymorphisms of these genes are associated with an increased risk of carcinogenesis, including ovarian cancer [6,7]. Specifically, polymorphisms of the interleukin-10 (IL-10) gene are correlated with different types of malignant neoplasms [8,9]. Nevertheless, there is no concrete evidence that these polymorphisms increase the risk of developing ovarian cancer. Hence, in the present study, a systematic review and meta-analysis of the existing literature was performed to assess whether a relationship between the IL-10 polymorphisms and ovarian cancer risk exists and whether the IL-10 gene can be used as a genetic marker of ovarian cancer.

Material and methods

The research protocol of the present systematic review and meta-analysis was not registered in any database.

Search strategy

The online databases PubMed, EMBASE and SCOPUS were searched systematically for articles from June 2023 till inception using the keywords "IL-10", "Interleukin-10", "IL10", "Ovarian cancer", "Ovarian tumour", "Polymorphisms", "Polymorphic", "SNP" and a combination of Boolean operators, excluding review articles, letters and commentaries.

Using the citation manager EndNote, duplicates were removed, and citations were subsequently screened based on their titles and abstracts. Inclusion criteria were case-control studies studying the frequencies of IL-10 gene polymorphisms in healthy individuals and patients with ovarian cancer. All articles reporting polymorphisms of other genes in ovarian cancer and articles reporting polymorphisms of IL1–0 in other diseases other than malignant ovarian cancer were excluded. The final selection was made after the two reviewers assessed the remaining studies based on their full text. Two independent reviewers (Stavri Totou and Datis Kalali) performed the selection process.

Data extraction and qualitative analysis

The following data was extracted from each study and included in the qualitative analysis by two reviewers (Stavri Totou and Datis Kalali):

- Number of ovarian cancer patients and controls enrolled in the study,
- The genetic polymorphisms studied and their respective genotypes,
- The frequency of genotypes in cases and controls,
- The odds ratios of the polymorphisms (cases vs controls) and their respective p-values.

The qualities of the included studies were assessed by two independent reviewers (Stavri Totou and Datis Kalali) using the Newcastle-Ottawa scale for case-control studies [10]. No disagreements arose between the reviewers during the quality assessment.

Quantitative analysis

Initially, the odds ratios of the polymorphisms were extracted or calculated separately (in case the study did not report the ratio) alongside their respective 95% confidence intervals under four different inheritance models: dominant, recessive, co-dominant and allele. An alpha value of 0.05 was used. Thus, the ratios are considered statistically significant if their 95% confidence intervals do not contain the number 1, or their respective p-value is less than 0.05 [11]. The Higgins and Thompson I^2 statistic was used to assess the heterogeneity between the studies, where an I^2 value than 50% indicates the presence of statistical heterogeneity [12]. If heterogeneity is present, a random effects model is preferred for performing a meta-analysis, or a fixed effects model is used. Subsequently, a meta-analysis of all included studies was undertaken to create forest plots and calculate a pooled odds ratio for all studies under the four different inheritance models. A funnel plot was constructed, and Egger's

test was performed to assess whether significant publication bias existed within the meta-analysis. All statistical analyses were performed using STATA release version 17.0 (StataCorp LL, College Station, Texas, USA) and Review Manager release version 5.4.1 (RevMan, Cochrane, London).

Results

Included studies

The database search on the internet retrieved a total of 55 citations (12 citations from PubMed, 20 citations from SCOPUS and 23 citations from EMBASE) and an addition of another two citations were retrieved manually through other sources. After removing duplicates, a total of 32 citations remained, amongst which 21 were excluded after screening since irrelevance to the research question was evident from their titles or abstracts. Among the remaining 11 studies, which were assessed based on their full texts, a total of two studies were reviewed, and four did not contain relevant data for the research. Thus, a total of 5 studies were included in the present meta-analysis. **Figure 1** provides a graphical overview of the literature identification, screening and inclusion process. **Table 1** contains a summary of the characteristics of all the studies that were included.

Quality analysis

The Newcastle-Ottawa scale was used to assess the qualities of all included studies, and **Table 2** contains the recorded results of this assessment. Generally, the studies were of adequate quality, so they did not carry a high risk of biased results.

Meta-analysis

Two polymorphisms were identified in the studies: IL-10–1082 G>A (rs1800896) and IL-10–819 C>T (rs1800871). The meta-analysis did not indicate any statistically significant result relating the IL-10–1082 G>A polymorphism to the risk of ovarian cancer. However, regarding the IL-10–819 C>T polymorphism, it was found that the CT and TT genotypes were significantly related to the risk

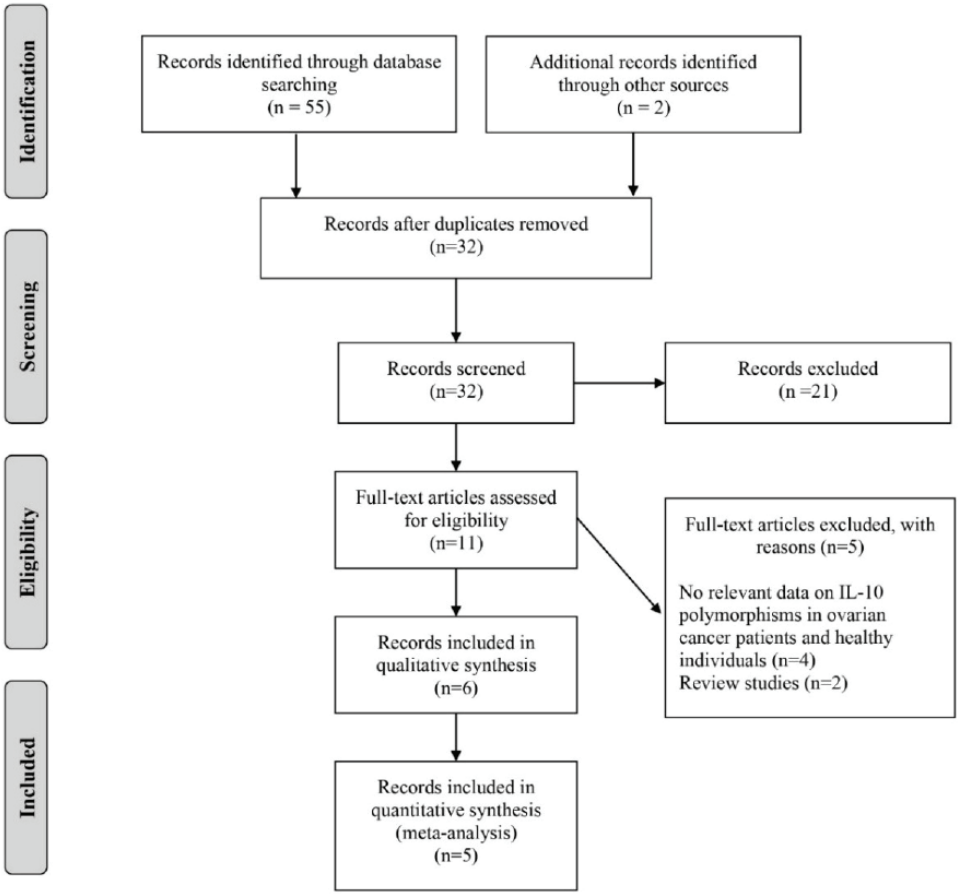

Figure 1. PRISMA diagram of the search strategy and inclusion process.

of ovarian cancer (OR = 2.67; p = 0.02), indicating that the T-allele of the polymorphism is related to ovarian cancer under a dominant inheritance model. **Tables 3** and **4** summarize the pooled odds ratios and other statistical measures retrieved in the meta-analysis. **Figure 2** shows a forest plot that explores the association between ovarian cancer risk and the IL-10–819 C>T polymorphism under a dominant inheritance model. Forest plots of the meta-analysis of all other genetic models for both polymorphisms have been provided in the Supplementary material.

Publication bias

The symmetrical shape of the funnel plot (see **Figure 3**) indicated no evident bias under the dominant model, confirming the reliability of the retrieved results. A P-value of 0.36 (greater than

Table 1. Characteristics of included studies.

Study (Author, year)	Country	Participants (cases/ controls)	Age of participants (cases/ controls)	Sample type	Genotyping method	Polymorphisms studied
Almolakab et al., 2022 [13]	Egypt	48/48	45.3/50.6 (Mean)	Blood	SSP-PCR	IL-10-819 C>T and IL-10-1082 G>A
Briacu et al., 2007 [14]	Germany	147/129	45.5/55 (Median)	Blood	Pyrosequencing™	IL-10-819 C>T and IL-10-1082 G>A
Bushley et al., 2004 [15]	USA	180/218	54.7/54.7 (Mean)	Blood	SSP-PCR	IL-10-819 C>T and IL-10-1082 G>A
He et al., 2008 [16]	China	33/90	Unknown	Blood	SSP-PCR	IL-10-819 C>T
Kutikhin et al., 2014 [17]	Russia	74/168	55.3/58.3 (Mean)	Blood	SSP-PCR	IL-10-1082 G>A

Table 2. Quality assessment of studies in the meta-analysis.

Study (Author, year)	Newcastle-Ottawa scale scores			
	Selection	Comparability	Exposure	Total
Almolakab et al., 2022 [13]	3	1	3	7
Briacu et al., 2007 [14]	3	1	2	7
Bushley et al., 2004 [15]	3	2	3	8
He et al., 2008 [16]	3	1	1	5
Kutikhin et al., 2014 [17]	3	1	3	7

Table 3. Pooled odds ratios for IL-10–819 C>T polymorphism (cases vs. controls).

Genetic model		Odds ratio [95% CI]	Meta-analysis model	I-squared %	P-value
Co-dominant model	CC	1			
	CT	1.59 [0.99, 2.55]	Random effects	56	0.05
	TT	1.18 [0.84, 1.66]	Fixed effects	37	0.34
Dominant model	CT + TT vs. CC	2.67 [1.17, 6.12]	Random effects	70	0.02
Recessive model	CC + CT vs. TT	0.81 [0.57, 1.14]	Fixed effects	48	0.12
Allele model	C vs. T.	1.12 [0.88, 1.42]	Random effects	81	0.35

Table 4. Pooled odds ratios for IL-10–1082 G>A polymorphism (cases vs. controls).

Genetic model		Odds ratio [95% CI]	Meta-analysis model	I-squared %	P-value
Co-dominant model	GG	1			
	GA	1.21 [0.94, 1.57]	Fixed effects	6	0.14
	AA	0.80 [0.50, 1.29]	Random effects	60	0.36
Dominant model	GA + AA vs. GG	0.87 [0.48, 1.59]	Random effects	71	0.66
Recessive model	GG + GA vs. AA	1.30 [0.84, 2.00]	Random effects	52	0.24
Allele model	G vs. A	1.14 [0.95, 1.36]	Random effects	66	0.16

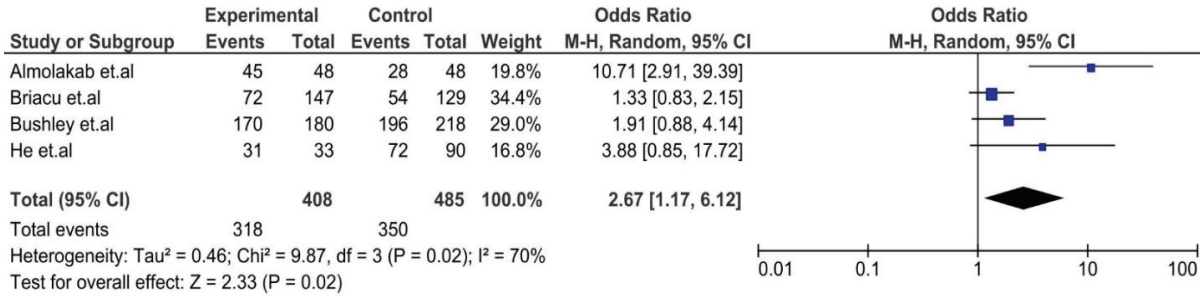

Figure 2. Forest plot of meta-analysis of the IL-10-819 C>T polymorphism (dominant model).

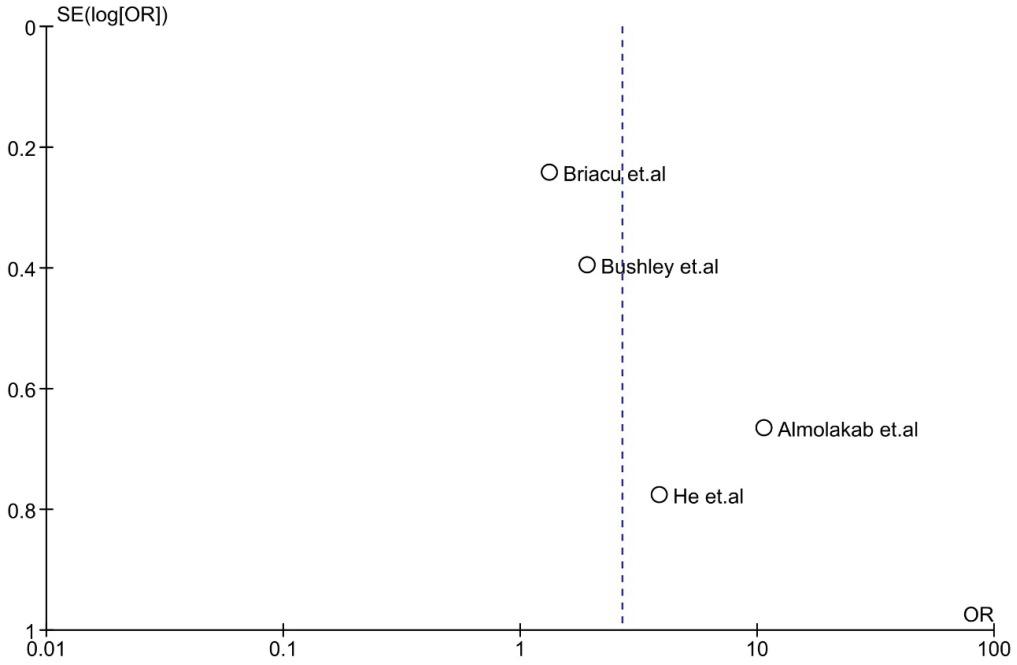

Figure 3. Funnel plot of meta-analysis of the IL-10–819 C>T polymorphism (dominant model).

0.1) in Egger's test further verified these results. Egger's regression test showed an intercept of 3.58 (95% CI:[-4.07,11.23]). Funnel plots of the meta-analysis of all other genetic models for both polymorphisms have been provided in the Supplementary material.

Discussion and conclusions

The results of the present systematic review indicate that the IL-10–819 C>T gene polymorphism is directly related to ovarian cancer. A previous network meta-analysis by Hu et al., screening different genetic markers, did not find IL-10 polymorphisms related to ovarian cancer [18]. Simultaneously, a 2015 meta-analysis investigating the IL-10–1082 G>A polymorphism with cancer did not obtain any significant results for ovarian cancer [19]. Nevertheless, one meta-analysis conducted in 2013, which assessed the relation of the IL-10–819 C>T polymorphism with cancer. However, it contained only three studies relating to ovarian cancer [20]. Thus, the present updated meta-analysis further verifies the latter result. It is worth mentioning that Il-10 is known to be an anti-inflammatory cytokine and thus can contribute to an increased risk of tumourigenesis and tumour aggressiveness [21]. Specifically, an increased expression of IL-10 induces a decreased expression of the pro-inflammatory cytokines IL-1a, IL-1b, IL-6, IL-12 and TNF-alpha and regulates the expression of the BCL-2 protein [21,22]. Interestingly, the IL-10–819 C>T polymor-

phism of the promoter region is known to correlate to higher gene expression, possibly explaining the results of the meta-analysis [13,23]. Overall, more studies must be conducted to verify our obtained results further and assess whether other polymorphisms of the IL-10 gene are related to the development of ovarian cancer.

Limitations

Even though the present meta-analysis was performed according to PRISMA guidelines and all means of assessment indicated a low risk of bias, it contains some substantial limitations. First, only a few studies were eligible for inclusion in the meta-analysis, decreasing the statistical power for calculating a pooled odds ratio [24]. Moreover, a moderate level of statistical heterogeneity was found, possibly due to the differences in the number of participants included in each study and the differences between the characteristics of the included participants [25]. Unfortunately, due to the lack of sufficient data on genotype distribution according to gender, age and environmental factors, a meta-analysis on subgroups based on these factors could not be performed in order to assess the latter assertation. It is also worth mentioning that most studies were performed in countries with Caucasian and Asian populations, indicating that the meta-analyses did not include a broad range of ethnicities. Finally, the literature search was limited to articles written in English; thus, articles in other languages may have been missed in this meta-analysis.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Pros and cons of continous glucose monitoring

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ABSTRACT

Introduction. Diabetes mellitus is a metabolic disorder that might result in short and long-term health complications and even death if not properly managed. This disease affected 451 million people in 2017 worldwide and these figures are expected to increase to 693 million by 2045. Currently, there is no cure for diabetes. However, self-management, especially keeping BG in the recommended range, is crucial to the treatment.

Aim. The aim of this paper is to offer an overview of current literature regarding CGM technologies. We outline mechanism of action, current use of CGM and discuss pros and cons of using this method in DM management.

Material and methods. A review of the literature available in PubMed and Google Scholar databases was conducted.

Results and conclusions. Blood glucose measurement using a glucometer is an invasive method, not very comfortable for the patient, it detects only one temporary blood glucose level. This method does not reflect glucose fluctuations and trends, which makes effective diabetes management difficult. Even supplementing

this method with HbA1c measurement does not bring as much relevant information for making therapeutic decision as CGM. The abundance of data provided by CGM and the ability to analyze them in greater detail, provide additional information to help achieve glycemic goals. It is a discreet and minimally invasive method, and the reading of blood glucose values can be easily read from mobile device. Data storage allows the doctor to view the past course of the disease and modify treatment. Manufacturers are constantly improving their devices, eliminating flaws, and the benefits of CGM improve treatment outcomes, which should translate into a reduction in the long-term complications of diabetes. Further research is needed, leading to the development of CGM technology.

