Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2023, Volume 3, Page No: 191-206

Available online at: www.galaxypub.co/page/journals

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Immune-Regulatory Effects of Zingiber officinale Bioactives in Infants of Obese Mothers: Insights from a Network Pharmacology Study

Amina El-Sayed[1]*, Karim Hassan[1], Nour Abdelrahman[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.
[2]Department of Biotechnology, Faculty of Science, Ain Shams University, Cairo, Egypt.

***E-mail** ✉ amina.elsayed@gmail.com

Received: 04 October 2022; **Revised:** 28 January 2023; **Accepted:** 05 February 2023

ABSTRACT

Maternal obesity during pregnancy can elevate the likelihood of negative health consequences in offspring, including compromised immunity. At present, no established therapeutic protocol exists for pregnant women with obesity to mitigate risks to both mothers and babies. Zingiber officinale (ginger) is a widely utilized herbal remedy for managing symptoms of hyperemesis gravidarum. Its active constituents are known for possessing anti-obesity and anti-inflammatory effects. This research sought to examine potential interactions between ginger's plant-derived compounds and proteins associated with immune responses in children of obese mothers, using a network pharmacology methodology. Disease-related proteins were retrieved from GeneCards, while compound structures were obtained from PubChem. Cytoscape was employed to build protein-protein interaction networks. Compound-gene interactions were visualized, followed by pathway enrichment analysis and molecular docking simulations. Employing network pharmacology combined with molecular docking, 36 active ingredients from Zingiber officinale were found to influence critical proteins (TLR4, NF-κB, TNF-α) implicated in inflammatory processes and cytokine release. Key components, namely 6-gingerol and 6-shogaol, exhibited robust binding to TLR4 according to docking results, positioning them as potent ligands for this receptor. This is particularly relevant due to TLR4's involvement in inflammation linked to developmental origins of obesity. The findings highlight the capacity of Zingiber officinale's active ingredients, especially 6-gingerol and 6-shogaol, to modulate immune imbalances in offspring of obese mothers, targeting essential elements like TLR4, NF-κB, and TNF-α.

Keywords: Gingerol, Inflammation, Shogaol, TLR-4, TNF-α.

How to Cite This Article: El-Sayed A, Hassan K, Abdelrahman N. Immune-Regulatory Effects of Zingiber officinale Bioactives in Infants of Obese Mothers: Insights from a Network Pharmacology Study. Spec J Pharmacogn Phytochem Biotechnol. 2023;3:191-206. https://doi.org/10.51847/0oLX7X1pai

Introduction

Obesity represents a major public health challenge in developed and emerging countries alike. Its global incidence has risen dramatically, reaching pandemic proportions [1]. It contributes to numerous conditions, such as insulin resistance, high blood pressure, dyslipidemia, non-alcoholic fatty liver disease, and heart-related disorders [2, 3]. At its core, obesity stems from caloric excess leading to fat accumulation, especially in visceral areas. Excess nutrients trigger adipocyte enlargement and proliferation [4]. These alterations are accompanied by immune cell recruitment, resulting in chronic low-grade inflammation and extracellular matrix reorganization to accommodate growth [5, 6].

Obesity is increasingly common among women of reproductive age. Epidemiological data indicate that around 14% of pregnancies are affected by maternal obesity [1, 7]. This condition raises the chances of maternal complications, including gestational diabetes, hypertensive disorders of pregnancy, preeclampsia, chorioamnionitis, and urinary tract infections, with potential effects on fetal development [8, 9]. Offspring face higher risks of large birth weight, low Apgar scores, and neonatal hypoglycemia. Long-term issues in children

include obesity, altered blood pressure and lipids, insulin resistance, cardiovascular problems, and greater vulnerability to infections [10, 11].

Maternal obesity during gestation is associated with placental structural changes and impaired vascular function, hindering nutrient delivery to the fetus [9, 12]. Research by Challier *et al*. revealed placental modifications in obese pregnancies, including macrophage phenotype shifts and elevated pro-inflammatory cytokines [13]. Such placental inflammation influences systemic responses, heightening infection susceptibility in newborns [11, 13, 14]. Multiple investigations have documented higher rates of infections—covering respiratory, ear-nose-throat, and digestive tracts—in children of obese mothers during early childhood [7, 14, 15].

No approved standard therapy currently addresses obesity in pregnant women. Ginger (Zingiber officinale Roscoe), from the Zingiberaceae family and Zingiber genus, is frequently consumed by expectant mothers to ease nausea and vomiting in hyperemesis gravidarum [16, 17]. Ginger extracts are also valued for their effects against obesity, inflammation, oxidation, and microbial growth [18, 19]. Preclinical evidence shows that ginger derivatives or compounds like gingerol and shogaol can dampen inflammation in models of metabolic disorders and diabetes [20-22]. These properties position ginger as a candidate for maternal health applications. Although Zingiber officinale has been studied extensively, detailed mechanistic insights into its role in supporting immune function in offspring of obese mothers remain limited. This investigation applied network pharmacology to assess how Zingiber officinale's active elements might engage with proteins involved in immune alterations due to maternal obesity. We proposed that these plant compounds possess broad-spectrum regulatory effects on immunity in affected infants.

Materials and Methods

Ethical approval

The research received approval from the Ethics Committee of the Faculty of Medicine, Universitas Indonesia, under reference KET-1279/UN2.F1/ETIK/PPM.00.02/2024.

Compilation of zingiber officinale constituents database

Chemical components of Z. officinale were collected from existing literature on its phytochemical profile [23, 24]. Structures in SDF format were downloaded from PubChem for 2D representations [25]. These models were used to evaluate compound suitability as drugs. Druggability was assessed via the SwissADME platform (<http://www.swissadme.ch/>), incorporating Lipinski's rule of five criteria and oral bioavailability forecasts [26, 27].

Development of disease-associated protein database

Relevant proteins were extracted from GeneCards [28]. Searches in the disease category employed terms like "obesity AND pregnancy AND bacterial infection AND children AND innate immunity AND cellular immunity AND humoral immunity". Gene-disease associations were exported as XLSX files.

Identification of zingiber officinale compound targets

SMILES notations for Z. officinale components were inputted into SWISS Target Prediction (<http://www.swisstargetprediction.ch/>) for predicting binding proteins [29]. Results were restricted to Homo sapiens. Protein identifiers were matched to UniProt entries to standardize names and remove redundancies. Protein interconnections were then queried in the STRING database.

Ranking of key disease-related proteins

All predicted targets were uploaded to STRING (<https://string-db.org/>) via the multi-protein interface [30], limited to human species with a high confidence threshold (0.900). Interaction data were saved in TSV format. Networks were visualized and analyzed in Cytoscape 3.10.2 to rank nodes by degree centrality [31, 32].

Target prediction for zingiber officinale phytoconstituents and interactions with targets linked to disease pathogenesis

Results from SwissTargetPrediction revealed potential targets for every phytochemical constituent identified in Z. officinale. To gain deeper insights into the underlying mechanisms, compound-target associations were mapped

and displayed as a network using Cytoscape version 3.10.2, depicting relationships among the bioactive molecules and their corresponding proteins [31, 32]. This visualization was further combined with a protein-protein interaction (PPI) network to construct an integrated compound-target framework for Zingiber officinale, emphasizing pathways related to the immunological responses in offspring of mothers with obesity during gestation.

Enrichment analysis

Functional enrichment was performed to explore gene ontology (GO) annotations and associated pathways. Gene Ontology (GO) classifies gene functions and their associated products into three primary categories: molecular functions, biological processes, and cellular components. Molecular functions describe activities at the molecular scale executed by gene products. Biological processes involve coordinated molecular events leading to broader outcomes, whereas cellular components indicate the specific subcellular locations where these gene products are active [33]. In parallel, pathway enrichment was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [34]. Both GO and KEGG analyses were executed via the online platform Metascape [35].

Molecular docking

Three-dimensional structures of key target proteins were sourced from the RCSB Protein Data Bank for the docking studies. Ligand structures in 3D format were obtained from PubChem. Interaction assessments between proteins and ligands were conducted with AutoDockTools, involving the elimination of water molecules and the incorporation of polar hydrogens. Binding pockets were defined according to the coordinates of co-crystallized ligands in the original structures. The search space was set as a grid with dimensions of 60 × 60 × 60 Å across the x, y, and z directions. Docking simulations were run using AutoDockTools [36] and employed the Lamarckian Genetic Algorithm following parameter optimization. Validation was confirmed by re-docking the original ligand to its receptor, yielding a root-mean-square deviation (RMSD) below 2 Å, which verifies the reliability of the selected docking conditions [37].

Results and Discussion

A total of fifty-three bioactive constituents from Zingiber officinale were compiled based on existing literature reports [23, 24]. Their two-dimensional molecular structures were retrieved from PubChem. Drug-likeness properties and potential oral bioavailability of these constituents were assessed using SwissADME, applying Lipinski's Rule of Five as the criterion **(Table 1).**

Molecular weights varied between 192.21 and 467.60 g/mol, with dehydrozingerone as the smallest and 10-zingerine as the largest. Predictions indicated high gastrointestinal absorption for 50 of the 53 molecules. Compliance with Lipinski's Rule of Five was observed in 49 compounds, with the remaining four showing a single violation.

Target prediction via SWISS identified associations for 49 constituents of Z. officinale with 5,405 specific proteins. After deduplication, this yielded 861 unique target proteins across the 49 selected compounds **(Figure 1).** Protein interactions among these targets were then queried in the STRING database, identifying 622 proteins with interconnections at a 90% confidence threshold **(Figure 2).** Network visualization and analysis were performed in Cytoscape 3.10.2.

Table 1. Prediction of *Zingiber officinale* known phytoconstituents and their drugability

Molecule	Formula	MW (Dalton)	Compound CID	Lipinski Violation	GI absorption
4-Gingerol	C15H22O4	266.33	5317596	0	High
6-Gingerol	C17H26O4	294.39	442793	0	High
8-Gingerol	C19H30O4	322.44	168114	0	High
10-Gingerol	C21H34O4	350.49	168115	0	High
12-Gingerol	C23H38O4	378.55	118547702	0	High
Acetoxy-10-gingerol	C24H38O4	390.56	157009850	1	High
Zingerone	C11H14O3	194.23	31211	0	High
6-Paradol	C17H26O3	278.39	94378	0	High