Introduction

Diabetes mellitus (DM) is a metabolic disorder that causes abnormal blood glucose (BG) regulation that might result in short and long-term health complications and even death if not properly managed [1]. This disease affected 451 million people in 2017 worldwide and these figures are expected to increase to 693 million by 2045 [2]. Currently, there is no cure for diabetes. However, self-management of the disease, especially keeping BG in the recommended range, is crucial to the treatment [1].

Currently, patients with diabetes may choose between two major types of system for glucose measurement: blood glucose monitoring (BGM) systems measuring glucose within capillary blood and continuous glucose monitoring (CGM) systems measuring glucose within interstitial fluid. Although BGM and CGM systems offer different functionality, both types of system are intended to help users achieve improved glucose control [3]. Moreover, patients with diabetes may use HbA1C to trace the mean blood glucose in the past 2–3 mo.

Fingerstick blood glucose can detect only one instant blood glucose; therefore, it does not represent long term day-to-week blood glucose levels. Although the HbA1c level represents the mean blood glucose in the past 3 mo, it does not reflect the fluctuations of blood glucose. To solve these shortcomings, a continuous glucose monitor is a device developed to monitor interstitial glucose levels by a mini-invasive subcutaneous sensor [4]. In this paper we focus on CGM systems. We outline mechanism of action, current use of CGM and discuss pros and cons of using this method in DM management.

CGM description

The CGM system is mainly comprised of 3 components: a) biosensor, b) transmitter and c) monitor. The biosensor is a tiny cannula inserted into the subcutaneous fatty tissue and continuously measures glucose concentration in the interstitial fluid. The glucose sensor is based on a glucose oxidase (GOD). The biosensor must be changed every 7–14 d, and some biosensors can be used for a maximum of 180 d (Eversense). The transmitter is a small, reusable device that is connected to the biosensor to send the measured data of interstitial glucose levels wirelessly. Finally, the monitor receives the wireless real-time interstitial glucose signal. The monitor function can be performed by a special mobile device, smartphone using an dedicated application, and some systems allow data to be sent directly to the insulin pump. That provides easy mobile acces to real-time glucose levels and provide feedback with many smart features, such as arrows depicting the current glucose trends and smart alarms for impeding hypo- hyperglycemic events, improving patient self-management [5]. The smartphone can also send glucose readings to the cloud, and the medical staff can access them. The large amount of data of glucose levels can be analyzed to produce an output that combines the glucose readings and suggested medications, diet and exercise amount through the cloud system [5,6]. Currently, two different types of CGM systems are available on the market: real-time continuous glucose monitoring (rtCGM) systems and intermittently scanned continuous glucose monitoring (isc-CGM), flash glucose monitoring [FGM]) systems. rtCGM systems measure the glucose values and automatically display, every 5 min, a recent value.

In contrast, the sensor of iscCGM systems measures glucose levels every minute and stores one value every 15 min. iscCGM systems need to be actively scanned to obtain glucose information and to show it on the device display. The scans have to be performed at least every 8 h to retain the whole daily glycemic data [7]. Scanned glucose values of iscCGM systems can be either downloaded to a personal computer or uploaded to a cloud-based system [8,9].

Mechanism of action

Glucose concentration is estimated based on the production of hydrogen peroxide by GOD and the associated release of electric current, which is directly proportional to the concentration of glucose in the interstitial fluid. In detail, GOD and its cofactor, which works as the initial electron acceptor, catalyze the oxidation of glucose to hydrogen peroxide (H_2O_2) and gluconic acid, whereas the cofactor is reduced: glucose + GOD – $cofactor_{(oxidized)}$ → gluconic acid + GOD – $cofactor_{(reduced)}$. The cofactor is regenerated in a reaction with oxygen (O_2), which leads to the formation of H_2O_2:GOD – $cofactor_{(reduced)}$ + O_2 → GOD – $cofactor_{(oxidized)}$ + H_2O_2. H_2O_2 is oxidized at a catalytic electrode where the amount of transferred electrons is detected: $H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$. This electron flow is proportional to the glucose concentration in the interstitial fluid [8].

Accuracy and precision

High-quality performance of medical devices for glucose monitoring is important for a safe and efficient usage of this diagnostic option by patients with diabetes. In the literature, BGM system accuracy is assessed mainly according to ISO15197:2013 accuracy requirements, nor requirements to determine and compare the accuracy of CGM systems reproducibly [8]. CGM accuracy has hitherto mainly been assessed by MARD. The mean absolute relative difference (MARD) parameter is used most often to characterize the measurement performance of CGM systems. Many patients with diabetes routinely use CGM systems as the diagnostic cornerstone of their diabetes treatment and they make insulin dosing decisions based on the determined readings. It's important to note that MARD is just one of several metrics used to evaluate CGM systems performance. Other metrics, such as mean absolute difference (MAD), time-in-range (TIR), and continuous glucose error grid analysis (CG-EGA), provide complementary information and a more comprehensive understanding of device performance. In this paper we focus only on MARD and we briefly describeTIR [10].

MARD is calculated by averaging the absolute values of relative differences between CGM/BGM system measurement results and corresponding comparison method results. In this case, "absolute" means each individual relative difference value is considered a positive value, irrespective of whether the calculated difference with respect to the comparison result is positive or negative. Reported as a percentage, MARD is the average of the absolute difference between these values. The less the MARD is, the closer are the CGM readings to the comparison values. Current CGM systems reach MARD values in the range of approximately 8%–12%. Using CGM for insulin dosing decisions is feasible below a certain level of sensor error, estimated at MARD = 10%. Further accuracy improvement did not contribute substantively to better glycemic outcomes [10].

Accuracy and precision have improved dramatically [7,11,12]. For a wide range of glucose values, CGM data are accurate enough to use for self-adjustment of insulin dosage, detection of hypoglycemia, and evaluating response to therapy. Accuracy is strongly dependent on the glucose level [13], rate of change of glucose and number of other factors [9]. MARD should be considered alongside other metrics and clinical outcomes to gain a comprehensive understanding of a device's performance [3,8,14].

The systems currently available on the market offer a MARD of 8%–12%, examples:

- Dexcom G6: The Dexcom G6 CGM system has been reported to have a MARD value of around 9%–10%.
- Medtronic Guardian Sensor 3: The Medtronic Guardian Sensor 3 CGM system has reported MARD values in the range of 8%–12%.
- Abbott FreeStyle Libre: The FreeStyle Libre CGM system by Abbott has reported MARD values ranging from approximately 9%–11%.
- Eversense CGM system had reported MARD values in the range of 9%–12%.

Pros

Approval for non-adjuvant use

There has been steady improvement in the accuracy of glucose sensors (±10% MARD), which has led to greater acceptance by patients and physicians and has enabled users of CGM to reduce the number of measurements of capillary blood glucose (CBG). It is proved that a 10% MARD should be sufficient to permit self-adjustment of insulin dosage without the need for a confirmatory CBG. Thus, CGM is ready for non-adjuvant use—no longer just an adjuvant to self-monitoring of blood glucose (SMBG) [10,15,16]. The ability of CGM devices to accurately collect and document glucose levels is accepted by the clinical community [17,18]. Several CGM devices are authorized by regulators to replace SMBG testing for diabetes treatment decisions, which is the so-called non-adjunctive use of these devices. In addition, a specific category of FDA class 2 device type, known as an integrated CGM (iCGM) device [23], is used by the FDA to refer to CGM devices that are suitable for use with digitally connected medical devices, including automated insulin delivery systems [19,20].

Better insight into the disease and smart features

Use of CGM continues to expand in clinical practice. As a component of diabetes self-management, daily use of CGM provides the ability to obtain immediate feedback on current glucose levels as well as direction and rate of change in glucose levels [21]. Smart features such as alert, alarms and trend arrows which warn of impending or occurring hypo- or hyperglycemia events. As a result, rapidly increasing or decreasing glucose levels can be noticed and subsequently counteracted. Through the early perception of changing glucose levels, the probability of nocturnal hypoglycemic events [22], as well as missed bolus insulin injections for meals, can be reduced. Nonetheless, excessive occurrence of alarms can also lead to reduced compliance in patients ("alarm fatigue") [23]. Also trend arrows in CGM systems serve as an early warning for impending hypoglycemia and hyperglycemia events. Downward trend arrows appear when glucose level is falling, whereas upward arrows appear when it is rising. Consequently, the trend arrows may indicate the need to ingest carbohydrates or for correcting insulin dose. CGM provides a much larger number of glucose readings than occasional SMBG, whereby a comprehensive picture of daily glucose course is obtained. Up to 288 glucose measurement results every day (within a 5-min interval) make the use of easy understandable and standardized data readouts and graphical presentations necessary. Retrospective CGM data enable patients to enhance their glycemic management by adjustment of their therapy and behavior with the help of their clinicians under consideration of supplementary disclosures, such as insulin dosing and carbohydrate intake. These data, for example, can enable insights into the patterns of hypo- and hyperglycemia events that occur over time and lead to a change in their therapy to avoid such events in the future [24]. This information allows people with diabetes to optimize dietary intake (e.g. adjustments in pre-bedtime snacks to reduce nocturnal hypoglycemia) and exercise, make informed therapy decisions regarding mealtime and correction of insulin dosing, and, importantly, react immediately and appropriately to mitigate or prevent acute glycemic events [25–27].

Sleep Quality

Many factors contribute to insufficient sleep duration and poor sleep quality in people with T1D. Nocturnal diabetes management tasks, such as glucose testing and insulin administration, may be necessary for routine diabetes care. In addition, hypoglycemia, rapid changes in glucose levels, and fear of hypoglycemia can delay sleep onset and cause frequent night awakenings.

CGM use has been associated with improved subjective sleep quality, especially for parents of children with T1D and spouses/partners of people with T1D [28,29]. Sharing of real-time glucose data, has transformed T1D care for many people. This is especially true in the pediatric population because parents can view their child's glucose levels at all times including overnight, without disrupting the child's sleep. In addition to improved sleep, fear of hypoglycemia, health-related quality of life, stress, and anxiety, have been shown to be better among parents of children using a CGM [30].

However, CGM devices can also disrupt sleep due to alarms and increased anxiety which can lead to nocturnal awakenings [31,32]. Fre-

quent alarms, whether nocturnal or throughout the day, often lead people to discontinue use of CGM, an experience known as "alarm fatigue" [32]. Although many parents have benefitted from their child's use of CGM, some parents of young children with T1D may continuously monitor their child's CGM glucose level due to fear of hypoglycemia. This can result in parents having greater sleep disturbances than the child, whose sleep disruptions are decreased due to fewer finger stick glucose tests during the night [33].

Integrating the CGM system with an insulin pump and an internal algorithm allows to create a hybrid closed-loop system that automatically adjusts insulin delivery based on glucose values and trends from the CGM sensor. HCL system studies highlighted significant improvement in nocturnal glucose levels [34–37].

A number of controlled clinical trials have evaluated sleep outcomes in patients using HCL systems. Although objectively assessed sleep (e.g., actigraphy) has shown neither improved or impaired sleep with the use of HCL systems compared to sensor augmented pumps or the previously used diabetes regimen [38–42], multiple studies have found improvements in subjective sleep quality [38,40,43–45], likely related to trust in the system to manage blood glucose levels and decreased fear of hypoglycemia [46]. In a qualitative study, participants reported that overnight increase in time spent in range (between 70 mg/dL and 180 mg/dL) and improved sleep quality led to reported improvements in daily functioning (improved energy level, feeling better) and overall glucose regulation [47].

Overwhelmingly, patients and families report improvements in diabetes glycemic outcomes with device use; however, there remain concerns about how devices impact sleep, with CGM alarms as a common reason for nocturnal disruptions. Use of a device often requires weighing the benefits versus the burden, which can vary greatly from person to person [48].

Decreased HbA1c with lower risk of hypoglycemia

HbA1c is currently recognized as the key surrogate marker for the development of long-term diabetes complications in people with type 1 and type 2 diabetes and has been used as the primary end point for many CGM studies [49,50]. While HbA1c reflects average glucose over the last 2–3 months, its limitation is the lack of information about acute glycemic excursions and the acute complications of hypo- and hyperglycemia. HbA1c also fails to identify the magnitude and frequency of intra- and inter-day glucose variation [51,52]. Despite some limitations, HbA1c is the only prospectively evaluated tool for assessing the risk for diabetes complications, and its importance in clinical decision making should not be undervalued. Rather, the utility of A1C is further enhanced when used as a complement to glycemic data measured by CGM [21].

In randomized, controlled trial, was observed that the benefit associated with continuous glucose monitoring was strongly related to age. In patients 25 years of age or older, substantially tighter glycemic control was evident in the continuous-monitoring group in both glycated hemoglobin levels and sensor glucose results. More patients in the continuous-monitoring group than in the control group had a glycated hemoglobin level of less than 7.0% without having a severe hypoglycemic event. The results of this study indicate that continuous glucose monitoring improves glycated hemoglobin levels and may enhance the management of type 1 diabetes in adults who have the motivation to use this technology and the capability to incorporate it into their own daily diabetes management [53].

Another randomized trial among adolescents and young adults with type 1 diabetes showed a small but statistically significant lowering of HbA1c over 26 weeks of CGM use compared with standard BGM. This finding offers potential for clinical importance with a meaningful shift in the HbA1c distribution toward improved glycemic control; however, further research of longer duration and with clinical outcomes is needed before reaching definitive conclusions about the clinical value of the study's findings [54].

In another systematic review and meta-analysis of RCTs comparing CGM with conventional therapy, use of CGM led to a modest 0.17% reduction in HbA1c, with a 70.74 min increase of time spent in the target range. Moreover, CGM provided additional benefits in glycemic control, including the significant reduction of TBR, TAR, and CV, thus suggesting an improvement of glucose variability compared with usual care. Such a result may appear insufficient for the great majority of

people with diabetes; on the other hand, it may also reflect a more intense effect in reducing hypoglycemia, thus expressing the effort in ameliorating glucose control while reducing glucose variability [55].

TIR

In clinical practice, time in rangeis both appropriate and useful as clinical targets and outcome measurements that complement HbA1C for a wide range of people with diabetes and that the target values should be considered an integral component of CGM data analysis and day-to-day treatment decision making. To streamline data interpretation, the ATTD [56] consensus panel identified "time in range" as a metric of glycemic control that provides more actionable information than HbA1C alone. The metric includes three key CGM measurements: percentage of readings and time per day within target glucose range (TIR), time below target glucose range (TBR), and time above target glucose range (TAR). The primary goal for effective and safe glucose control is to increase the TIR while reducing the TBR. The consensus group agreed that expressing time in the various ranges can be done as the percentage (%) of CGM readings, average hours and minutes spent in each range per day, or both, depending on the circumstances [21].

Cons

Cost

Continuous glucose monitoring devices are costly, with inconsistent reimbursement across government bodies. Many countries, including Australia and America, offer reimbursement for people with type 1 diabetes mellitus, with limited subsidization for people with type 2 diabetes mellitus. Germany reimburses real-time CGM for all types of diabetes, whereas Spain offers no reimbursement at all. Most CGM systems require sensor changes every 6–14 days, which generates significant costs [57]. CGM appears to be a cost-effective intervention for individuals with type 1 diabetes. Key drivers of CGM cost-effectiveness include reduction of chronic complications through improvement in glycemic management, and reduction in frequency and duration of hypoglycemic episodes [58]. These studies also highlight the rapidly evolving nature of CGM which has driven down usage costs and may continue to do so with further advances [59]. One also needs to consider costs to society for failure to implement CGM, including costs of emergency management of severe hypoglycemic episodes (emergency room visits, hospitalizations, mortality, and morbidity), the costs of failure to achieve the optimal level of glycemic control in terms of quality of life, and long-term complications [9].

Lag time of interstitial fluid glucose relative to blood glucose

There is a delay as glucose is transported from blood to interstitial fluid. This delay could be appreciable in early forms of CGM (e.g., 15 min). Largely because of improvements in algorithms for computing glucose from the raw electrical signal from the sensor, this problem has been dramatically reduced to only a few minutes for several systems [9]. The new glucose algorithm reduces the time lag for FreeSyle Libre System to about two minutes (2.4 minutes for adults and 2.1 minutes for pediatric population) compared to the previous-generation the product (4.5 minutes, in a study without glucose manipulation) [60,61] the drop in CGM lags behind the drop in blood glucose during prolonged aerobic exercise by 12 ± 11 min, and MARD increases to 13 (6–22)% during exercise as well. Therefore, if hypoglycemia is suspected during exercise, individuals should confirm glucose levels with a capillary glucose measurement [62].

Calibration

The improvement in accuracy of CGM sensors has been accompanied by a reduced need for frequent calibration (Eversense – one per day [63]) or any calibration (Abbott FreeStyle Libre, Dexcom G6, Medtronic Guardian Sensor 4) by the user [61,64,65].

Sensor lifetime

Sensor lifetime is another factor that contributes to cost, inconvenience, and slow user acceptance. Even the durability of the adhesive used for attachment of the sensor to the skin is a matter of concern. One can expect that user acceptance will continue to improve as sensor lifetime increases and ease of sensor insertion improves [9]. Companies are trying to meet customer expectations,

and currently available sensors on the market offer operating times from 7 (Medtronic Guardian 3) to even 180 days (Eversense) [5].