7-Paradol	C18H28O3	292.41	13733135	0	High
8-Paradol	C19H30O3	306.44	213821	0	High
10-Paradol	C21H34O3	334.49	51352076	0	High
Methyl-6-paradol	C18H28O3	292.41	85807832	0	High
8-Paradyl monoacetate	C21H32O4	348.48	131752880	0	High
1-Dehydro-6-gingerdione	C17H22O4	290.35	9796015	0	High
1-Dehydro-8-gingerdione	C19H26O4	318.41	44610342	0	High
1-Dehydro-10-gingerdione	C21H30O4	346.46	14999388	0	High
12-Dehydrogingerdione	C23H34O4	374.51	154791045	0	High
6-Gingerdione	C17H24O4	292.37	162952	0	High
10-Gingerdione	C21H32O4	348.48	5317591	0	High
4-Shogaol	C15H20O3	248.32	9794897	0	High
6-Shogaol	C17H24O3	276.37	5281794	0	High
8-Shogaol	C19H28O3	304.42	6442560	0	High
10-Shogaol	C21H32O3	332.48	6442612	0	High
12-Shogaol	C23H36O3	360.53	9975813	1	High
Methyl-6-shogaol	C18H26O3	290.40	91721066	0	High
Methyl-8-shogaol	C20H30O3	318.45	91721121	0	High
4-Gingerdiol	C19H28O6	352.42	5318274	0	High
6-Gingerdiol	C17H28O4	296.40	11369949	0	High
10-Gingerdiol	C21H36O4	352.51	101572265	0	High
Diacetoxy-4-gingerdiol	C19H28O6	352.42	5318274	0	High
Diacetoxy-6-gingerdiol	C21H32O6	380.48	57341725	0	High
Methyl diacetoxy-6-gingerdiol	C22H34O6	394.50	5319662	0	High
6-Dihydroparadol	C17H28O3	280.40	10378937	0	High
4-Isogingerol	C15H22O4	266.33	11482504	0	High
6-Zingerine	C22H29N5O3	411.50	101798901	0	High
8-Zingerine	C24H33N5O3	439.55	101798902	0	High
10-Zingerine	C26H37N5O3	467.60	101798903	0	Low
6-Isoshogaol	C17H24O3	276.37	11694761	0	High
Dehydrozingerone	C11H12O3	192.21	5354238	0	High
Beta-Sitosterol	C29H50O	414.71	222284	1	Low
Tetracosanoic	C24H48O2	368.64	11197	1	Low
Hexahydrocurcumin	C21H26O6	374.43	5318039	0	High
Octahydrocurcumin	C21H28O6	376.44	11068834	0	High
Zerumbone	C15H22O	218.33	5470187	0	High
Gingerenone A	C21H24O5	356.41	5281775	0	High
2-Gingerol	C13H18O4	238.28	59305567	0	High
8-Gingerdiol	C23H36O6	408.53	156373480	0	High
Methyl-6-gingerol	C18H28O4	308.41	70697235	0	High
Methyl-8-gingerol	C20H32O4	336.47	86217089	0	High
Methyl-10-gingerol	C22H36O4	364.52	86181346	0	High
Methyl-6-gingerdiol	C22H34O6	394.50	101564407	0	High
8-Gingerdione	C19H28O4	320.42	14440537	0	High
6-Gingesulfonic acid	C17H26O6S	358.45	126890	0	High

A total of 240 proteins were found to be associated with the immune responses in offspring of mothers experiencing obesity during gestation. These proteins were mapped for interconnections via the STRING database, yielding 194 proteins that demonstrated reliable interactions at a 90% confidence threshold **(Table 2).** The resulting protein-protein interaction (PPI) network was then evaluated in Cytoscape 3.10.2 to assess node connectivity degrees. The outcomes of this evaluation are presented in **Figure 3.** Proteins with elevated degree scores are indicative of greater centrality and influence in the underlying disease mechanisms. The proteins exhibiting the highest degrees included IFNG, TNF, IL6, CD4, IL10, CD8A, IL1B, TLR4, IL2, and IL4.

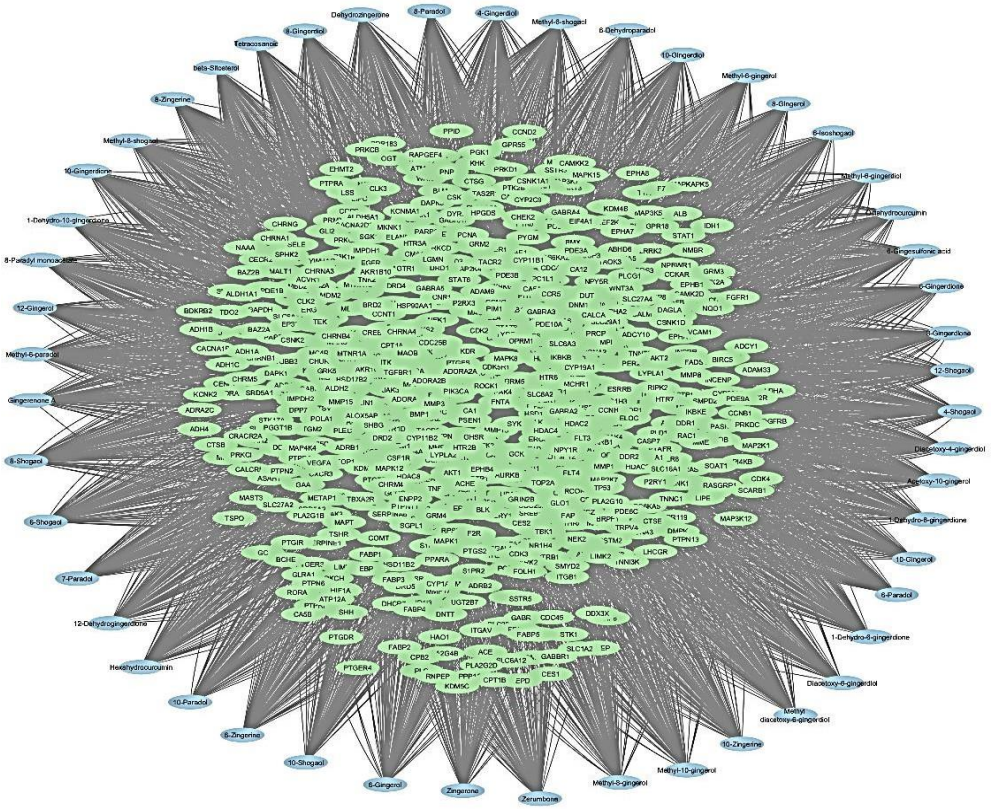


Figure 1. Different target proteins (861) from 49 phytochemical compounds from *Zingiber officinale*, as displayed with Cytoscape

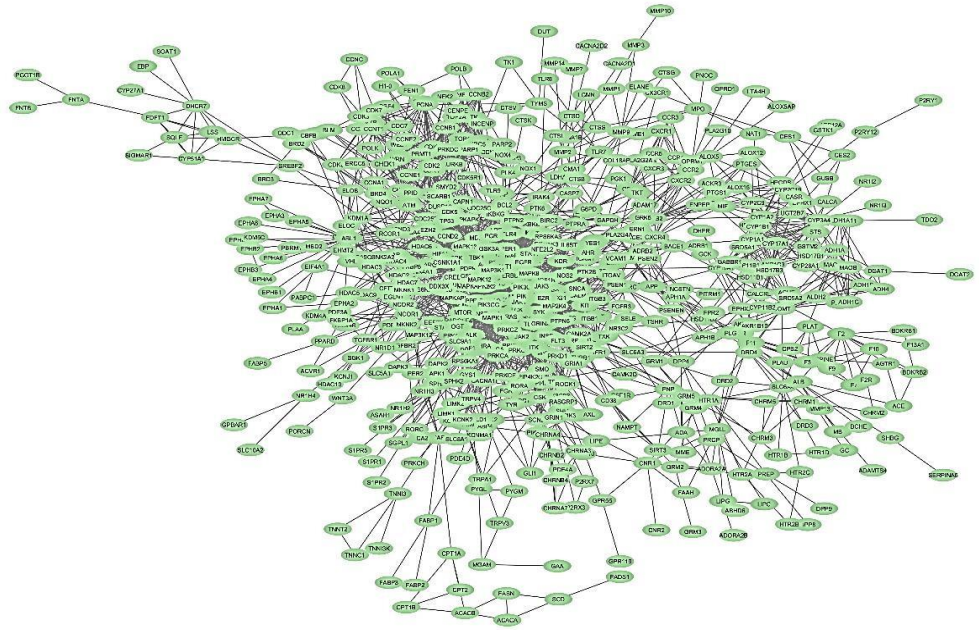


Figure 2. Interactions of 622 target proteins associated with the immunological response of children from obese mothers at a 90% confidence level

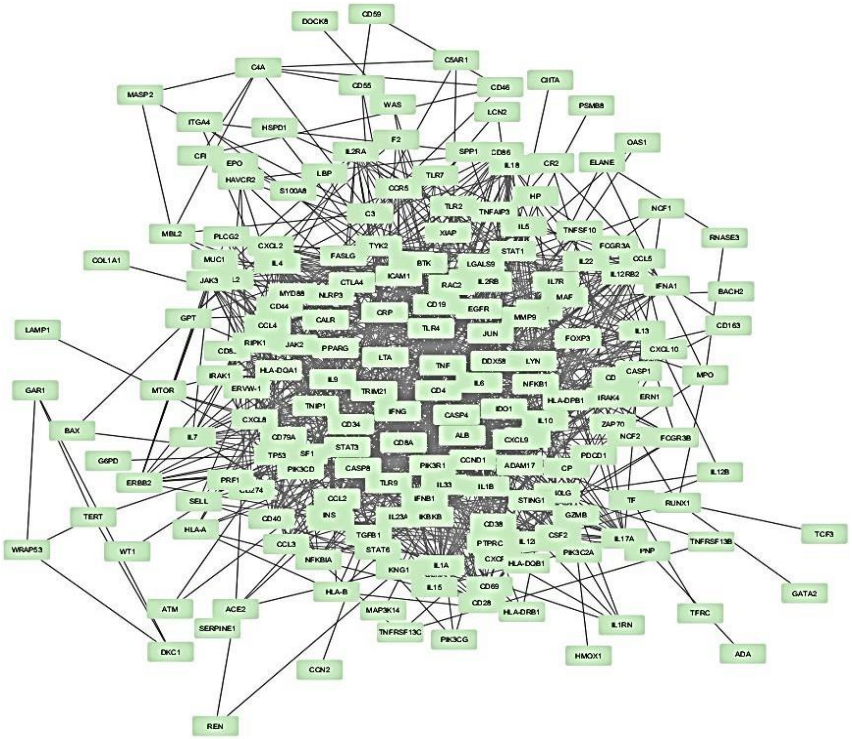


Figure 3. Protein-protein interaction network related to the immune response of children born to obese mothers during pregnancy

Table 2. Target proteins with a 90% confidence level identified using the STRING database