Poor adhesion, sweating and skin irritation

When we talk about medical devices for diabetes treatment, the focus is usually on scientific aspects and clinical efficacy. Safety issues are largely discussed in terms of hypoglycemic events, devices failures, and so on. However, in practice other aspects, like rashes, itching, site reactions, pulling off, falling off, sweating off, losing a transmitter or receiver and so on are often of concern for patients and diabetologists. One area that does not get much attention involves the adhesives used to attach devices to the human skin. There is a trend for the extension of glucose sensor wearing time of continuous glucose monitoring systems (CGM). Longer wearing time means less injuries of the skin, less hassle for sensor change and lower sensor costs per day. However, longer wearing time of glucose sensors or insulin infusion sets means also higher challenges for the adhesive material used. The consequence of longer usage time might be that we see in more patients allergic skin reactions (contact dermatitis) [66]. This is especially significant for individuals with skin sensitivities, pediatric patients, and those who use devices chronically. Dermatological complications are often cited as a barrier to device use and a reason for device discontinuation. Furthermore, it is a frequent topic of discussion in diabetes follow-up visits, although little evidence-based literature exists to guide providers in managing skin integrity issues [67].

Conclusions

CGM has emerged as a valuable tool to assess the effectiveness and safety of treatment in many patients with type 1 diabetes and in selected patients with type 2 diabetes treated with intensive insulin regimens [68]. Applying these technologies to diabetes management results in immediate information regarding glucose levels to the user, as well as glucose trend, its current direction, and rate of change, leading to an increased time in the target glucose range by reducing hyperglycemia and minimizing the occurrence of hypoglycemia [69,70]. In previous meta-analyses of randomized controlled trials conducted in patients with both type 1 [71–73] and type 2 diabetes [74,75], the use of CGM provided a reduction in HbA1c of ~0.3%, with less hypoglycemia [71,72], compared with usual care. The large quantity of glucose readings collected by CGM allows users to obtain a more complete profile of the glycemic status over the entire day, including the time spent in the target ranges and the time spent in hypo- and hyperglycemia, as well as measures of glucose variability, adding some useful information for assessment of the current glycemic profile in addition to what is provided by the HbA1c [56,76]. A recent international consensus on the use of CGM highlighted the importance of assessing and reporting the percentages of TIR, TBR and TAR in conjunction with measures of glucose variability as key metrics for the evaluation of glucose control in clinical studies [55,56]. The pace of development in diabetes technology is extremely rapid. New approaches and tools are available each year. It is hard for research to keep up with these advances because by the time a study is completed, newer versions of the devices are already on the market. The most important component in all of these systems is the patient. Technology selection must be appropriate for the individual. Simply having a device or application does not change outcomes unless the human being engages with it to create positive health benefits. This underscores the need for the health care provider to assist the patient in device/program selection and to support its use through ongoing education and training. Expectations must be tempered by reality—we do not yet have technology without flaws that completely eliminates the self-care tasks necessary for treating diabetes, but the tools described in this paper can make it easier to manage [77].

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

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Plants: past and present in the battle against diabetes

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ABSTRACT

From ancient times, when medicine was based on folk knowledge, to the present era of advanced science, the beneficial effects of plants on various diseases, including diabetes, have been discovered. Approximately 537 million people worldwide have diabetes, and forecasts indicate further increases. Hence, there is a need to develop new effective therapies and interventions to support diabetes treatment. Many plants impact carbohydrate metabolism, and the amount of in vitro and in vivo research on animals and humans continues to grow, updating our knowledge about their potential applications in diabetes treatment and its complications. This review discusses six plant sources with proven anti-diabetic activity. The study serves as a literature review on plants and their derived compounds that exhibit hypoglycemic effects, which are significant in managing prediabetic conditions and diagnosed diabetes.

Introduction

From ancient times, when medicine relied on folk knowledge, to the present era of advanced science, numerous plants have been studied for their beneficial impact on diabetes [1]. Both in the past and today, plants play a significant role in regulating blood sugar levels. The International Diabetes Federation reports that approximately 537 million people worldwide are living with diabetes, and it is projected that by 2045, approximately 783 million individuals will be living with diabetes [2]. Hence, there is a need for new effective therapies in the fight against diabetes and supportive interventions. Plant compounds often take the forefront in this battle, not only against diabetes itself but also against prediabetic conditions. They aim to support the patient as an adjunct to a proper diet and physical activity, serving as the first step in combating unhealthy habits that lead to later disease. We no longer rely on folklore and traditions regarding the use of herbs in specific disease entities. Contemporary scientific research focuses on identifying active plant constituents and investigating their mechanisms of action and potential applications in treating diabetes and blood sugar regulation. Some active ingredients from the plants described in this

study have been isolated and utilized to produce dietary supplements or antidiabetic medications. This review aims to systematize the current knowledge regarding selected plants and the compounds derived from them that demonstrate hypoglycemic effects. Approaching the mechanisms of action of medicinal plants in diabetes and supporting it with clinical evidence may help specialists to implement new procedures in standard diabetes therapy.

Plants with hypoglycemic properties

In this review of the latest scientific literature, we focused on the discoveries of six commonly used medicinal plants: *Aloe vera* Linnaeus, *Cinnamomumverum* J. Presl, *Momordicacharantia* Linnaeus, *Morus alba* Linnaeus, *Trigonellafoenum-graecum* Linnaeus, and *Zingiberofficinale* Roscoe. The scientific studies discussed in this work focus on identifying active plant compounds, their mechanisms of action, and potential applications in treating diabetes and blood sugar regulation. Many active plant components have been isolated and used in appropriate concentrations to produce dietary supplements and anti-diabetic medications. The discussed plant products enhance their health-promoting effects when appropriately processed and combined with other plants. Additionally, other health benefits of these mentioned plants have been highlighted. All the information has been presented clearly in **Tables 1**, **2** and **Figure 1**.

Aloe vera (L) Burm. f. (syn *Aloe barbadensis Mill*), *Asphodelaceae*

Aloe verais widely distributed in hot and arid regions of North Africa, the Middle East, Asia, the southern Mediterranean, and the Canary Islands [3]. The use of *Aloe vera* dates back to ancient times when it has been utilized for generations as a medicinal and cosmetic remedy. *Aloe vera* is renowned for its application in treating various skin issues, such as burns and wounds [4].

Table 1. Phytochemicals – mechanisms of action influencing glucose metabolism of medicinal plants.

Medicinal Plants	Phytochemicals with potential action in diabetes	Potential mechanism of action in diabetes
Aloe vera	Polysaccharides Chromium Biotin	– improving glucose transport – improving the morphology and functioning of pancreatic islets [5] – reducing the toxic effect of fat on the liver [6] – improving insulin sensitivity [7]
Cinnamomum verum	Cinnamaldehyde Cinnamicacid Cinnamateesters Polyphenols	– improving insulin sensitivity [15] – increased GLUT4 translocation [16] – inhibits the production of glucose in the liver [18] – inhibition of alpha-amylase and alpha-glucosidase [19]
Momordica charantia	Charantin Polypeptide-p Lectins Momordicosides	– increased glucose uptake [28] – gluconeogenesis inhibition [29] – inhibition of alpha-amylase and alpha-glucosidase [30] – improving insulin sensitivity [29]
Morus alba	Mulberry leaf alkaloids Flavonoids 1-Deoxynojirimycin (DNJ) Chlorogenicacid	– inhibition of alpha-amylase [42,43] – improving insulin sensitivity [45] – promoting insulin secretion [46] – antioxidant properties [46–47] – protection of the liver and pancreas [46–47]
Trigonella foenum-graecum	Fenugreek saponins Fenugreek fiber Trigonelline 4-Hydroxyisoleucine	– improving the morphology and functioning of pancreatic islets [56] – improving insulin sensitivity [57] – inhibits the production of glucose in the liver [57] – stimulates the insulin signaling cascade [59] – antioxidant properties [61]
Zingiber officinale	Gingerols Shogaols Zingerone Zerumbone	– increase in the activity of glycolytic enzymes [71] – antioxidant properties [72] – increasing the expression of the glucose transporter (GLUT-4) [73]

Abbreviations: GlUT-4 – Glucose Transporter 4

Table 2. Medical plants and their impact on glucose metabolism.

Medicinal plants	Authors	Features of the study	Dose	Time of taking the drug	Number of study participants	Intervention group	Control group	Results
Aloe vera	Choi et al. [10]	A randomized controlled trial	Processed aloe vera gel 147 mg/cap and aloesin powder 3 mg/cap	8 weeks	136	Allocated to experimental group: 68 Lost to follow-up: 8 Analysed: 60	Allocated to placebo group: 68 Lost to follow-up: 6 Analysed: 62	Decrease of: body weight, BFM, Insulin resistance
	Huseini et al. [11]	A randomized, double-blinded, and placebo-controlled trial	2x300 mg	2 months	60	30	30	Decrease of: FPG, HbA1c, TC, LDL-C
	Alinejad-Mofrad et al. [12]	A randomized, double-blinded, and placebo-controlled trial	n1 – 2x300 mg n2 – 2x500 mg	8 weeks	72	n1–24 n2–24	n3–24	Decrease of: FPG
Cinnamomum verum	Zare R et al. [20]	A triple-blind placebo-controlled randomized clinical trial	2x500 mg	3 months	140	BMI < 27 (n = 33) BMI ≥ 27 (n = 37, loss of 1 participant in the intervention group – leaving 36 in the intervention group)	BMI < 27 (n = 33, loss of 1 participant in the control group – leaving 32 in the control group) BMI ≥ 27 (n = 37)	Decrease of: BMI, adipose tissue, visceral fat, FPG, 2hpp, HbA1C, insulin resistance, TC, LDL-C, HDL-C
	Akilen et al. [21]	A randomized, double-blinded, and placebo-controlled trial	2 g	12 weeks	58	30	28	Decrease of: HbA1c, SBP, DBP
	Neto et al. [22]	A triple-blind placebo-controlled randomized clinical trial	3 g/day	90 days	160	Allocated to experimental group: 80 Lost to follow-up: 9 Analysed: 71	Allocated to placebo group: 80 Lost to follow-up: 11 Analysed: 69	Decrease of: HbA1C, FPG
Momordica charantia	Cortez-Navarrete et al. [34]	A randomized, double-blinded, and placebo-controlled trial	2000 mg/day	3 months	24	Allocated to experimental group: 12 Lost to follow-up: 2 Analysed: 10	Allocated to placebo group: 12 Lost to follow-up: 2 Analysed: 10	Decrease of: weight, BMI, fat percentage, WC, HbA1C, 2-h glucose in OGTT, AUC of glucose Increase of: AUC of insulin, total insulin secretion, first phase of insulin secretion
	Kim et al. [35]	A randomized, double-blinded, and placebo-controlled trial	2380 mg/day	12 weeks	96	Allocated to experimental group: 66 Lost to follow-up: 4 Analysed: 62	Allocated to placebo group: 30 Lost to follow-up: 2 Analysed: 28	Decrease of: FPG
Morus alba	Qu et al. [50]	A Multicenter, Randomized, Double-Blind, Double-Dummy, and Parallel Controlled Clinical Trial	???	24 weeks	600	360	240	Decrease of: HbA1c
	Thaipitakwong et al. [51]	A randomized controlled clinical study	12 mg/day	12 weeks	59	Allocated to experimental group: 29 Lost to follow-up: 1 Analysed: 28	Allocated to placebo group: 30 Lost to follow-up: 4 Analysed: 26	Decrease of: FPG, HbA1c, insulin resistance
Trigonella foenum-graecum	Gupta et al. [62]	A randomized, double-blinded, and placebo-controlled trial	1 gm/day hydroalcoholic extract of fenugreek seeds	2 months	25	12	13	Decrease of: (AUC) of blood glucose, TG Incrase of: insulin sensitivity, HDL-C
	Verma et al. [63]	A multicenter, randomized, placebo-controlled, double-blind, add-on clinical study	2x500 mg (Fenfuro)	90 days	154	???	???	Decrease of: FSG, FPG, HbA1c Increase of: fasting and post-prandia, l C-peptide levels
	Hadi et al.[64]	A parallel randomized clinical trial	3x5 g fenugreek seed powder	8 weeks	50	Allocated to experimental groups: 25 Lost to follow-up: 1 Analysed: 24	Allocated to placebo group: 25 Lost to follow-up: 1 Analysed: 24	Decrease of: FPG, SBP Increase of: some liver and kidney function
Zingiber officinale	Carvalho et.al. [74]	A randomized, double-blinded, and placebo-controlled trial	1,2 g	90 days	144	Allocated to experimental groups: 72 Lost to follow-up: 24 Analysed: 47	Allocated to placebo group: 72 Lost to follow-up: 15 Analysed: 56	Decrease of: FBS, TC, LDL
	El Gayar et al. [75]	A randomized, single blind, placebo-controlled clinical trial	3x600 mg	8 weeks	80	40	40	Decrease of: BMI, HbA1c, FBG, FSI, TC, LDL-C
	Mahluji et al. [76]	A randomized, double-blinded, and placebo-controlled trial	2 g	2 months	64	Allocated to experimental groups: 32 Lost to follow-up: 4 Analysed: 28	Allocated to placebo group: 32 Lost to follow-up: 2 Analysed: 30	Decrease of: LDL-C, TG, HOMA Increase of: QUICKI
	Arablou et al. [78]	A randomized, double-blinded, and placebo-controlled trial	2x800 mg	12 weeks	70	Allocated to experimental groups: 35 Lost to follow-up: 2 Analysed: 33	Allocated to placebo group: 35 Lost to follow-up: 5 Analysed: 30	Decrease of: FPG, HbA1C, insulin, HOMA, TG, TC, CRP, PGE
	Rostamkhani et al. [80]	A randomized, double-blinded, and placebo-controlled trial	2 g	8 weeks	44	Allocated to experimental groups: 22 Lost to follow-up: 2 Analysed: 20	Allocated to placebo group: 22 Lost to follow-up: 1 Analysed: 21	Decrease of: FBG, HOMA-IR, urea
	Mozaffari-Khosravi et al. [79]	A randomized, double-blinded, and placebo-controlled trial	3x1 g	8 weeks	88	Allocated to experimental groups: 44 Lost to follow-up: 4 Analysed: 40	Allocated to placebo group: 44 Lost to follow-up: 3 Analysed: 41	Decrease of: FBS, HbA1c Increase of: QUICKI

Abbreviations: BFM – body fat mass; FPG – fasting plasma glucose; HbA1c – Glycated Hemoglobin; TC – triglicerydes; LDL-C – low density lipoprotein cholesterol; BMI – Body Mass Index; 2hpp – Two hours postprandial glucose; HDL-C – high density lipoprotein cholesterol; SBP – systolic blood pressure; DBP – diastolic blood pressure; WC – Waist Circumference; AUC – Area Under the Curve; TG – triglycerides; FSG-fasting serum glucose; FBS – fasting blood sugar; FSI – fasting serum insulin; HOMA-IR – homeostatic model assessment; QUICKI – Quantitative Insulin Sensitivity Check Index; PGE – Prostaglandin E2

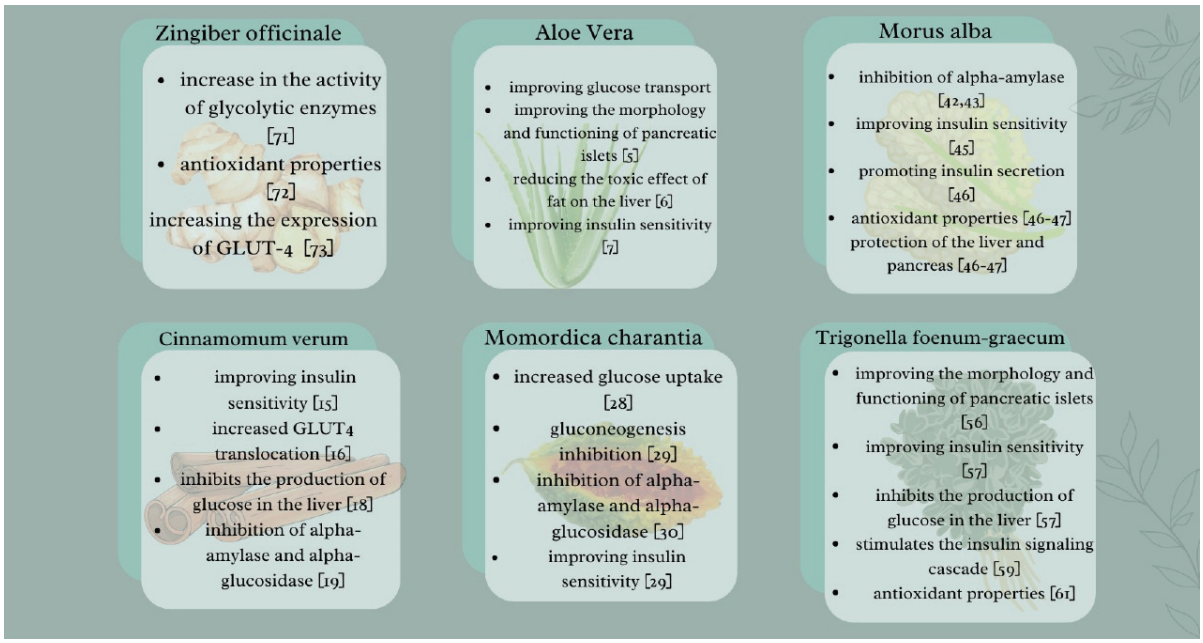

Figure 1. Plants – graphical summary of their impact on glucose metabolism.

Phytochemicals – Mechanisms of hypoglycemic action of Aloe vera

Among the compounds found in *Aloe vera*, notable ones include vitamins (such as vitamins A, C, E, and B12), enzymes (e.g., amylase, catalase, and peroxidase), minerals (e.g., zinc, copper, selenium, and calcium), sugars (including monosaccharides like mannose-6-phosphate and polysaccharides like glucomannans), anthraquinones (aloin and emodin), tritrpenes (f.ex. lupeol) and phytosterols (f.ex. campesterol), hormones (auxins and gibberellins), and others (like salicylic acid, lignin, and saponins) [4]. The influence of *Aloe vera* on glucose and lipid metabolism can be explained through several mechanisms. One involves the action of high-molecular-weight polysaccharides and phytosterols in the aloe gel (prepared from the leaves). These components can affect glucose transport by regulating the markers responsible for its uptake and lower cholesterol levels by reducing its absorption from the gastrointestinal tract. *Aloe vera leaf* extract has been shown to normalize fasting plasma glucose (FPG) and insulin levels in the serum of rats. Furthermore, *Aloe vera* supplementation contributed to the improvement of pancreatic islet morphology and function [5]. Another mechanism involves the reduction of the toxic effects of fat on the liver and the improvement of cellular insulin sensitivity [6–7]. Additionally, researchers suggest that *Aloe vera* may decrease adipose tissue mass and enhance insulin sensitivity by activating a muscle protein kinase known as AMP-activated protein kinase, which plays a crucial role in regulating glucose and lipid metabolism [8].

Clinical evidence of the hypoglycemic effects of Aloe vera

Aloe vera appears to possess anti-diabetic properties. Some studies have focused on *Aloe vera*, demonstrating its ability to reduce glucose and fructosamine levels [9]. A randomized controlled trial involving 136 obese patients with prediabetic conditions and early untreated diabetes confirmed that supplementation with Aloe QDM complex (comprising processed aloe gel at a dose of 147 mg/capsule and aloe powder at a dose of 3 mg/capsule) for eight weeks not only reduced insulin resistance but also body weight and adipose tissue mass [10]. A study conducted by Huseini et al. found that applying *Aloe vera* leaf gel twice daily at 300 mg for two months decreased fasting blood glucose levels, HbA1c, total cholesterol, and LDL-C [11]. Furthermore, *Aloe vera*can be used in the prevention of diabetes. Consumption of pure powdered of *Aloe vera extract* (300 mg twice daily for four weeks) reduced fasting blood glucose levels in individuals with prediabetic conditions [12].

Cinnamomum verum J. Presl (syn C. zeylanicum Blume), Lauraceae

Cinnamon is a spice derived from the bark of trees belonging to the Cinnamomum genus. The most common species are *Cinnamomumverum* (Ceylon cinnamon) and *Cinnamomum cassia* Siebold (also known as Chinese cinnamon or cassia). Known initially primarily in Southeast Asia, the Portuguese introduced cinnamon to Europe from Sri Lanka in the early 16th century. It has been used for its health-promoting properties as a traditional remedy [13].

Phytochemicals – Mechanisms of hypoglycemic action of Cinnamomum verum

The hypoglycemic properties of cinnamon are primarily attributed to proanthocyanidins (epicatechin polymers), cinnamaldehyde, and cinnamic acid contained in the leaves and the bark [14]. Cinnamon extract regulates genes associated with insulin sensitivity, inflammation, and cholesterol metabolism/lipogenesis [15]. In a study on streptozotocin-induced diabetic rats, cinnamon extract exhibited antidiabetic effects independent of insulin. Cinnamon (aqueous cinnamon extract) upregulated mitochondrial UCP-1 and increased GLUT4 translocation in muscle and adipose tissue [16]. Cinnamaldehyde is responsible for the effect on GLUT4 [17]. Cinnamon extract (aqueous cinnamon extract and cinnamon polyphenol-enriched defatted soy flour) inhibits glucose production in the liver and reduces the expression of key regulators of gluconeogenesis in the liver, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase [18]. Additionally, cinnamon affects the absorption of carbohydrates by inhibiting the enzymes alpha-amylase and alpha-glucosidase. [19].

Clinical evidence of the hypoglycemic effects of Cinnamomum verum

In a clinical study conducted in 2019, 140 patients with type 2 diabetes were divided into four groups: cinnamon (BMI ≥ 27, BMI < 27) and placebo (BMI ≥ 27, BMI < 27). Supplementation with cinnamon (bark powder) at a dose of 500 mg twice daily for three months resulted in improvements in anthropometric parameters (BMI, adipose tissue, visceral fat), glycemic parameters (FPG, Two hours postprandial glucose -2hpp, HbA1c, fasting insulin, and insulin resistance), and lipid parameters (total cholesterol, LDL-c, and HDL-c) (except for triglyceride levels). All observed changes (except for total cholesterol and LDL-c) were significantly more pronounced in patients with higher baseline BMI (BMI ≥ 27) [20]. Another clinical study conducted by Akilen et al. with 58 patients taking 2 g of cinnamon (500 mg of bark powder four a day) or a placebo found that cinnamon supplementation resulted in a decrease in the average HbA1c values in the cinnamon group (8.22% to 7.86%) compared to the placebo group (8.55% to 8.68%). Additionally, systolic and diastolic blood pressure (HbA1c) also decreased significantly in the cinnamon group (SBP: 132.6 to 129.2 mmHg and DBP: 85.2 to 80.2 mmHg) compared to the placebo group (SBP: 134.5 to 134.9 mmHg and DBP: 86.8 to 86.1 mmHg) [21]. Beneficial effects in type 2 diabetes are also demonstrated by the results obtained by Neto et al., where after 90 days of consuming 3 g of cinnamon bark powder, patients had a statistically significant reduction in glycated hemoglobin by 0.2% and fasting venous glucose by 0.55 mmol/l compared to the placebo group [22]. However, no effect of cinnamon (3 g of cinnamon extract in wholes) intake on the reduction of NF-kB, sirtuin 1 (SIRT1), High-Sensivity C Reactive Protein (hs-CRP), IL-6, and TNF-α was found in patients with type 2 diabetes, which play a significant role in the development of atherosclerosis [23], nor on the adhesion molecules ICAM-1 and VCAM-1 [24].

Momordica charantia L., Cucurbitaceae

Momordica charantia, also known as bitter melon or bitter gourd, is commonly cultivated in warm regions of the world, where its immature fruits are used as a vegetable [25]. In addition to its culinary uses, *Momordica charantia* has a long history in traditional medicine [26]. It is used as a remedy for digestive problems, as a laxative, and as an anthelmintic. Most importantly, it is utilized to treat diabetes and its complications.

Phytochemicals – Mechanisms of hypoglycemic action of Momordica charantia

The mechanism of hypoglycemic action of *Momordicacharantia*is multifaceted. Among the active compounds, we can distinguish polysaccharides, peptides, proteins, lipids, terpenoids, saponins, and phenols [25]. Some of these com-

pounds have hypoglycemic potential. In a study by Hsiao et al., 15 cucurbitane-type triterpenoids from bitter melon fruits were investigated in C2C12 myoblasts. At a concentration of 10 μM, two tested compounds: 25-hydroxy-5β,19-epoxycucurbita-6,23-dien-19-on-3β-ol3-O-β-D-glucopyranoside and 7β,25-dihydroxycucurbita-5,23 (E) -dien-19 -al 3-O-β-D-allopyranoside, increased glucose uptake by 50% and over 100%, respectively. The latter compound was a positive control, even more effective than insulin [27]. In a study conducted on the FL83B liver cell line, evaluating the activity of three triterpenoids isolated from the stem at a dose of 5 μg/ml, all three compounds [(23E)-cucurbita-5,23,25-triene-3β,7β-diol, 3β,25-dihydroxy-7β-methoxycucurbita-5,23 (E) -diene and 3β,7β,25-trihydroxycucurbita-5,23 (E) -dien-19-al] increased glucose uptake compared to cells treated with insulin. The mechanism of action was associated with AMPK activation [28]. AMPK has been recognized as a potential target in treating metabolic diseases, including obesity and type 2 diabetes. It is a cellular energy sensor that promotes ATP-producing catabolic pathways, such as glucose uptake, and inhibits ATP-consuming processes. Chen et al. also demonstrated the impact of cucurbitane-type triterpenoids from the fruits of *Momordica charantia* on glucose production in H4IIE liver cells. At a concentration of 100 μM, four out of the tested triterpenoids inhibited gluconeogenesis by approximately 50% [29]. In a study by Pera et al., triterpenoids isolated from the *Momordica charantia* fruits, were examined for their inhibition of α-amylase and α-glucosidase enzymes at concentrations of 0.87 mM and 1.33 mM. The compounds showed similar α-amylase inhibition activity to acarbose (0.13 mM), a positive control (68.0–76.6%). In the α-glucosidase inhibition test, karavilose VIII (56.5%) was the most active compound, while the activity of other compounds ranged from 24% to 40% [30]. So far, the inhibitory effect of several compounds contained in *Momordica charantia* on glucose absorption through the inhibition of α-amylase and α-glucosidase has been proven [30,31]. The results of a study conducted by Lee et al. suggest the influence of *Momordica charantia* on the inhibition of PTPN2, an enzyme associated with insulin resistance. At a concentration of 20 μM, nine out of the twenty-seven tested compounds from the *Momordica charantia* fruits exhibited inhibitory activity ranging from 72% to 93% [32]. Additionally, saponins contained in fruits of *Momordica charantia* can improve the morphology and viability of pancreatic β-cells and increase insulin secretion concentration-dependent. This will likely occur through the PI3K/Akt/FoxO1 signaling pathway [33].

Clinical evidence of the hypoglycemic effects of Momordica charantia

Cortez-Navarrete et al. evaluated the effect of administering *M. charantia* on insulin secretion and sensitivity. The clinical study was conducted on 24 patients who received *M. charantia* (2000 mg fruit powder per day) or placebo for three months. The *M. charantia* group showed significant decreases in body weight, BMI, percentage of body fat, WC, A1C, fasting glucose, and glucose AUC. *M. charantia* administration increased the AUC of insulin, first-phase insulin secretion, and total insulin secretion [34]. In a study on 90 patients who took bitter melon extract (bitter melon powder in capsules) for 12 weeks, hypoglycemic effects were observed in patients with type 2 diabetes [35]. Furthermore, the extract from bitter melon acts synergistically with oral hypoglycemic drugs and enhances their effects on NIDDM [36]. However, the hypoglycemic effect of bitter melon is weaker, as evidenced by comparing the effects of a daily dose of 2000 mg with 1000 mg of metformin [37]. Additionally, a study comparing the effects of bitter melon and glibenclamide showed that the hypoglycemic effect of bitter melon is inferior to glibenclamide. However, bitter melon may be more effective in alleviating cardiovascular risk factors associated with diabetes [38]. Bitter melon reduces elevated fasting serum glucose levels in individuals with prediabetes. This is demonstrated by the results of a study in which the effect of consuming 2.5 g of powdered bitter melon was evaluated over eight weeks. The CROS analysis ($t = -2.23$, $p = 0.031$, $r = 0.326$) showed a significant difference in the change in FPG of 0.31 mmol/L (5.6 mg/dL) with a tendency ($R2 = 0.42387$). This indicates the potential use of *Momordica charantia* as an adjunctive therapy [39]. Additionally, changes in sialic acid were examined in patients with NIDDM after treatment with bitter melon (55 mL of juice per 24 h) and rosiglitazone (4 mg/24 h). In diabetes, there is an increase in serum sialic acid levels, which

is a strong predictor of cardiovascular mortality. Each experimental group consisted of a total of 25 patients of both genders. Patients treated with bitter melon maintained sialic acid levels comparable to healthy individuals, while rosiglitazone increased serum sialic acid levels [40].

Morus alba L., Moraceae

Morus alba, also known as white mulberry, is a fruit tree native to Asia with a long history of use in traditional medicine [41]. Its fruits, leaves, and roots have been utilized to treat various ailments. White mulberry contains diverse phytochemicals that contribute to its medicinal properties. Among them are flavonoids such as rutin, quercetin, isorhamnetin, phytosterols, and phenolic acids, including chlorogenic acid, caffeic acid, and protocatechuic acid. However, its anti-diabetic properties are attributed to moranolins, mulberrochromenes, and alkaloids [41].

Phytochemicals – Mechanisms of hypoglycemic action of Morus alba

Alkaloids from mulberry twigs (Sangzhi alkaloids [SZ-A]) consist mainly of 1-deoxynojirimycin (1-DNJ), phytomoleculefagomine (FA), 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), and other polyhydroxyalkaloids. In mulberry twigs, the 1-DNJ is the dominant alkaloid, accounting for 50% of the iminosugars. In a study conducted by Ye et al., the impact of water extract of Shangzhi (SZ) on rats and mice with standard and alloxan-induced diabetes was evaluated, and the results were compared with those of acarbose, an alpha-glucosidase inhibitor. It was shown that SZ-A reduced fasting and postprandial blood glucose levels and prolonged the peak glucose concentration, similar to acarbose, indicating an impact on alpha-glucosidase [42,43]. Unabsorbed phytochemicals from *Morus alba* compete with glucose for intestinal glucose transporters [43]. Studies indicate that DNJ, besides inhibiting alpha-glucosidase, alleviates hyperglycemia by improving insulin sensitivity and affecting the activation of the PI3K/AKT insulin signaling pathway in skeletal muscles [44]. Animal experiments have shown that SZ-A improves insulin resistance, increases basal insulin levels, and enhances glucose-stimulated insulin secretion [45]. Phytochemicals (e.g., quercetin 3-(6-malonylglucoside) present in white mulberry twigs exhibit antioxidant activity, improving the oxidative state of the body and protecting liver and pancreatic cells from damage [46,47]. Even a single intake of mulberry leaf extract (300 mg) with a meal reduces the digestion and absorption of carbohydrates [48]. In addition, a beneficial effect on the lipid profile in patients with T2DM should also be mentioned [49].

Clinical evidence of the hypoglycemic effects of Morus alba

Alkaloids from mulberry twigs (Sangzhi alkaloids [SZ-A]) demonstrate equivalent hypoglycemic effects to acarbose in patients with T2DM. In a 24-week study involving 600 patients, HbA1c decreased by 0.93% (10.2 mmol/mol), comparable to the result obtained with acarbose (50 mg three times daily). Furthermore, SZ-A administration resulted in a lower incidence of adverse effects and gastrointestinal disturbances [50]. Another study conducted by Ling et al. also recognized SZ-A as effective and safe in treating Type 2 Diabetes [51]. In a 12-week study by Thaipitakwong et al., it was determined that 12 mg of mulberry DNJ represented the minimum effective dose for alleviating postprandial hyperglycemia. Mulberry leaves reduced fasting plasma glucose (FPG) levels by 3.86 ± 5.99 mg/dL ($p = 0.002$) and glycated hemoglobin (HbA1c) by 0.11 ± 0.22% ($p = 0.011$) compared to baseline values. Additionally, mulberry leaves alleviated insulin resistance ($p = 0.057$) [52]. Furthermore, the mulberry leaf aqueous extract (dried mulberry leaves and was standardized to 3.6 mg/g of DNJ) improves postprandial glucose response in individuals with prediabetes, indicating the potential use of white mulberry in diabetes prevention [53]. In a study by Takahashi et al., the optimal timing of mulberry leaf extract (DNJ, 1 mg per tablet) intake was determined. An internal clock controls glucose tolerance and is worse in the evening. From the perspective of chrono-nutrition, the prophylaxis of diabetes requires the evaluation of the anti-diabetic effects of functional components and nutrients at different times of intake. Consuming mulberry leaf extract in the evening, but not in the morning, effectively improves glucose tolerance [54].

Trigonella foenum-graecum L., Leguminosae

Fenugreek is a native plant of Eastern Europe and parts of Asia, but it is now cultivated worldwide

for its leaves and seeds. Fenugreek seeds have traditionally been used as an expectorant, to alleviate cold symptoms, as a laxative, to aid digestion, and to support lactation [55].

Phytochemicals – Mechanisms of hypoglycemic action of Trigonella foenum-graecum

Among the most studied bioactive compounds influencing carbohydrate metabolism in fenugreek, diosgenin, 4-hydroxyisoleucine, and soluble dietary fiber fractions can be mentioned [56]. Diosgenin improves the function of pancreatic β-cells, downregulates enzymes involved in hepatic gluconeogenesis and glucose export, upregulates hepatic glucokinase, and increases the levels of hepatoprotective and antioxidant enzymes [56]. In studies on rats, 4-hydroxyisoleucine improved insulin sensitivity by increasing peripheral glucose utilization and reducing hepatic glucose production [57]. Research has shown that fenugreek's soluble dietary fiber fraction (SDF) does not exhibit hypoglycemic activity alone but acts during glucose perfusion. When SDF was administered concurrently with an oral glucose load, it significantly reduced the rise in blood glucose levels in healthy rats at 75 minutes and in rats with diabetes at 30 minutes. Additionally, SDF improved glucose uptake by adipocytes [58]. In studies conducted by Vijayakumar et al., the effect of fenugreek seed extract (FSE) (seeds were ground and dialyzed into extract) was evaluated in alloxan-induced diabetic mice, and it was found that the action of FSE was associated with the activation of the insulin signaling pathway. FSE improved GLUT4 translocation from intracellular spaces to the cell membrane [59]. Pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK) are two key enzymes involved in glycolysis and gluconeogenesis, respectively, and their activity is impaired in diabetes. In a study by Mahammada et al., in addition to the beneficial effect on GLUT4, the influence of fenugreek (fenugreek seed powder) on the restoration of PK and PEPCK activity was demonstrated [60]. The antioxidant activity of fenugreek (25 mg of Trigonella foenum-graecum seed powder solution twice a day per 1 month) protects the liver and pancreas from oxidative damage induced by diabetes [61].

Clinical evidence of the hypoglycemic effects of Trigonella foenum-graecum

In a study conducted by Gupta et al., patients with newly diagnosed type 2 diabetes received 1 g of aqueous-alcoholic extract of fenugreek seeds daily. After two months of the study, an improvement in glycemic control and a reduction in insulin resistance was observed in the intervention group [62]. The commercial product 'Fenfuro', which contains an extract from fenugreek seeds (patented with water–ethanol extraction), lowers fasting and postprandial blood glucose levels, allowing for a reduction in the dose of anti-diabetic medications [63]. Consumption of fenugreek seeds (5 g fenugreek powder mixed with water three times daily) has a beneficial effect on fasting plasma glucose (FPG), systolic blood pressure (SBP), and certain liver and kidney function tests in patients with type 2 diabetes mellitus (T2DM) [64]. Additionally, fenugreek positively impacts lipid metabolism in patients with type 2 diabetes mellitus (DM2) [61]. Interestingly, substituting fenugreek flour for standard flour used in bread-making enables the production of bread that retains the beneficial properties of fenugreek in reducing insulin resistance [65].