No	Gene Symbol	Description	Degree	UniProt ID
1	IFNG	Interferon gamma	58	P01579
2	TNF	Tumor necrosis factor	54	P01375
3	IL6	Interleukin 6	53	P05231
4	CD4	CD4 molecule	48	P01730
5	IL10	Interleukin 10	44	P22301
6	CD8A	CD8a molecule	43	P01732
7	IL1B	Interleukin 1 beta	43	P01584
8	TLR4	Toll-like receptor 4	33	O00206
9	IL2	Interleukin 2	30	P60568
10	IL4	Interleukin 4	30	P05112
11	CXCL8	C-X-C motif chemokine ligand 8	29	P10145
12	IL1A	Interleukin 1 alpha	28	P01583
13	NFKB1	Nuclear factor kappa B subunit 1	28	P19838
14	STAT3	Signal transducer and activator of transcription 3	26	P40763
15	CCL2	C-C motif chemokine ligand 2	22	P13500
16	CXCL10	C-X-C motif chemokine ligand 10	22	P02778
17	CCL5	C-C motif chemokine ligand 5	21	P13501
18	CSF2	Colony stimulating factor 2	21	P04141
19	JAK2	Janus kinase 2	21	O60674
20	CSF3	Colony stimulating factor 3	20	P09919
21	IL17A	Interleukin 17A	20	Q16552
22	MYD88	MYD88 innate immune signal transduction adaptor	20	Q99836
23	CD40	CD40 molecule	19	P25942
24	IL13	Interleukin 13	19	P35225
25	JAK3	Janus kinase 3	19	P52333
26	CD28	CD28 molecule	18	P10747
27	CD80	CD80 molecule	18	P33681
28	CXCR4	C-X-C motif chemokine receptor 4	18	P61073
29	ICAM1	Intercellular adhesion molecule 1	18	P05362
30	PIK3R1	Phosphoinositide-3-kinase regulatory subunit 1	18	P27986
31	CCL4	C-C motif chemokine ligand 4	17	P13236
32	CD86	CD86 molecule	17	P42081
33	CTLA4	Cytotoxic T-lymphocyte associated protein 4	17	P16410

34	EGFR	Epidermal growth factor receptor	17	P00533
35	FOXP3	Forkhead box P3	17	Q9BZS1
36	IL5	Interleukin 5	17	P05113
37	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	17	P05412
38	STAT1	Signal transducer and activator of transcription 1	17	P42224
39	TLR2	Toll-like receptor 2	17	O60603
40	TP53	Tumor protein p53	17	P04637
41	CCL3	C-C motif chemokine ligand 3	16	P10147
42	IL2RA	Interleukin 2 receptor subunit alpha	16	P01589
43	IL7	Interleukin 7	16	P13232
44	PIK3CD	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta	16	O00329
45	CD44	CD44 molecule (Indian blood group)	15	P16070
46	CXCL2	C-X-C motif chemokine ligand 2	15	P19875
47	IL15	Interleukin 15	15	P40933
48	IL18	Interleukin 18	15	Q14116
49	IL7R	Interleukin 7 receptor	15	P16871
50	CCR5	C-C motif chemokine receptor 5	14	P51681
51	CASP8	Caspase 8	13	Q14790
52	CD19	CD19 molecule	13	P15391
53	CD40LG	CD40 ligand	13	P29965
54	ERBB2	Erb-b2 receptor tyrosine kinase 2	13	P04626
55	IFNB1	Interferon beta 1	13	P01574
56	IRAK1	Interleukin 1 receptor associated kinase 1	13	P51617
57	PTPRC	Protein tyrosine phosphatase receptor type C	13	P08575
58	CD274	CD274 molecule	12	Q9NZQ7
59	CXCR3	C-X-C motif chemokine receptor 3	12	P49682
60	ICOS	Inducible T-cell costimulator	12	Q9Y6W8
61	IKBKB	Inhibitor of nuclear factor kappa B kinase subunit beta	12	O14920
62	IL2RB	Interleukin 2 receptor subunit beta	12	P14784
63	IL9	Interleukin 9	12	P15248
64	LYN	LYN proto-oncogene, Src family tyrosine kinase	12	P07948
65	MMP9	Matrix metallopeptidase 9	12	P14780
66	TYK2	Tyrosine kinase 2	12	P29597
67	CASP1	Caspase 1	11	P29466
68	CXCL9	C-X-C motif chemokine ligand 9	11	Q07325
69	ALB	Albumin	10	P02768
70	BTK	Bruton tyrosine kinase	10	Q06187
71	C3	Complement C3	10	P01024
72	IFNA1	Interferon alpha 1	10	P01562
73	IL12RB1	Interleukin 12 receptor subunit beta 1	10	P42701
74	NFKBIA	NFKB inhibitor alpha	10	P25963
75	PDCD1	Programmed cell death 1	10	Q15116
76	RIPK1	Receptor interacting serine/threonine kinase 1	10	Q13546
77	FASLG	Fas ligand	9	P48023
78	FCGR3A	Fc gamma receptor IIIa	9	P08637
79	FCGR3B	Fc gamma receptor IIIb	9	O75015
80	IL12RB2	Interleukin 12 receptor subunit beta 2	9	Q99665
81	IRAK4	Interleukin 1 receptor associated kinase 4	9	Q9NWZ3
82	PLCG2	Phospholipase C gamma 2	9	P16885
83	TGFB1	Transforming growth factor beta 1	9	P01137
84	TLR9	Toll-like receptor 9	9	Q9NR96
85	BCL2	BCL2 apoptosis regulator	8	P10415
86	CRP	C-reactive protein	8	P02741
87	DDX58	DExD/H-box helicase 58 (RIG-I)	8	O95786
88	STAT6	Signal transducer and activator of transcription 6	8	P42226
89	C4A	Complement C4A (Chido/Rodgers blood group)	7	P0C0L4
90	CD34	CD34 molecule	7	P28906
91	CD69	CD69 molecule	7	Q07108
92	GZMB	Granzyme B	7	P10144
93	INS	Insulin	7	P01308
94	RAC2	Rac family small GTPase 2	7	P15153
95	SELL	Selectin L	7	P14151
96	TLR7	Toll-like receptor 7	7	Q9NYK1