Zingiber officinale Rosc., Zingiberaceae

Ginger, known as *Zingiber officinale*, has a rich history and wide medical application. Its usage dates back thousands of years and has roots in ancient China and India [66]. In traditional medicine, ginger has been widely used to treat gastrointestinal disorders such as nausea, vomiting, and indigestion. It is also appreciated for its anti-inflammatory properties and potential for pain relief and reducing inflammation [67].

Phytochemicals – Mechanisms of hypoglycemic action of Zingiber officinale

[6]-Gingerol, which is one of the main components of the rhizome of *Zingiber officinale*, influences the reinforcement of the glucose-stimulated insulin secretion pathway mediated by GLP-1 in pancreatic β-cells [68]. Additionally, [6]-Gingerol increases the membrane presentation of GLUT4 transporters in the skeletal muscles of diabetic mice [68]. In a study on rats with induced type 2 diabetes, ingesting ginger extract at a 4 ml/kg body weight significantly reduced blood glucose levels after six weeks [69]. Treatment with *Zigniber officinale* significantly increases insulin levels and decreases fasting blood glucose levels in diabetic rats treated with

ginger juice [70]. In studies conducted by Abdulrazaq et al., ginger juice increased the activity of glucokinase, phosphofructokinase, and pyruvate kinase and exhibited anti-hyperglycemic effects [71]. In vitro studies on mouse myoblast and myotube cell lines demonstrated the influence of ginger extract on the increased expression of GLUT4 transporters on the cell surface compared to the control [72]. Furthermore, *Zingiber officinale* protects against diabetes-induced kidney damage by alleviating oxidative stress, inflammation, and apoptosis [73].

Clinical evidence of the hypoglycemic effects of Zingiber officinale

In a study conducted by Carvalho et al., the effectiveness of ginger in lowering blood glucose levels, total cholesterol, and LDL cholesterol (LDL-C) was demonstrated [74]. In a study conducted in 2019 on a group of newly diagnosed, obese (BMI > 30 kg/m^2) diabetic patients, El Gayara et al. showed that daily consumption of 3 capsules, each containing 600 mg of powdered ginger (dried, finely ground rhizomes of ginger in gelatin capsules), for eight weeks resulted in a reduction in BMI, HbA1c, FBG, FSI, triglycerides, TC, and LDL-C [75]. In another study, ginger supplementation (dried and ground rhizomes of ginger in tablets containing 1 g ginger in each) significantly lowered insulin levels, LDL-C, triglycerides, and HOMA index while increasing the value of the Quantitative Insulin Sensitivity Check Index (QUICKI index) compared to the control group [76]. Shidfar et al. examined the effect of administering 3 g of powdered rhizomes of ginger (in capsules) daily for three months, which resulted in improved glycemic indices, total antioxidant capacity (TAC), and PON-1 activity in patients with type 2 diabetes, confirming its antioxidant properties [77]. Further studies confirm the impact of ginger (powdered rhizomes of ginger in 800 mg capsules [78] or one-gram capsules containing ginger powder [79] intake on reducing fasting plasma glucose levels, HbA1c, insulin, HOMA, triglycerides, total cholesterol, CRP, prostaglandin E2 (PGE_2), and improving insulin resistance indices such as the QUICKI index [78,79]. In patients with diabetes and end-stage renal disease, ginger (2000 mg of rhizomes ginger powder) lowers blood glucose levels, increases insulin sensitivity, and reduces serum urea levels [80].

Conclusions

Diabetes is a global health problem affecting millions worldwide, and forecasts indicate further increases in cases. Therefore, searching for new, effective therapies and interventions to combat this disease is necessary. The modern approach to managing diabetes increasingly turns to traditional medicine and explores the use of natural plant remedies. Scientific research focuses on identifying active plant compounds, studying their mechanisms of action, and exploring their potential applications in diabetes treatment and blood sugar regulation. Many active plant ingredients have been isolated and used to produce dietary supplements and anti-diabetic medications at appropriate concentrations. Combinations of different plant products are often used to enhance their effects synergistically [81]. The mechanisms of action of plant-derived compounds summarized in the publication represent a consolidated compilation of scientific achievements thus far regarding the anti-diabetic properties of plant preparations. They include improvements in glucose transport, influence on glucose metabolism, inhibition of carbohydrate-digesting enzymes, promotion and enhancement of insulin secretion, reduction of insulin resistance, improvement of pancreatic islet morphology and function, and liver protection. These pieces of information, presented clearly, are summarized in **Table 1**. The potential of plant-based anti-diabetic agents is only partially discovered, thereby encouraging further research and discoveries aimed at understanding their mechanisms of action and utilizing them within contemporary health movements.

Abbreviations

FPG – Fasting Plasma Glucose, HbA1c – Glycated Hemoglobin, UCP-1 – Uncoupling Protein, PEPCK – Phosphoenolpyruvate Carboxykinase, BMI – Body Mass Index, NF-kB – Nuclear Factor Kappa B, SIRT1 – Sirtuin 1, hs-CRP – High-Sensitivity C-Reactive Protein, IL-6 – Interleukin-6, TNF-α – Tumor Necrosis Factor-Alpha, ICAM-1 – Intercellular Adhesion Molecule 1, VCAM-1 – Vascular Cell Adhesion Molecule 1, AMPK – AMP-Activated Protein Kinase, WC – Waist Circumference, NIDDM – Non-Insulin Dependent Diabetes Mellitus, 1-DNJ – 1-Deoxynojirimycin, FA – PhytomoleculeFagomine, DAB – 1,4-Dideoxy-1,4-Imino-D-Arabinitol, T2DM – Type 2 Diabetes Mellitus, SZ-A – Sangzhi Alkaloids, SDF – Soluble Dietary Fiber, FSE – Fenugreek Seed Extract, PK – Pyruvate Kinase, GLP-1 – Glucagon-Like Peptide 1, FBG – Fasting Blood Glucose, TAC – total antioxidant capacity

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPERS

JMS Journal of Medical Science

Pandemic potential of henipaviruses

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ABSTRACT

Introduction and purpose. Hendra and Nipah are two highly dangerous zoonotic viruses belonging to the group of henipaviruses. Although they have been known for over 20 years, no human drug or vaccine has been invented. This paper aims to to describe the epidemiology of the reported paramyxoviruses, the pandemic potential of henipaviruses, and a standardised action plan to counter their spread. This paper reviews scientific articles from 2012-2023 published in scientific databases such as Pubmed, Researchgate, and Google Scholar. The keywords used were pandemic potential of henipaviruses, Hendra virus, Nipah virus, and henipavirus epidemics.

State of knowledge description. The mortality rate of henipaviruses varies between 50 and 100%. The Nipah virus is particularly dangerous, with epidemics recurring virtually every year in Asia since 1998. The Hendra virus situation may be manageable because there is an effective vaccine for horses most vulnerable to infection. Due to human activity, the habitats and climate of the animals serving as virus reservoirs are changing. Because of frequent henipavirus outbreaks in Asia and Australia, extensive efforts are being made to contain and neutralise them rapidly.

Conclusions. As henipaviruses pose a high pandemic threat, more research into drugs and vaccines is required. It is also essential to develop effective bio-assurance plans, introduce controls on their operation and educate the population on the issue. Reservoir animals, through anthropogenic environmental changes, are changing habitats and feeding sites, making more and more territories vulnerable to the disease. New species of henipaviruses constantly emerge and pose an epizootic challenge to public health. Hence, an essential action is to increase the amount of research into the virus's epidemic development and conduct it as widely as possible.

Introduction

Paramyxoviruses are a group of single-stranded RNA viruses with negative polarity. They belong to the family Paramyxoviridae and the order Mononegavirales [1,2]. The Henipavirus genus viruses, Hendra (HeV) and Nipah (NiV) are a severe public health concern. They cause local epidem-

ics of Hendra and Nipah viral diseases with high mortality rates. Therefore, unique bio-assurance plans are being implemented in vulnerable areas to protect against the potential development and spread of the disease. The lack of defined treatment and vaccines qualifies them as biosafety level 4 pathogens. NiV has been recognized by the WHO as a global health problem and listed as an epidemic threat and biological weapon [3,5]. Henipaviruses can be a high-risk threat due to their lack of a human vaccine, zoonotic disposition and confirmed cases of human-to-human transmission [4].

Fruit bats, particularly Pteropus, are natural reservoirs of pathogens. In Australia, all four species of flying foxes that were studied (Pteropus alecto, Pteropus poliocephalus, Pteropus conspicillatus, and Pteropus Scapulatus) were found to carry the virus, with a particular emphasis on Pteropus alecto and Pteropus conspicillatus. The virus asymptomatically circulates between individuals of bats thereby maintaining continuity of existence and replication [5]. There are two mechanisms of infection, one exemplified by the outbreak in Malaysia – transmission of the virus through animals, from bats to horses to pigs to human infection or transmission of the virus straight from bat to human observed in Bangladesh and India. In the Philippines, transmission has been seen through the consuming contaminated, unwashed, raw date palm fruit [6,7].

Disease and symptoms of henipavirus infection

Nipah virus disease is a zoonosis. Frugivorous bats transmit the pathogens causing it. The possibility of transmitting the virus through close contact with infected body fluids of infected animals has been documented, and very rarely among humans. However, the transmission of the infection via droplets is doubtful. It has been experimentally found that the amount of virus in saliva, throat secretions, and urine is small. It was first recorded in Kampung Sungai Nipah in Malaysia in 1998 among pigs. It manifested mildly in animals with respiratory and nervous system syndromes. At the same time, in humans, high fever was observed, and ARDS were observed in approximately 50–60%; a few days after infection, mental status changes, visual paralysis, areflexia and limb weakness appeared. Patients' condition often deteriorates rapidly, with symptoms suggestive of brain stem involvement, leading to coma and death within a few days. In the cerebrospinal fluid, lymphocytic pleocytosis and raised proteins with normal glucose levels are observed [16,47]. Nipah disease observed among the Malaysian and Singaporean populations began with a sudden increase in body temperature, headaches and dizziness, vomiting and thinner stools – mild, unusual symptoms of a viral infection. Nervous system symptoms such

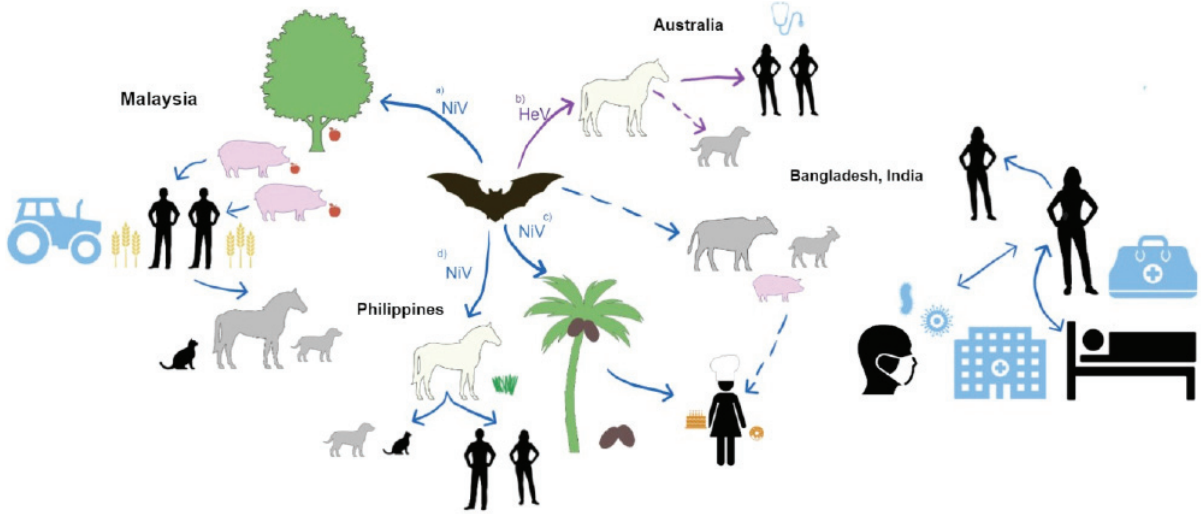

Figure 1. Virus transmission pattern by country based on available studies [8,9,11,12,20,22,24,25].

as loss of consciousness, areflexia, and a drop in blood pressure followed, and some patients also suffered from epileptic seizures. The most severe post-infection symptoms are encephalopathy and atrophy of the white matter of the brain. Behavioural changes and motor paralysis of some muscles are also observed [17–19]. The infection has been reported to be asymptomatic in 8% of infected people [20]. The clinical manifestations described vary depending on disease location. The epidemic in Malaysia had a lower mortality rate than epidemics in India or Bangladesh [21]. In the Malaysian Nipah disease epidemic, nervous system syndromes played a key role. In contrast, during the epidemics in India and Bangladesh, respiratory failure, acute cough and atypical pneumonia symptoms were observed among patients [22,23]. Nipah virus infection, regardless of region, most often leads to severe encephalitis and death [24]. Nipah should be differentiated between Japanese encephalitis, malaria of the nervous system and rabies. Magnetic resonance imaging and laboratory tests are helpful in differential diagnosis [3,27].

Epidemic in Malaysia and Singapore

In September 1998, the first infections of unknown aetiology were observed among pigs and farmers near the town of Ipok. Subsequent cases were registered in the towns of Sikamat and Bukit Pelandok. At first, the illness was believed to be caused by the Japanese encephalitis virus. However, it was soon discovered that a different pathogen, the Nipah virus, was the actual cause of the disease, as mainly adults and people who had been vaccinated for Japanese encephalitis were still contracting the illness [24,25]. Nipah virus is primarily transmitted through contact with the bodily fluids of infected pigs, such as faeces, urine, and saliva. Adult males associated with swine farms were most at risk of contracting the disease. Interestingly, native Malays, as followers of Islam, are not allowed to have close contact with pigs and also are not allowed to consume pork, so among their population, no case of infection has been reported [8]. The disease has spread to Singapore through numerous negligence in controlling exported goods, and most likely through uncontrolled pork and pig livestock shipments. Initially, illnesses were reported among slaughterhouse workers, but then the disease developed in pig farmers who also participated in pig transits from infected Malaysia [25]. In order to eradicate the epidemic, the transportation of pigs was banned, preventive culling of exposed or sick animals was used, and educational activities and national sanitary surveillance were carried out. In Malaysia, the primary industry is pork production. The preventive culling of more than a million animals caused substantial financial losses. However, the measures taken in both countries brought good results, and the epidemic was halted. The last disease was found in May 1999; no case has been found since then [4,19].

Epidemic in India and Bangladesh

In early 2001, numerous cases manifesting as acute fever combined with impaired cognition and concentration were observed near the city of Siliguri in India. Pathogen isolates that had been previously tested were compared to samples collected during a viral outbreak in Bangladesh. It was noted that there was a similarity between the two as both samples showed the presence of NiV henipavirus. Additionally, another outbreak was identified in West Bengal, where five cases were reported, and each turned out to be fatal. The largest outbreak of the Nipah virus in India was reported in Kerala in 2018; 23 patients were reported, of whom 18 died; thus, the disease mortality rate was 91% [25–27].

In contrast to Malaysia and Singapore, in Bangladesh and India, the leading infectious agent of NiV was the consumption of date palm juice or contact with a sick person. In India, the contagion's spread mechanism relied mainly on the zoonotic potential of the virus, bat-to-human, human-to-human transmission. The danger of this case was also posed by domestic animals, which could transmit the virus. The last Nipah virus infection was reported in the second week of September 2023 in the southern Indian state of Kerala. Six cases were reported, including two deaths. Kerala faced the presence of the virus for the fourth time. The region's authorities took immediate action to prevent the spread of

the virus. Schools, offices and public transport in Kozhikode district were closed, and wearing masks in public places was imposed. No further cases of the disease have been reported since September 15 [44,45]. The epidemic in Bangladesh began in Mehepur district in 2001, with 13 cases reported. Since then, numerous outbreaks have been observed yearly in different parts of the country until 2015, and the virus' mortality rate has remained at 76.2%. During the outbreak in Bangladesh, 261 cases, including 199 deaths, were reported. Cases of person-to-person transmission have also been reported among the Bangladeshi community, although the risk of such transmission is very low [11,22,24,27,49].

Epidemic in Australia

In late 1994, in the Brisbane area of Hendra, previously unknown respiratory symptoms with hemorrhagic symptoms were observed among horses in a suburban stable [10]. Twenty horses became ill, of which 13 died and their trainer, who successively lost his life as a result of respiratory and kidney failure. The next epidemic case was reported in another part of Australia – Queensland – it involved two horses and one human who died of recurrent encephalitis [8,9]. The virus has been identified in 50 outbreaks. By 2021, 105 horses had died of HeV in Australia. Several bio-assurance measures have been taken to control the outbreaks; these have mainly consisted of increasing the hygiene of watering holes, regular cleaning, changing the water as frugivorous bat secretions could be found there, regular decontamination of stables, testing horses for HeV and preventive culling in case of illness [12]. Hendra virus infection may carry the stigma of an occupational infection because, in Australia, the most common source of infection was the transmission of the virus from horses to humans, which mainly exposed horse breeders and veterinarians [18,31,41].

Virus detection

According to WHO, the preferred diagnostic method is qRT-PCR due to its high sensitivity and specificity. Immunochemical tests, ELISA antibody detection tests, and virus neutralization tests, which can be performed in high-class BSL 3+ and BSL L4 safety laboratories, are also used. These tests are the reference standard in serological diagnosis of NiV and HeV. To increase the scale of testing and enable lower-class laboratories to perform them by being able to work on irradiated viral antigens that are thus neutralized and come from cell cultures [24,28,46–48].