97	HLA-A	Major histocompatibility complex, class I, A	6	P04439
98	IL23A	Interleukin 23 subunit alpha	6	Q9NPF7
99	IL33	Interleukin 33	6	O95760
100	PRF1	Perforin 1	6	P14222
101	CCND1	Cyclin D1	5	P24385
102	ERVW-1	Endogenous retrovirus group W member 1, envelope	5	Q9UQF0
103	FAS	Fas cell surface death receptor	5	P25445
104	HAVCR2	Hepatitis A virus cellular receptor 2	5	Q8TDQ0
105	HLA-B	Major histocompatibility complex, class I, B	5	P01889
106	HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	5	P04440
107	HLA-DQA1	Major histocompatibility complex, class II, DQ alpha 1	5	P01909
108	HLA-DRB1	Major histocompatibility complex, class II, DR beta 1	5	P01911
109	HP	Haptoglobin	5	P00738
110	IL1RN	Interleukin 1 receptor antagonist	5	P18510
111	IL22	Interleukin 22	5	Q9GZX6
112	LGALS9	Galectin 9	5	O00182
113	MBL2	Mannose-binding lectin 2	5	P11226
114	MPO	Myeloperoxidase	5	P05164
115	MTOR	Mechanistic target of rapamycin kinase	5	P42345
116	MUC1	Mucin 1, cell surface associated	5	P15941
117	NLRP3	NLR family pyrin domain containing 3	5	Q96P20
118	PPARG	Peroxisome proliferator-activated receptor gamma	5	P37231
119	RUNX1	RUNX family transcription factor 1	5	Q01196
120	TNFSF10	TNF superfamily member 10	5	P50591
121	XIAP	X-linked inhibitor of apoptosis	5	P98170
122	ZAP70	Zeta chain of T cell receptor associated protein kinase 70	5	P43403
123	ACE2	Angiotensin converting enzyme 2	4	Q9BYF1
124	CALR	Calreticulin	4	P27797
125	CD38	CD38 molecule	4	P28907
126	CD46	CD46 molecule	4	P15529
127	CD55	CD55 molecule (Cromer blood group)	4	P08174
128	CD79A	CD79a molecule	4	P11912
129	CD81	CD81 molecule	4	P60033
130	CP	Ceruloplasmin	4	P00450
131	CR2	Complement C3d receptor 2	4	P20023
132	F2	Coagulation factor II, thrombin	4	P00734
133	GPT	Glutamic-pyruvic transaminase	4	P24298
134	TERT	Telomerase reverse transcriptase	4	O14746
135	TNFAIP3	TNF alpha induced protein 3	4	P21580
136	C5AR1	Complement C5a receptor 1	3	P21730
137	CASP4	Caspase 4	3	P49662
138	CD163	CD163 molecule	3	Q86VB7
139	CFI	Complement factor I	3	P05156
140	CSF1	Colony stimulating factor 1	3	P09603
141	DKC1	Dyskerin pseudouridine synthase 1	3	O60832
142	ELANE	Elastase, neutrophil expressed	3	P08246
143	GAR1	H/ACA ribonucleoprotein complex subunit GAR1	3	P28007
144	HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1	3	P01920
145	IDO1	Indoleamine 2,3-dioxygenase 1	3	P14902
146	IL12B	Interleukin 12B	3	P29460
147	ITGA4	Integrin subunit alpha 4	3	P13612
148	KNG1	Kininogen 1	3	P01042
149	MASP2	MBL associated serine protease 2	3	O00187
150	NCF1	Neutrophil cytosolic factor 1	3	P14598
151	NCF2	Neutrophil cytosolic factor 2	3	P19878
152	PIK3CG	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma	3	P48736
153	SPP1	Secreted phosphoprotein 1	3	P10451
154	TNFRSF13C	TNF receptor superfamily member 13C	3	Q96RJ3
155	TNIP1	TNFAIP3 interacting protein 1	3	Q15025
156	WAS	WASP actin nucleation promoting factor	3	P42768
157	WRAP53	WD repeat containing antisense to TP53	3	Q9BUR4
158	ATM	ATM serine/threonine kinase	2	Q13315
159	BAX	BCL2 associated X, apoptosis regulator	2	Q07812

160	CD59	CD59 molecule (CD59 blood group)	2	P13987
161	EPO	Erythropoietin	2	P01588
162	HSPD1	Heat shock protein family D (Hsp60) member 1	2	P10809
163	LCN2	Lipocalin 2	2	P80188
164	MAF	MAF bZIP transcription factor	2	O75444
165	OAS1	2'-5'-Oligoadenylate synthetase 1	2	P00973
166	PNP	Purine nucleoside phosphorylase	2	P00491
167	REN	Renin	2	P00797
168	RNASE3	Ribonuclease A family member 3	2	P12724
169	TF	Transferrin	2	P02787
170	TNFRSF13B	TNF receptor superfamily member 13B	2	O14836
171	TRIM21	Tripartite motif containing 21	2	P19474
172	ADA	Adenosine deaminase	1	P00813
173	ADAM17	ADAM metallopeptidase domain 17	1	P78536
174	BACH2	BTB domain and CNC homolog 2	1	Q9BYV9
175	CCN2	Cellular communication network factor 2	1	P29279
176	CIITA	Class II major histocompatibility complex transactivator	1	P33076
177	COL1A1	Collagen type I alpha 1 chain	1	P02452
178	DOCK8	Dedicator of cytokinesis 8	1	Q8NF50
179	ERN1	Endoplasmic reticulum to nucleus signaling 1	1	O75460
180	G6PD	Glucose-6-phosphate dehydrogenase	1	P11413
181	GATA2	GATA binding protein 2	1	P23769
182	HMOX1	Heme oxygenase 1	1	P09601
183	LAMP1	Lysosomal associated membrane protein 1	1	P11279
184	LBP	Lipopolysaccharide binding protein	1	P18428
185	LTA	Lymphotoxin alpha	1	P01374
186	MAP3K14	Mitogen-activated protein kinase kinase kinase 14	1	Q99558
187	PIK3C2A	Phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha	1	O00443
188	PSMB8	Proteasome 20S subunit beta 8	1	P28062
189	S100A8	S100 calcium binding protein A8	1	P05109
190	SERPINE1	Serpin family E member 1	1	P05121
191	STING1	Stimulator of interferon response cGAMP interactor 1	1	Q86WV6
192	TCF3	Transcription factor 3	1	P15923
193	TFRC	Transferrin receptor	1	P02786
194	WT1	WT1 transcription factor	1	P19544

Network analysis for prediction involved combining two distinct networks: one linking phytochemical components from Zingiber officinale to their target genes, and another comprising protein-protein interactions associated with the disease mechanism. This integrated network was analyzed using Cytoscape software (version 3.10.2). Clustering was carried out with the ClusterOne plugin, yielding a cluster of 47 nodes, out of which 20 key nodes were selected based on rankings from CytoHubba. The top 20 nodes identified were IL1B, IL6, IL10, IFNG, TNF, IL1A, IL4, IL2, CXCL8, CD4, TLR4, IL13, IL17A, CSF2, IL6, CD8A, NFKB1, CXCL10, CSF3, and IL2RA **(Figure 4).**

Furthermore, pathway enrichment analysis was performed utilizing the Metascape platform. The Gene Ontology (GO) results for these targets encompassed 740 terms in biological processes, 14 in cellular components, and 35 in molecular functions **(Figure 5).** The biological process category highlighted involvement in cellular responses to cytokines, cytokine signaling pathways, and inflammatory processes. For cellular components, the proteins were localized to structures such as endosomal membranes, phagocytic vesicles, and the canonical inflammasome complex. Molecular function terms primarily indicated roles in receptor binding, cytokine activity, and receptor-ligand interactions.

A schematic overview depicting the connections among the bioactive compounds in Zingiber officinale, the target proteins related to immune responses in offspring of obese mothers during gestation, and the corresponding enrichment results is presented in **Figure 6.**

Among the 49 phytochemicals identified in Zingiber officinale, 36 were found to interact with the 47 protein nodes. These active compounds are 10-gingerdiol, 10-gingerdione, 10-gingerol, 10-paradol, 10-shogaol, 12-dehydrogingerdione, 12-gingerol, 12-shogaol, 1-dehydro-10-gingerdione, 1-dehydro-6-gingerdione, 1-dehydro-8-gingerdione, 4-gingerdiol, 4-shogaol, 6-dehydroparadol, 6-gingerol, 6-gingesulfonic acid, 6-paradol, 6-shogaol, 6-zingerine, 7-paradol, 8-gingerdiol, 8-paradyl monoacetate, 8-zingerine, acetoxy-10-gingerol, beta-sitosterol,

dehydrozingerone, diacetoxy-4-gingerdiol, diacetoxy-6-gingerdiol, gingerenone a, hexahydrocurcumin, methyl-6-gingerdiol, methyl-6-gingerol, methyl-6-paradol, methyl-8-gingerol, octahydrocurcumin, and zingerone.