Treatment and prevention methods

Testing serum IgM levels has detected the virus, and diagnostic methods such as polymerase chain reaction-PCR and real-time PCR performed on tissue material or cerebrospinal fluid have also been used [24,28].

Treatment of Hendra virus is problematic due to the need for more effective drugs. Studies have been conducted on hamsters using ribavirin and chloroquine and their combinations but have yet to show positive results [21]. Currently, research is being conducted on recombinant monoclonal antibodies, but the efficacy of this therapy is low [9,12]. In 2012, Zoetis Australia launched Equivac® HeV vaccine for horses. This vaccine is administered in two doses three weeks apart. Three weeks after the second dose, antibodies that effectively protect against the disease are formed. A booster dose is given after six months, with subsequent booster doses every 12 months. Equivac® HeV has side effects but are mainly local and occur in 0.001% of horses tested [10,29]. Breeders are often reluctant to vaccinate horses because they believe the vaccine negatively affects horses' athletic performance, a myth debunked by a large study [30]. The vaccine is effective for non-human monkeys, although more research is needed to test its effectiveness in humans [12].

Currently, there is no targeted treatment for Nipah virus infection. Ribavirin is used as an adjuvant treatment. During the 1998–1999 outbreak in Malaysia, it was administered to some infected patients, and it was found that among them, the mortality rate was 36% lower than the control group [2]. During the 2018 outbreak in Kerati, India, ribavirin gave patient treatment results, but the study group was too small to draw firm conclusions [23]. Studies in animal models do not support the efficacy of ribavirin [7].

There have been many attempts to invent a drug targeting the Nipah virus, and the most promising studies involve monoclonal antibodies against the G protein of Henipavirus (m102.4) [13,23]. The m102.4 monoclonal antibody neutralizes HeV, NiV-M and NiV-B viruses. In animal model studies, administration of the antibody after exposure to the virus protected against disease. The antibody was administered to 14 people, and no side effects were registered. There are other hope-rising ongoing studies on other antibody h5B3.1 [31].

Currently, there is no registered vaccine against the Nipah virus. Much research is underway on different types of vaccines, many of which are effective for animal models [31–34]. Recently, an HeV vaccine against the Hendrach virus was found to protect African Green Monkeys from the Nipah virus [24,35].

Discussion

The world has been facing Henipavirus epidemics for about 30 years. After the COVID-19 pandemic, more attention is being paid to the threat posed by the viruses, and the scientific world is more focused on developing new strategies to combat epidemics [36]. They are particularly dangerous because their mortality rate oscillates between 50–100%, and no specific treatment has yet been developed. Particular attention should be paid to the Nipah virus, which has caused epidemics for over 20 years, almost yearly [5]. It has a high pandemic potential, as no drug or vaccine has been developed for it; the bats that spread it are found in almost all of Asia, and pigs, which are raised practically all over the globe, may also be involved in spreading the virus [1,31,33,36]. When considering the pandemic nature of the Nipah virus, it is worth focusing on bats since they migrate seasonally over long distances, contributing to the spread of the disease [26]. Through deforestation of the land, bat roosting and foraging sites are changing. It has been postulated that climate warming may change their roosting areas, contributing to outbreaks in places where they did not previously occur [22,37,38]. Only for the Hendra virus is there an effective horse vaccine that effectively stops outbreaks from developing [8,9].

Nipah and Hendra are the two main species of henipaviruses, but not the only ones. Mutations always lead to the emergence of new types [2,39]. In 2021, a new henipavirus, Langay, appeared in China; its reservoir is shrews, from which humans are infected. There is no evidence that the disease can be transmitted from human to human, but it is not excluded [15,40]. Its symptoms are usually not dangerous, but fatal cases have occurred. Somewhat reminiscent of the symptoms of COVID-19, LayV manifests mainly with fever, fatigue, muscle aches and respiratory symptoms – mainly cough and shortness of breath [39,41].

Due to the lack of effective treatment, available vaccines and high mortality rates, henipaviruses are a significant public health challenge. The current state of knowledge on treatment and epidemiology is inadequate; so more research is essential [3,6,31,42].

Conclusions

1. Henipaviruses have a high pandemic potential, so research needs to focus on the epidemiology of individual species.
2. Climate change and deforestation are likely to change bat migration routes and areas, so changes in bat habitat should be studied.
3. More research is needed on numerous research and control groups on the treatment of henipaviruses.
4. Because of how deadly some henipaviruses are, it is crucial to develop a vaccine as soon as possible.
5. Due to the lack of targeted treatment for henipaviruses, it is imperative to raise awareness about them within the countries where they occur.

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REVIEW PAPER

JMS Journal of Medical Science

Impact of testosterone levels and testosterone replacement therapy on men's health

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ABSTRACT

Various studies have shown that testosterone levels have a heavy impact on areas of a man's health. Low serum testosterone (and, by analogy, late-onset hypogonadism) may be responsible for such conditions as type 2 diabetes, obesity in the abdominal area, and, most of all, heightened cardiovascular risk (CV). Among other outcomes, researchers have pointed out metabolic syndrome and dyslipidemia, as well as an increased risk of anxiety disorders and major depressive disorders. There have also been reports of testosterone's influence on fertility, bone mineral density, and the development of polycythemia. Low testosterone can have a variety of effects, all of which increase the risk of premature death by raising inflammatory marker levels.
Overly high testosterone, however, has been proven to have a notable influence on men's personalities, as well as other psychological and social traits, both in endogenously elevated testosterone levels and in patients with a history of anabolic-androgenic steroid use.
The last decade's research on testosterone's impact on the organism has yielded contradictory results. Therefore, examination and understanding of the influence of its abnormal levels prove essential to not only guarantee the best quality of hypogonadism treatment but also to efficiently prevent any side effects or complications associated with testosterone use.

Introduction

Testosterone (T) is a steroid hormone influencing men's physiology. It is responsible for developing male sexual characteristics and maintaining them later in life. With age, however, levels of testosterone decline [1], which in approximately

5% of cases might lead to the development of late-onset hypogonadism (LOH) [2,19].

The definition of testosterone deficiency varies among researchers, who may need help forming consistent diagnoses for men affected by it. Therefore, it is essential to understand and clearly define the issue. A recent study by Salter et al. compared multiple guidelines for testosterone therapy produced by some of the major medical societies specializing in urology, endocrinology and sexual medicine and presented a consensus that testosterone deficiency is defined by abnormal laboratory results as well as clinical manifestations. Laboratory thresholds vary from as low as <10.4 nmol/L (American Urological Association) to <12.1 nmol/L (European Association of Urology, International Society for Sexual Medicine, International Society for the Study of the Aging Male). Some societies do not propose detailed levels, either not including them in the recommendations (American Association of Clinical Endocrinologists) or describing the measurements as 'consistently low' (Endocrine Society). British Society of Sexual Medicine and the European Association of Urology emphasise the importance of measuring total and free testosterone levels. Clinical signs mentioned by Salter et al. include anaemia, osteoporosis, infertility and erectile dysfunction, psychological symptoms, diabetes mellitus and muscle mass loss, all of which we will describe later in our review. Patients presenting such symptoms should undergo further examination [3]. European Academy of Andrology (EAA) highlights the importance of considering LOH a functional instead of an organic form of hypogonadism (caused by obesity and medication). It, therefore, suggests excluding organic causes of hypogonadism before diagnosing the patient [4].

Various studies have shown that LOH heavily impacts many areas of a man's health [5–10]. It is important to remember that as a functional disease, hypogonadism should be initially treated with lifestyle changes such as weight loss or withdrawal of drugs impairing the production of testosterone, if possible. EAA suggests testosterone replacement therapy's (TRT) positive influence on sexual function in hypogonadal men, but there is insufficient evidence regarding its positive impact on other outcomes [4].

In the face of the last decade's contradictory results of testosterone research [11], this review aims to analyse the most recent studies regarding testosterone level impact on male cardiovascular (CV), metabolic and psychological health. Moreover, we tried to assess the benefits as well as side effects of TRT, such as a suspected increase in CV event risk [12], prostate cancer or metabolic syndrome [13].

For this review, the terms 'functional hypogonadism' and 'testosterone deficiency' will be used interchangeably with 'late-onset hypogonadism' (currently considered an outdated term), depending on the name used by cited authors.

Material and methods

Publications from 1987 to August 2023 were searched via PubMed and GoogleScholar using the terms "testosterone", "hypogonadism", "LOH", "TRT", "cardiovascular diseases", "cardiovascular risk", "cardiovascular events", "Testosterone Trials", "metabolic syndrome", "muscle loss"; "diabetes", "prostate cancer", "psychology", "anabolic androgenic steroids", and "depression". Additional papers were found and studied through the references of those papers. Only articles in English were considered.

Testosterone and its functions in men's organism

Testosterone is the principal sex hormone produced and secreted by Leydig cells stimulated by luteinising hormone (LH). Its influence on the organism begins as early as in the first weeks of gestation when it starts conditioning the process of genital virilization, such as phallic enlargement, the development of seminal vesicles and prostate (however, these processes are influenced more by dihydrotestosterone than testosterone). Another organ influenced by testosterone is the brain, where during foetal growth, the aromatase converts testosterone to estradiol, causing masculinization and organisation of neural pathways characteristic of male behaviours [14].

Later on, in the third trimester, testosterone, along with the anti-Müllerian hormone (AMH), stimulates the development of sex by promot-

ing the degeneration of the Müllerian duct and growth of the Wolffian duct [15].

Before puberty, testosterone conditions the adrenarche, which manifests physically by developing adult body odour, axillary and pubic hair growth or an increase in testicular size. During puberty, masculine development stimulated by testosterone continues, resulting in the progressing appearance of tertiary sexual characteristics (for example, modification of skull shape, growth of facial hair, Adam's apple appearance, voice deepening) [16].

In adulthood, testosterone is necessary for maintaining spermatogenesis [17], muscle growth and inducing sexual activity [14]. It also seems to have a significant impact on motivation and ambition [18], as well as cognitive functions, which is especially noticeable in older men suffering from hypogonadism [19].

Finally, testosterone has a significant anti-inflammatory effect on multiple tissues. Low testosterone levels correlate with increased C-reactive protein (CRP), macrophage inflammatory proteins 1-α and 1-β and TNF-α, and many other pro-inflammatory cytokines and adipokines. This prolonged inflammation is one of the main reasons why testosterone's low levels may lead to metabolic syndrome, cardiovascular diseases, neurodegeneration and increased mortality risk [20], which our study will further discuss.

Cardiovascular diseases

Testosterone deficiency's influence on cardiovascular risk is a controversial and problematic topic. The available research lacks adequately powered randomized trials and does not provide sufficient longitudinal studies regarding TRT's safety in men with hypogonadism.

Meta-analyses considering the effect of endogenous T levels on CV risk have shown conflicting results. Corona et al. conducted a random effect meta-analysis using data from 37 observational studies published between 1988 and 2017 [21]. The analysis included 43,041 men at a mean age of 63.5 years, with a mean follow-up time of 333 weeks. The presented study showed that low T in ageing men is a marker of CV risk. However, the authors noted that the possible benefits of T treatment in reducing this risk should be examined in longer-term, specifically designed trials. Marriott et al. did not report the same results, who analyzed data on 20,180 men aged 64.9 ± 3.3 years with a mean T concentration of 15.4 ± 0.7 nmol/L measured using mass spectrometry [22]. This meta-analysis demonstrated no significant effect of a 5 nmol/L increase in T level on the risk of all-cause mortality or death from CV disease.

Although some studies conducted in the past have suggested that TRT may be associated with increased CV risk [23–26], several recently published meta-analyses have not supported such conclusions. Corona et al. gathered data from 15 pharmaco-epidemiological studies and 93 randomized placebo-controlled trials (RCT). They also found no indication of increased CV risk caused by correctly applied T therapy (TTh) with data from pharmaco-epidemiological studies suggesting that TTh reduces overall mortality and CV morbidity [27]. Fallara et al. analyzed data regarding 179,631 hypogonadal adult men (≥18 years old) and found that those treated with TTh had a lower all-cause mortality risk as compared to control groups without increased CV risk [28]. Hudson et al. also found no evidence that TTh increased short- or medium-term CV risk in men with hypogonadism [29]. This study involved RCTs, which included individual participant datasets (IPD), and investigated the effect of incorporating data from trials that did not provide IPD. Thirty-five primary studies were analyzed with a total of 5,601 participants at a mean age of 65. Finally, Ayele et al. conducted a systematic review analyzing the association between TRT and the risk of venous thromboembolism (VTE), which included 13 RCTs and a total of 5,050 men aged ≥18 years. The results suggested that TRT is not associated with an increased risk of VTE [30].

Several studies also analyzed the safety of TTh in patients with comorbidities. Mangolim et al. gathered data from 16 RCTs, including men with low T levels and obesity. Researchers found that in these men, TRT slightly improved lean body mass and LDL; however, its impact on CV events was unclear [31]. Another study conducted by Cannarella et al. aimed to assess the risk of TRT on cardiac function and angina in patients with low T levels coexisting with heart failure (HF) or coronary heart disease [32]. Seven RCTs, including 140 patients of NYHA class II and III,

were analyzed. Upon analysis, no effect of TRT on death and rehospitalization rates of patients with HF was found, and in patients with chronic angina, a significant delay in time to ischemia was observed.

Even though none of the meta-analyses described above suggested that TTh might be associated with increased CV risk when used as indicated, most concluded that further research is needed to confirm its safety.

On June 16, 2023, the results from the Testosterone Replacement Therapy for Assessment of Long-term Vascular Events and Efficacy Response in Hypogonadal Men (TRAVERSE) trial were presented at ENDO 2023. TRAVERSE trial is a randomized, double-blinded, placebo-controlled study conducted at 316 clinical trial sites in the United State [33]. A total of 5246 men between the ages of 45 and 80 were enrolled in the experiment and assigned in a 1:1 ratio to receive daily transdermal 1.62% testosterone gel or matching placebo gel. All participants had preexisting or a high risk of CV disease. Moreover, participants had to present at least one of the symptoms of hypogonadism, including decreased sexual desire or libido, decreased spontaneous erections, fatigue or decreased energy, low or depressed mood, loss of axillary or pubic body hair or decreased frequency of shaving, or hot flashes; and had to have two fasting serum testosterone levels of less than 300 ng/dL.

The primary safety end-point of the trial was the first occurrence of a major CV event (including death, nonfatal myocardial infarction, and nonfatal stroke). Upon analysis, researchers identified 182 patients (7.0%) in the testosterone group and 190 patients (7.3%) in the placebo group who suffered a major CV event (hazard ratio, 0.96; 95% confidence interval, 0.78 to 1.17; $P < 0.001$ for noninferiority). After censoring the data on events that occurred more than 365 days after the last dose of TRT or placebo, a primary safety end-point event occurred in 154 patients (5.9%) in the testosterone group and 152 patients (5.8%) in the placebo group (hazard ratio, 1.02; 95% CI, 0.81 to 1.27; $P < 0.001$ for noninferiority). It is also worth noting that the mean (±SD) duration of treatment and follow-up, respectively, were 21.7 ± 14.1 and 33.0 ± 12.1 months, and thus, there is still a need for longer-term studies with greater follow-up to ascertain the safety of TRT use [33].

TRAVERSE trial provides strong evidence regarding CV safety of adequately prescribed TRT; it is still essential to remember that this therapy may result in adverse effects. A higher incidence of nonfatal arrhythmias, atrial fibrillation and acute kidney injury has been reported among patients who received testosterone than among those who received placebo. Therefore, TRT should only be prescribed to middle-aged and older men with symptomatic androgen deficiency in whom testosterone is clinically indicated [33].

Diabetes

Testosterone, as an endogenous sex hormone, is an important factor in glucose metabolism and maintaining glucose homeostasis. However, it is not sure whether testosterone deficiency promotes the development of pre-diabetes and T2D or if it is the other way around [34]. There is no doubt a correlation that primary care doctors should be aware of, and all male patients with T2D should be screened for hypogonadism [35]. It is even more important when considering the results of the International Diabetes Federation. In 2019, 9.3% of the global population was estimated to have type 2 diabetes, and this percentage is expected to increase steeply in the following years. Moreover, half of the people with diabetes are not aware that they suffer from it [36].

A recent study found that among all age groups mean, testosterone levels were significantly lower in patients with diabetes than in corresponding non-diabetic groups. In addition, a longer duration of diabetes correlated with decreased testosterone levels [37]. Men in prediabetic states also have been found to have lower levels of testosterone than their healthy counterparts [38].

A study by Gouda et al. found that in non-obese (BMI below 30) men with T2D, one of the risk factors of testosterone deficiency is an elevated visceral adiposity index. This index could be used to predict testosterone deficiency in men without obesity with T2D [39]. In contrast, higher levels of testosterone have been associated with lower risks of developing T2D [5]. More so, increased testosterone is related to better outcomes of T2D development markers (better insulin sensitivity and lower levels of stimulated glucose) [38]. As a possible mechanism, Navar-

ro et al. showed that testosterone enhances the effect of glucagon-like peptide 1 (GLP-1) and the function of beta-cells [40].

TRT has been found to completely prevent the progression from prediabetes to type 2 diabetes in men with LOH [41]. Moreover, improvements in glycemia and lipid ratios were also observed [41, 42]. Additionally, the mortality rates were lower for patients with T2D whose testosterone levels were normal or who were on TRT compared with patients with T2D who suffered from testosterone deficiency [32].

Therefore, as TRT positively affects men with LOH, it might be used as a treatment for better T2D control and improvement in men's health.

Obesity, metabolic syndrome and muscle loss

There is strong evidence that low testosterone levels are associated with a higher prevalence of metabolic syndrome (MetS) in men of any age [9]. However, it has yet to be proven if the connection goes only through hyperinsulinism or if there is an independent association. A study with an 11-year follow-up has shown that even in men with normal BMI, there are significantly higher odds of developing MetS for men in the lower quartile of serum testosterone levels [44].

The exact role of testosterone and other sex hormones on human metabolism has not been clearly defined yet. However, the usually emphasised aspects are, among others, defective lipid uptake, lipolysis stimulation, and decreasing lipogenesis. In a 1999 study in which almost 400 males aged >20–85 were examined, there was a positive correlation found between age and BMI and fat mass (measured by impedance) and a negative correlation of age with levels of free testosterone and free insulin-like growth factor 1 (IGF-I) [45]. The role of testosterone in changes occurring during a lifetime may also be caused by its anabolic activity. Higher testosterone levels correlate with a lower lean mass loss in men over 65, as shown in a study that examined nearly 6000 men [46]. There was also a trial testing TRT in men with spinal cord injury and low testosterone serum levels that showed that after 12 months of TRT, there was a significant increase in the patients' lean tissue mass in comparison with the control group [47]. TRT has also proven effective in preventing muscle mass loss in HIV-infected patients [48]. The positive effect was most pronounced when intramuscular injections administered testosterone.

Prostate cancer

For a long time, scientists have not been able to conclude whether testosterone levels affect the development of cancer in men. The most likely and frequent possible cancer that depends on testosterone levels is prostate cancer.

In 2019, Kaipainen et al. conducted a study to test whether testosterone is a cancer growth factor through ligand-mediated androgen receptor activation. The study concluded that in addition to metabolism and transport of aldo-keto reductase family 1-member C3 (AKR1C3) – a hormone responsible for the conversion of androstenedione (AED) to testosterone – by tumour epithelium, testosterone can also be produced by components of the tumour microenvironment [49].

A meta-analysis conducted by Claps et al. in 2018 led to the conclusion that, depending on androgen deprivation therapy (ADT) intake and different clinical conditions, the association between circulating testosterone and prostate carcinoma (PC) prognosis varies [50]. For instance, in early PC, testosterone levels did not influence overall survival (OS). However, in advanced PC the prognosis and risk of death depended on the employment of ADT. Before ADT, higher testosterone levels correlated with a reduced risk of death, while during the said therapy, it was the low levels that reduced the risk of progression and death.

Also, in 2018, Walsh et al. conducted a retrospective initial cohort study of male veterans aged 40 to 89 years with laboratory-defined low testosterone levels between 2002 and 2011 and a recent prostate-specific antigen test. At first, the study found that only 313 out of the total number of men who were treated with testosterone had aggressive prostate cancer. However, upon adjusting for factors such as age, location, and other medical conditions, it was revealed that the incidence rate of aggressive prostate cancer was 0.57 per 1000 patients among untreated men and 0.58 per 1000 among treated men. Therefore, it was concluded

that there is no correlation between the cumulative dose or formula of testosterone and the development of prostate cancer [51].

Morales and Black described the case of a 71-year-old man who had previously undergone treatment for adenocarcinoma of the prostate gland. As a consequence the patient experienced a decrease in androgen levels and hypogonadism, as well as a decrease in libido, for which the patient decided to start testosterone replacement therapy. After half a year of testosterone treatment, the patient's prostate-specific antigen (PSA) increased, and therefore, he was advised to discontinue further testosterone treatment, which resulted in PSA's normalization. After the patient's death, caused possibly by a previously discovered hemorrhagic epidural mass and multiple pulmonary nodules, an autopsy was ordinated and revealed the prostate gland to be small with tumour cells histologically matching the ones found in the spine lesion and not originating from the prostate [52].

Based on such a small number of studies and articles, it cannot be claimed that testosterone affects the development of cancer in men, specifically prostate cancer. Many factors must be considered when conducting research, such as: age, comorbidities, place of work, and much more. Figuring this out is still a very relevant issue.

Osteoporosis

Bone demineralization is most commonly associated with estrogen deficiency (especially in the female population) – this mechanism is more frequently described in the literature and, therefore, better understood. Testosterone's influence on bone metabolism is relatively underrepresented in research but needs to be more important [53]. Osteoporosis is therefore often considered a "female" issue, with the male population presenting significantly lower prevalence and experiencing clinical manifestations much later (i.e. osteoporotic fractures occurring even ten years later in men's life than in women's), possibly because of a more gradual drop of testosterone levels. Despite that, morbidity and mortality of hip fractures are higher in men than in women [54], and only 10% of men receive proper treatment [55].

Traditional views suggest that testosterone deficiency in men has analogous effects on bone density as estrogen deficiency in women [56]. In a study from 1989, Stepan et al. showed a rapid decrease in bone density in castrated men, which led them to conclude that estrogen was the primary regulator of bone metabolism in women and testosterone in men. This theory was later modified over time by multiple studies, resulting in the currently accepted thesis that estrogens and testosterone play an important part in influencing men's bone metabolism [53].

Golds et al. mention in their review that due to the androgen receptors' presence in osteoblasts, osteocytes and osteoclasts, it is likely that testosterone has a direct influence on these cells, and therefore, on bone metabolism. However, based on a large Osteoporotic Fractures in Men Study (MrOS), the authors of this review point out that despite a clear correlation between hypogonadism and osteoporosis, the connection is possibly not based exclusively on free testosterone levels; the main factor influencing bone demineralization in men still seems to be low bioavailable estrogen (BioE2) and high sex hormone-binding globulin (SHBG). Free testosterone's effect on bone mineral density (BMD) seems to be nonsignificant, with only some (though not independent) effects on increased fracture risk [54].

Still, almost half of male osteoporosis cases have a secondary cause, one of the most frequently observed being hypogonadism [55]. Therefore, all cases of osteoporosis or idiopathic fragility fractures in older men should be investigated for possible secondary causes and treated accordingly.

Polycythemia

Polycythemia is an abnormally increased red blood cell mass. It is defined by the mass of red blood cells, which in men should be between 26 and 32 mL/kg [57]. That mass usually increases when hematocrit and haemoglobin values are above the norm, 51% and 185 g/L in men, respectively [58]. Secondary polycythemia is caused by chronic hypoxemia (obstructive sleep apnea, congestive heart failure) that stimulates the production of erythropoietin [59]. Testosterone also stimulates erythropoietin, increasing the haemoglobin

level and hematocrit [60]. Therefore, the low testosterone level in hypogonadism correlates with lower haemoglobin levels and lower haematocrit.. Introducing TRT can stop this decrease. However, in 4–40% of men, TRT may cause polycythemia and an increase of hematocrit, which, by increasing blood viscosity, may worsen already existing cardiovascular diseases [61].

At risk of developing erythrocytosis are especially older men treated with injections of testosterone preparations [62]. Injections have a higher chance of causing erythrocytosis than topical preparations [60].

Before starting TRT, the presence of conditions causing secondary polycythemia should be ruled out. Haematological assessment is needed to prevent hematocrit over 51% during TR, before starting the treatment, in 3–4 months, after a year of therapy and then annually. Moreover, to keep the hematocrit below the threshold of 52% dose adjustment or phlebotomy may be needed [63].

Impact of TRT on men's fertility

In men wanting to preserve fertility TRT is contraindicated [4]. TRT suppresses spermatogenesis, and that suppression may last even up to a year after the treatment is discontinued [64]. Instead of TRT, gonadotropin therapy is recommended for men with secondary hypogonadism who desire to preserve fertility. Functional secondary hypogonadism testicular function is intact and should respond well to stimulation with exogenous gonadotropin [4]. Rastrelli et al., in their meta-analysis, showed that in three-fourths of patients with hypogonadism, gonadotrophin therapy-induced sperm output [65]. hCG is the most common preparation used in gonadotrophin therapy [4]. In cases of organic secondary hypogonadism, a combination of hCG and FSH was shown to have a better outcome in restoring fertility [65]. Before starting TRT, informing the patient about possible side effects and considering their decision are crucial.

Pathopsychological effects

Psychological effects of high testosterone levels are a relatively new subject in the researchers' area of interest, with the main hypotheses mainly focused on an increase in aggressive and violent behaviours [66, 67], as well as the development of antisocial personality disorder (APD). The results of such studies still seem to be inconsistent. While there is evidence of a significant correlation of APD with high testosterone levels in saliva [68], other studies prove this relationship uncertain [69]. Some authors have even proved that higher testosterone levels have no significant link to aggression but rather to venturesomeness and adventure-seeking in men who are already diagnosed with personality disorders [70].

Yildirim et al. suggest that the original reason behind antisocial, sociopathic and psychopathic personalities might be high levels of foetal and circulating testosterone, which influence the maturation of mesolimbic dopaminergic circuitry responsible for emotional processing and empathy [71]. However, the same authors consider this aspect heavily modulated by other biological and psychosocial factors and conclude that higher testosterone levels cannot be considered a singular risk factor but rather a determinant that other elements must amplify to occur [71].

Another research suggests that cortisol is one of such biological factors capable of stimulating testosterone's influence on the subject's personality. In this study, Welker et al. point out that the positive correlation between testosterone and psychopathy only occurs in men with increased cortisol levels, with no such relationship observable in subjects with low cortisol. This theory's main limitation is, however, that it is impossible to decide whether high testosterone and cortisol are a cause, or a result of psychopathy [72].

In addition to those reports, Dreher et al. explored the subject of testosterone's stimulation of prosocial behaviours in men. They found out that the participants who had had testosterone injected were more prone to confrontational behaviours than those who had received a placebo. However, they also happened to be more generous, but only if such behaviour could enhance their economic and social status [73].

Similar conclusions might be drawn from the reports regarding anabolic androgenic steroids (AAS) use and their effects on men's neural function. AAS are known to pass through the blood-brain barrier. Since most common doses are supra-physiological (even 5–100 times great-

er than natural male production of testosterone), they are hypothesised to affect the central nervous system much more visibly than endogenous high levels of testosterone [74]. According to Hauger et al., such correlation is most commonly seen in AAS-dependent men, who showed more aggressive and violent behaviours than those who use AAS but have not developed a dependency.

It has also been pointed out that AAS abuse can often be linked with moodiness, anxiety, psychotic and manic episodes, as well as depression and suicide. However, it is still a topic of discussion whether such psychological complications are a result of AAS use or if they are independent comorbidities [75]. While overly high levels of testosterone induced by the abuse of AAS is suggested to contribute to the development of major depressive disorder, its naturally correct levels seem to be a protective factor against it. This correlation is most noticeable in men with hypogonadism. This group of patients shows a higher prevalence of anxiety disorders and major depressive disorder compared to men with correct levels of testosterone. A similar connection has been pointed out in patients who received anti-androgen treatment for prostate cancer [76, 77]. In an interesting study, Li et al. noticed that one of the enzymes responsible for testosterone's degradation is 3β- hydroxysteroid dehydrogenase (3β-HSD); they pointed out that rats infected with 3β-HSD-producing Escherichia coli manifested depressive behaviour with correlating lower serum and brain testosterone levels [77].

In contrast, Zarrouf et al. pointed out that TRT administration in hypogonadal men helped with diminishing the symptoms and improving the mood, which further supports the thesis of testosterone's protective qualities [78].

Conclusions

This review explores the most important aspects of testosterone's influence on the male body. Due to the wide range of possible consequences of testosterone's various levels, it is vital to consider the side effects when treating patients with both low and high serum testosterone. Some of the negative repercussions of abnormalities in testosterone levels are more directly dangerous than others. However, they all heavily influence the organism and should be analyzed and discussed with the patient. Low serum testosterone levels correlate with a higher risk of heart failure, In contrast, TRT may be a factor in reducing it, as well as having a protective effect on the heart and preventing early cardiovascular deaths. Similarly, a relationship between lower testosterone and a higher risk of metabolic disease and obesity has been noticed, where defective lipid uptake, lipolysis stimulation or decreasing lipogenesis are considered to be the main factors. Diabetes is another consequence of low testosterone, which is confirmed by tests run on men with both a prediabetic state and T2D. It is also possible that diabetes itself causes the reduction of testosterone levels, considering how men with a long history of diabetes seem to have considerably low levels of this hormone. A possible result is personality disorders, such as APD or psychopathy, as well as an increased risk of TRT-induced infertility and polycythemia.

Interestingly, high testosterone levels might also have a negative influence on a man's health. One of possible results are personality disorders, such as APD or psychopathy, as well as increased risk of TRT-induced infertility and polycythemia.

Considering how significant correct testosterone levels seem for maintaining both somatic and psychological health and well-being, it is imperative to continue research in this area.

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The pivotal role of uridine modifications in the development of mRNA technology

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ABSTRACT

In 2023, Katalin Karikó and Drew Weissman were awarded the Nobel Prize in Physiology or Medicine for their nucleoside base modifications research that later enabled mRNA vaccine development against COVID-19. This paper briefly reviews these achievements in the context of the development of mRNA technology and its enormous potential for medicine in the prevention of various infectious diseases and cancer treatment, including personalised therapies. It is beyond any doubt that discoveries made by Karikó and Weissman were pivotal in overcoming one of the major hurdles in the practical application of mRNA molecules, i.e., the recognition of exogenous mRNAs by endosomal Toll-like receptors and downstream innate immune response, ultimately leading to the decreased translational activity of delivered mRNA and its degradation. Although the Nobel Prize for Karikó and Weissman is fully justified, it must be stressed that mRNA technology would never unfold its potential for public health without a collective scientific effort encompassing over 40 years of research.

Introduction

On October 2, 2023, the Nobel Assembly at Karolinska Institute awarded Katalin Karikó and Drew Weissman the Nobel Prize in Physiology or Medicine "*for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19*" [1]. However, this achievement will likely have a broader impact on contemporary medicine, both within and outside the prevention of infectious diseases. This article discusses the accomplishments made by Karikó and Weissman in the context of the development, achievements, and future of mRNA technology.

Brief history of practical use of mRNA molecules

To understand the impact of research conducted by Karikó and Weissman, one should first comprehend the history of mRNA technology. The mRNA molecules and their regulatory role in the synthesis of proteins in cells were described in 1961. The first attempt to introduce mRNA molecules into cells to induce the translation of the desired protein dates back to 1976 when duck globin mRNA was microinjected into human and avian cells [2]. In 1978, the rabbit globin mRNA was introduced into mouse lymphocytes using liposomes as vehicles [3]. Almost a decade lat-

er, in 1989, the efficient and reproducible method for RNA transfection, based on cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride, incorporated into a liposome, was developed as shown by *in vitro* introduction of *Photinus pyralic* luciferase mRNA, synthesized *in vitro*, into variety cell types, including human, that resulted in increased enzyme activity [4]. One year later, mRNAs encoding chloramphenicol acetyltransferase, luciferase, and beta-galactosidase were injected into mouse skeletal *in vivo*, leading to detectable protein expression [5]. Soon, this approach was attempted for immunization (e.g., against influenza) and led to the induction of humoral and cellular immunity in mice [6, 7].

Revolutionary nucleosides modifications

However, significant challenges arose: (1) the vehicles used for mRNA had unfavorable safety profiles, (2) the use of naked mRNA was prone to immune recognition and degradation by RNAse, and (3) using dendritic cells transfected with mRNA *ex vivo*, offered as the potential solution to issues described in point 1 and 2, was impossible to be implemented in mass vaccinology [8]. Works by Karikó and Weissman provided a solution to the issue described in point 2, i.e., sensing of exogenous RNA by endosomal Toll-like receptors (specifically, TLR3, TLR7, and TLR8), ultimately leading to the production of pro-inflammatory cytokines and type I interferons, which activate RNA degradation [9]. However, as shown in 2005, the incorporation of various modified nucleosides ablated this response to different extents, resulting in higher translational activity of mRNA. Specifically, using N^6-Methyladenosine and ^{2}-Thiouridine suppressed the ability of RNA to stimulate TLR3, whereas N^6-Methyladenosine, ^{5}-Methylcytidine, ^{5}-Methyluridine, ^{2}-Thiouridine, and pseudouridine (Ψ) modifications blocked stimulation of TLR7 and TLR8. The immune stimulation was also suppressed proportionally with the number of modified nucleosides incorporated in RNA, but even a few modifications were superior compared to unmodified RNAs [10]. Substituting uridine with Ψ (see **Figure 1**) was eventually evidenced to significantly increase the activity of exogenous mRNA introduced into cells by reducing their recognition by innate immunity and increasing the stability of the RNA molecule [11, 12]. Realization of this was pivotal for the further development of the mRNA platform. As postulated, the altered secondary structures in modified mRNAs cannot be recognized effectively by RNA-dependent protein kinase, which correlates with attenuated IF2α phosphorylation [13]. As shown later by other authors, the substitution of uridine by N^1-Methyl-pseudouridine ($m^1\Psi$) (see **Figure 1**) revealed an even better performance than the use of Ψ because, in addition to TLR7 and TLR8, it also decreased the activation of TLR3 [14]. As suggested, this superb translation activity of $m^1\Psi$-containing mRNA could result from increased ribosome density resulting from the deceleration of elongation [13]. Broader evasion of Toll-like receptors and downstream innate immune signalling improved mRNA's cellular viability and significantly increased translation [14].

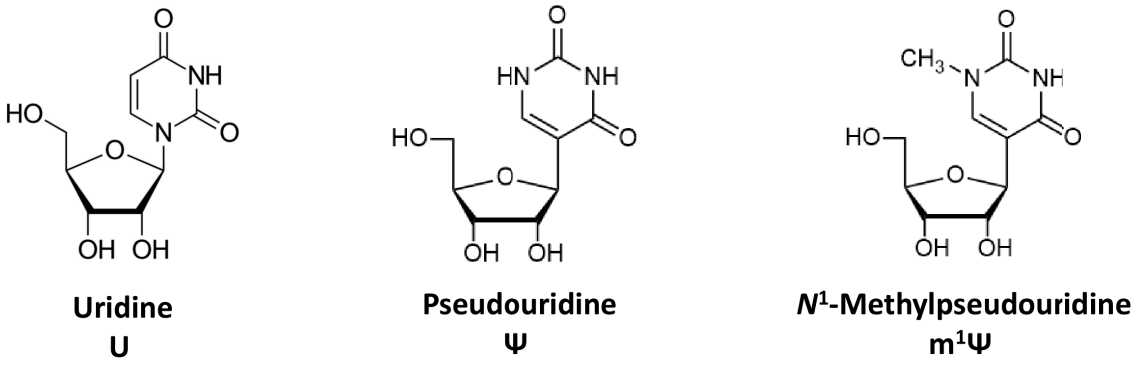

Figure 1. The uridine is a natural constituent of mRNA molecules. Pseudouridine is one of the modified nucleosides discovered by Katalin Karikó and Drew Weissman, 2023 Nobel Prize laureates in Physiology or Medicine, to evade Toll-like receptors recognition of exogenous mRNAs and increase the translational activity of these molecules. N^1-Methylpseudouridine was later demonstrated to be even more superior in this regard and was eventually used in mRNA vaccines against COVID-19.

Achievements of mRNA vaccines

Both authorized mRNA vaccines against COVID-19, i.e., BNT162b2 (BioNTech/Pfizer) and mRNA-1273 (Moderna), employed $m^1\Psi$ substituting each uridine [15]. Their use has been evidenced to be a life-saving intervention. COVID-19 vaccines, including mRNA vaccines given at over 2.5 billion doses, have averted an estimated 19.8 million deaths in the first year of the global COVID-19 vaccination campaign. The number of deaths averted per administered dose was more significant in high-income countries, and this phenomenon was attributed to better access to more immunogenic and efficacious mRNA vaccines [16]. In other words, vaccine equity, postulated numerous times throughout the COVID-19 pandemic, would save even more lives [16–19]. A Polish retrospective study also evidenced the high effectiveness of the mRNA vaccine, BNT162b2, in preventing COVID-19 deaths, with an estimated 61,803 deaths averted by vaccination in 2021 in Poland [20].

Future of mRNA technology

Beyond any doubt, such public health benefits would not be possible without previous discoveries made by Karikó and Weissman. However, their significance was not fully realized for years. The success of mRNA vaccines against COVID-19 led to continuous interest in further applications of the mRNA platform. As discussed recently, this technology provides various advantages, bypassing numerous issues that had long been slowing the progress of vaccine candidates when employing more traditional approaches [17]. As a result, various candidates developed using mRNA technology, i.e., against influenza viruses (including universal mRNA influenza vaccine), human immunodeficiency virus 1, respiratory syncytial virus, Nipah virus, Zika virus, human cytomegalovirus, and Epstein-Barr virus are currently on different stages of testing, including clinical studies [17].

Moreover, the mRNA platform is employed to develop novel cancer therapeutics with encouraging results from early clinical trials employing mRNA as monotherapy and in combination with checkpoint inhibitors [21]. The flexibility of mRNA technology allows the mRNA sequence to be quickly optimized to specific tumour-associated neoantigens that can vary widely between individuals, ultimately allowing the direction of the immune system in a highly personalized treatment approach [22]. Its potential has been recently shown in the phase 1 clinical trial of personalized mRNA neoantigen vaccine BNT122, expressing up to 20 neoantigens, for treating pancreatic ductal adenocarcinoma, a highly malignant form of cancer [23].

The collective research effort

The Nobel Prize in Physiology or Medicine for the achievements of Karikó and Weissman is fully justified. However, one should note that the mRNA technology would not unfold its potential for public health without a collective effort encompassing over 40 years of research. Pivotal discovery also included the development of nanoparticle carriers (formulated with PEGylated lipids, cholesterol, ionizable lipid, and phospholipids), which are characterized by an improved safety profile compared to cationic lipids used initially and enhance the cellular delivery of mRNA molecules [24]. Moreover, modifications of the 5′ cap and 3′ poly-A tail of mRNAs and selection of particular 5′UTR and 3′UTR also significantly stabilize mRNA molecules and increase their translational efficiency [25–27]. The critics highlight that the way the Nobel Prizes recognize individuals does not reflect the collaborative nature of modern research [28]. As Richard Feynman, a 1965 Nobel Laureate in Physics, once said, when asked about the meaning of this award: "*I don't like honours. I've already got the prize. The prize is the pleasure of finding the thing out, the kick in the discovery, the observation that other people use it. Those are the real things*". In the case of mRNA technology, the real thing is human health that has already been saved and can be saved in the future.

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The author declares no conflict of interest.

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Perspectives for better health: prepare for the exiting severe phase of the COVID-19 pandemic

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ABSTRACT

On May 5, 2023, the World Health Organisation declared that COVID-19 was no longer considered a public health emergency of international concern, and various governments are closing their healthcare facilities, meticulously prepared for the world's worst pandemic. However, the question arises of how to exit this pandemic's severe phase responsibly. The present communication discusses the follies of being underprepared to tackle COVID-19, particularly by the economically weaker nations, and it also outlines the necessary steps that are now needed for better management of future pandemics with international cooperation of the developed and developing countries. More attention is needed to address the paradox of digital access in excess but of little technical use, particularly in emergencies like the recent viral pandemic. If the scientific analyses of the healthcare issues had been carried out correctly, their predictions and strategies supported by authentic big data, managing the pandemic could have been better. Still, there is time and expertise developed recently that can help future generations. On the individual front, mass education, awareness of hygiene, and understanding and application of basic science, particularly in rural areas, needed to be safer rather than depend on traditional beliefs and expect miracles, a system prevalent in developing countries.

Introduction

The COVID-19 emergency in most parts of the world is at its fag end, and governments of the member nations of the World Health Organization are closing their healthcare facilities, which were meticulously prepared for the world's worst pandemic. Global efforts have controlled the coronavirus using state-of-the-art technological innovations [1]. Science has made it possible in a very short period. However, the world has lost millions, partly due to the mishandling of the viral attacks. Ultimately, the crisis has reminded us about the importance of health and well-being in our societies. Healthcare personnel worldwide should learn from earlier mistakes to mount more robust responses, as the transition from the pandemic to the endemic is relatively recent. The

successful control of the virus and its emerging lineages and sub-lineages has indicated some concealed failures that must be addressed now, requiring more vigilance, as even a bit of laxity can spoil the good work done. On societal and governmental parts, the utmost care and caution are required long-term. Healthcare systems battered by famines, droughts, and epidemics in low-income countries have been more affected by COVID-19, causing a great reverse in decades of economic and health sector improvements and reiterating the need for various national governments to prioritise issues of healthy living and well-being and work towards the goal of Sustainable Development Goals [2].

Post-COVID-19, several deluges follow, a mix of viral endemics asking to close one of the most outstanding global efforts very casually. For COVID-19, this means understanding the critical contradictions at the core of the pandemic in various parts of the developing and developed world, including the USA. The Global Health Security Index data shows that even high-income countries, which tend to respond well to pandemics, experienced over 6960783 confirmed deaths from COVID-19 [3]. However, this number may differ since correct data are unavailable for confirmed deaths due to the pandemic. Msemburi et al. [4] found it was 2.74 times higher globally than the figure reported till Dec 2021. The catastrophic destruction leading to deaths by COVID-19 in India was at its peak during the middle of 2021. The highest cumulative excess death numbers for India accounted for 4.74 million deaths. Conclusively, it is a cumulative loss of millions of human lives worldwide, enough to point out that mismanaged causalities cannot be counted for sure in an uncontrolled pandemic.

Getting Prepared

People from low-income countries with mediocre healthcare efficiencies can only partially rely on government healthcare help. They must be ready to pool resources to make a robust platform for providing essential health services. The European Union and the high-income countries have different healthcare systems, as fewer of the population are financially supported by their governments. Most of these countries have a system of tightly regulated, competing private health insurance companies, with government subsidies available for citizens who cannot afford coverage. The problem lies more with economically weaker nations. For instance, in India, bearing hospital costs has been typically out of pocket for decades, particularly in economically weak nations. However, the government has provided some help by increasing health budgets and doling out freebies at the cost of taxpayers, which is not enough to cater to the needs of millions. More than allocated government funds will be required to improve health care; alternate resources like out-of-pocket or pooled pockets must be worked out for the escalating health expenditures.

Governments with administrative transparency as their thrust area can plan to look after their people better with long-lasting solutions for delivering health. Creating viable joint financial strategies is needed for the real-time execution of essential public health functions, which are bound to magnify the ongoing challenges of existing and emerging infectious disease outbursts. The robust digitalisation of health metadata and its judicious use after proper scientific interpretation in populous nations is an important area requiring immediate attention. People who need the most medical attention are least known to us.

The COVID-19 pandemic has revealed the limitations of digital access to health data, despite having technology at hand. If we had used this state-of-the-art scientific analysis of COVID-19, its predictions and strategies supported by authentic healthcare data things would have been different in managing the pandemic. Still, expertise developed recently by regional and global efforts can help the health systems of future generations.

There is a need to establish and strengthen early identification and reporting epidemic systems, like a set of index systems, which can be established to analyse and evaluate the situation of infectious disease, judge the probability and severity of the crisis, and decide whether to send a crisis alarm. Health administrators must find sensitive and effective early warning indicators and establish a complete monitoring system for controlling future epidemics, helping us to identify leading threats and develop state-of-the-art therapies in advance.

The global vaccination programs and other urgent measures were accomplished in record time by coming together. These sincere efforts demonstrate how, in an emergency, the world's healthcare systems can be saved by using advanced technologies and helping each other. The effects of global vaccination and access to mRNA technology offered rapid vaccine adaptation to the rapidly changing viral variants, one of the best tools to control COVID-19 [5].

In this regard, various techniques have been employed to develop new vaccines. Among these, the COVID-19 messenger RNA (mRNA) vaccine has drawn significant attention due to its tremendous application prospects and advantages, which include a short development cycle, easy industrialization, a simple production process, flexibility to respond to new variants, and the capacity to induce better immune responses. Consequently, scientists are creating rapid plug-and-play technical platforms using mRNA technology or adenovirus vectors that can be quickly modified to combat a specific emerging threat [5,6].

There is also a necessity to access stronger and therapeutically effective antivirals, which can directly decrease the risk of severe infections through emerging new variants. These drugs should include broad-spectrum antivirals such as umifenovir, protease inhibitors like lopinavir /ritonavir RNA-dependent RNA polymerase inhibitors, remdesivir, and favipiravir. Other drugs that have been used include nucleosidase inhibitors and polymerase acidic endonuclease inhibitors, which are approved to prevent influenza infections. While some drugs appear promising in small case series and reports, more clinical trials are required to provide higher-quality evidence [7].

Omicron lineage is dominant worldwide, with infections driven by its emerging sub-lineages. Although the current understanding of the virus is improving, its evolution is inherently unpredictable, and a likely future scenario is the emergence of new potential variants that may be antigenically and phenotypically distinct from the early forms. Healthcare specialists must remain prepared for such evolutionary behaviour of emerging variants that could threaten the future by developing more specifically targeted antivirals. It is, therefore, of public health and clinical importance to understand the drivers of the changing virulence of the causative agents [8].

The instinctive survival attitudes also showed that strict compliance with protocols and guidelines can control the worst of calamities. The efficient and disciplinary handling of the waves of infectious diseases, including COVID-19, using advanced technology by the government of Saudi Arabia is an interesting example to illustrate that successful healthcare management strategies can save human lives. Even during the COVID-19, a multidisciplinary Saudi team from governmental sectors, including the Global Center for Mass Gatherings Medicine, shared in the assessment, planning, execution, and success of this holy event to prevent the spread of disease, and almost zero cases were reported during the Hajj pilgrimage of 2020 amid peak corona pandemic worldwide [9].

This instance highlights the success of the risk mitigation plan in place during the Hajj pilgrimage in 2020 during the COVID-19 pandemic and the efforts of the Saudi government to prevent associated outbreaks. Keeping the health care system fully prepared and even visualizing for the coming years, Saudi Arabia, under its recent Vision 2030 [10], wisely opted for thoughtful plans of action for its fellow citizens, saving unnecessary deaths to a great extent, proving the fact that investment in health never goes in vain in the long term.

Thus, health policy experts have to be more pragmatically prepared for the rapidly expanding burden of chronic conditions arising out of rapid viral mutations and their clinical severities all over the globe, in particular in developing economies. Intensive and active surveillance of susceptible animal species is needed as reverse zoonosis is documented, compounding the problem for inadequate facilities of genetic sequencing in many economically weak countries. There are also places with previously good surveillance that are decreasing or phasing out sequencing altogether as maintenance of high-tech facilities depends upon funds.

A lack of genomic surveillance will mean future variants will be detected much later or could be circulating at low levels before eventual detection. There is an urgent need for widespread and equitable local and regional surveillance coverage to rapidly detect potential new variants among infected communities before they spread more widely. In this regard, policy-

makers worldwide must strengthen their remote primary healthcare systems, taking lessons from such countries that have dedicated significantly despite the paucity of funds. We must gather rapid international commissioned support to secure the health of the world populations across the life course, using and maintaining new advanced technologies necessary for surveillance and survival [11,12].

One of the essential preparatory things that must be done at all levels to battle emerging pandemics is maximising the merger of private and government financial sources to achieve complex healthcare goals. The power of such partnerships between the private and government sectors must be worked out, needing more support. During this pandemic, most of the underprivileged parts of the world have seen the philanthropy of many international funders, both government and private, who have contributed to the cause of humanity with significant transparency and remarkable results. If respective government agencies across the continents tie up financially with known charitable trusts, the management of pandemics will be better.

Another area needing attention is local governments of developing countries coming forward to waive off taxes for long-term health care settings or provide significant tax reliefs. For instance, in India, the government can relieve the middle class by not paying substantial yearly increasing premiums for health insurance, either by reducing or exempting the taxes. With a transparent monitoring system, government sharing of healthcare insurance schemes like micro-financing on very low premiums can be made for the financially weak, who are more vulnerable to out-of-pocket expenses. Though several national and state-run schemes are available in most Asian countries, they are either extravagant or misused due to corruption, causing grief rather than relief to the people.

The governments of developing countries, who already suffer from workforce shortages and other issues in healthcare systems, should take advantage of the lessons learned during this crisis and build resilience to combat future health pandemics and achieve progress towards Universal Health Coverage. International organizations must scale up financing for pandemic preparedness while strengthening the financing of the World Health Organization. They must empower multilateral development banks to play a more prominent role in financing global public goods, as recommended by the OECD, to prepare for the next pandemic [13,14].

On the individual front, mass education, hygiene awareness, and basic science application are necessary for rural areas to be safer rather than depend on traditional beliefs and expect miracles, a system rampant in developing nations. In these countries, more attention is required to monitor animal-transmitted diseases by ensuring scientific and hygienic conditions [15].

Due to the financial crisis, many countries must prepare to enact plans for future pandemic prevention or response. They face numerous challenges in providing universal health coverage for citizens. They cannot allocate adequate investment or resources towards, for example, an increased workforce with low resources equipped to deal with current or future pandemics. Globalization starting from local areas is required with international cooperation.

India can contribute by providing affordable, effaceable, and cost-effective maintainable vaccines to the developing world, addressing the cold-chain storage issues faced in developing nations. However, as urged numerously by different groups of scientists [6,16–18] the vaccine inequality must be removed, as presently, the low-income countries get minimal supplies. COVID-19 revealed the fragility of vaccine production. The world heavily depends on just a few manufacturers. Vaccines and therapeutics must be made more equitably, with regional hubs ready to mass produce high-quality medical products in an emergency. It is pivotal to ensure the ability of lower-income regions to manufacture and distribute vaccines based on technologies, such as the mRNA platform, which allow a rapid response to the epidemiological threat and, subsequently, decrease the burden of infectious diseases more effectively [19,20].

Building local manufacturing capacity requires waiving intellectual property rights, and training people to work in them, often in low-resource settings, which necessitates the participation of the private sector alongside governments, given its role in vaccine research, production and distribution. The COVID-19 crisis has tested the resilience

and agility of various countries' health systems in an unprecedented way, shedding harsh light on its strengths and weaknesses; in several cases, a lack of preparedness, equipment and infrastructure to deal with an event of these proportions. However, the pandemic has also highlighted great solidarity, inventiveness, and resilience, not least on the part of the health workforce, which has led the way in fighting the pandemic on the ground. It has reminded us that health threats know no borders and that these challenges can only be faced if we work together across borders and sectors. Industrialised economies can significantly contribute in this regard, which will be a massive step in humanitarian equity and will work as a genuinely efficacious vaccine, much needed for global healthcare systems.

Summary

- In order to be better prepared for future pandemics, which are bound to magnify, pooling resources to make a strong platform for providing essential health services is recommended.
- Governments with transparency can look after their people better by creating joint financial strategies with private organisations for the real-time execution of essential public health functions.
- Robust digitalization of health metadata and its judicious use after proper scientific interpretation in populous nations to better manage outbursts of global infections requires immediate attention.
- More attention must be focused on easy access to effective anti-virals, which can directly decrease the risk of severe infections through emerging variants. Scaling up global vaccination programs with equity can avert millions of deaths. On the individual front, hygiene awareness, monitoring zoonotic diseases and applying basic science, particularly in rural areas, is needed.

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Data Availability
Relevant collected data are available on reasonable request to the corresponding author.

Conflict of interest statement
The authors declare no conflict of interest.

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Some examples

Standard journal articles

1. Petrova NV, Kashirskaya NY, Vasilyeva TA, Kondratyeva EI, Marakhonov AV, Macek Jr M, Ginter EK, Kutsev SI, Zinchenko RA. Characteristics of the L138ins (p.Leu138dup) mutation in Russian cystic fibrosis patients. JMS [Internet]. 2020 Mar 31;89(1):e383. doi: 10.20883/medical.383.

Books

Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

TABLES: Tables should be typed on sheets separate from the text (each table on a separate sheet). They should be numbered consecutively with Arabic numerals. Tables should always be cited in text (e.g. table 2) in consecutive numerical order. Each table should include a compulsory, concise explanatory title and an explanatory legend. Footnotes to tables should be typed below the table body and referred to by superscript lowercase letters. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript (e.g. in figures).

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