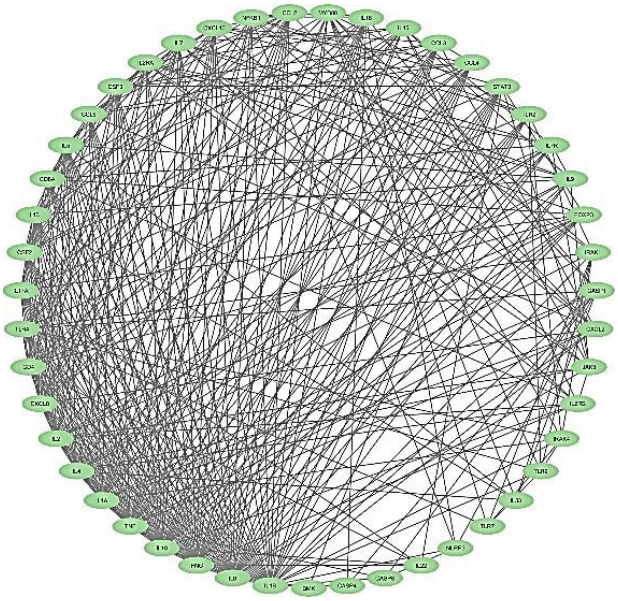


Figure 4. Results of a 47 nodes cluster utilizing ClusterOne

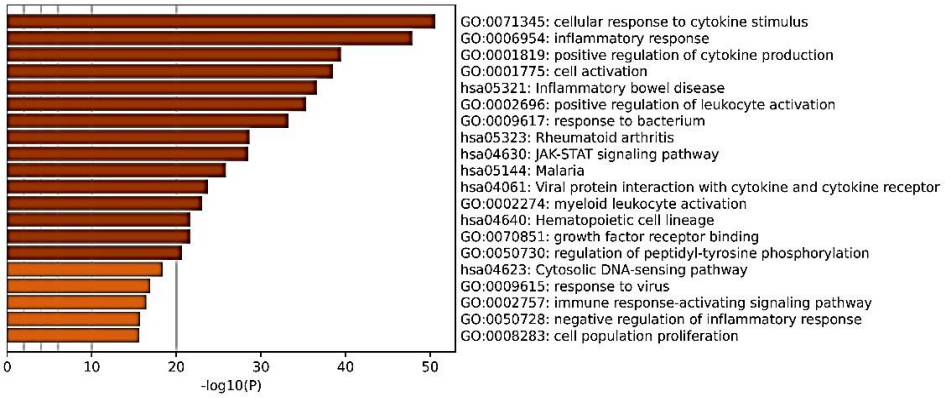


Figure 5. Result of enrichment analysis using gene ontology (GO) and pathways

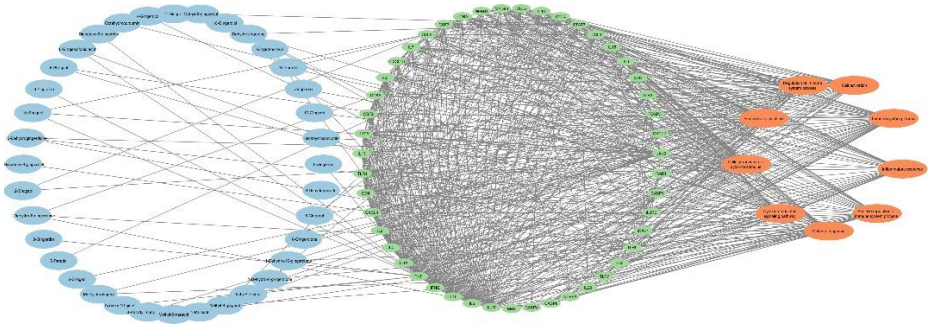


Figure 6. Pharmacological network analysis of the immunological response in children born to obese mothers during pregnancy, focusing on target genes of *Zingiber officinale* phytoconstituents

The selection of compounds for molecular docking was based on drug-likeness evaluations and their prominent interactions highlighted by CytoHubba in the network pharmacology analysis. From the 36 phytochemicals uncovered via network pharmacology, the two key constituents—6-gingerol and 6-shogaol—exhibited direct binding to the top 20 hub nodes determined through CytoHubba. Two receptor proteins, TLR4 (PDB ID: 2Z65) and TNF-α (PDB ID: 6OOY), sourced from the RCSB Protein Data Bank (https://www.rcsb.org/), were prioritized due to their frequent involvement in the core nodes enriched in the gene ontology results **(Figure 7).**

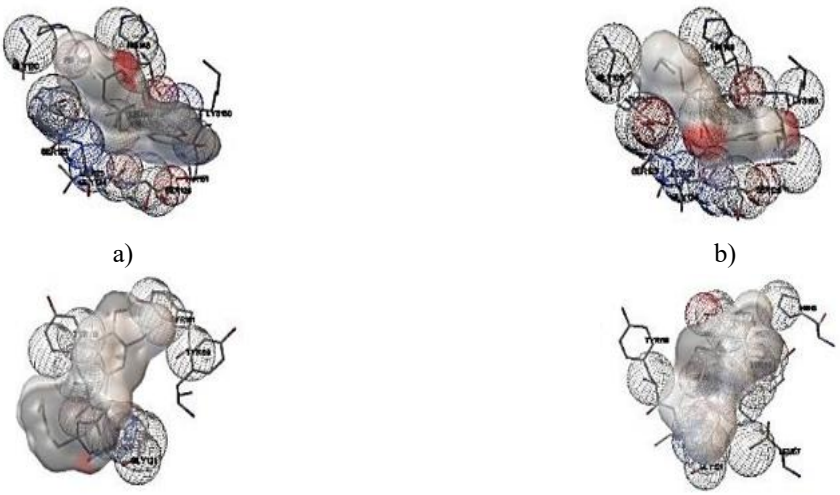
Molecular docking was initially carried out between each protein and its co-crystallized native ligand. The binding energy was -5.07 kcal/mol for TLR4 with its native ligand NAG, and -3.65 kcal/mol for TNF-α with its native ligand MRD. These native ligand dockings served as a reference for defining the grid box parameters during redocking of the Zingiber officinale compounds. All docking scores for the plant-derived ligands exceeded -5.0 kcal/mol in absolute value, suggesting robust binding to the targets. Specifically, 6-gingerol showed a binding affinity of -5.26 kcal/mol with TLR4 and -5.52 kcal/mol with TNF-α, while 6-shogaol recorded -6.04 kcal/mol with TLR4 and -5.45 kcal/mol with TNF-α.

Root-mean-square deviation (RMSD) values for the redocked native ligands were 1.89 Å for TLR4-NAG and 1.90 Å for TNF-α-MRD. Achieving RMSD below 2 Å upon redocking the native ligands under the defined conditions confirmed the reliability and validity of the docking protocol.

Zingiber officinale may modulate the immunological responses in offspring of obese mothers during gestation. It was chosen as a potential intervention for pregnant women largely owing to its established role in managing nausea and vomiting in hyperemesis gravidarum [16, 17]. Moreover, multiple preclinical investigations have demonstrated that ginger extracts or their active ingredients, including gingerol and shogaol, can attenuate inflammation in models of metabolic syndrome or diabetes [19, 38]. Such evidence positions ginger as a compelling option for advancing maternal health applications. Nevertheless, the specific interactions between Z. officinale phytochemicals and proteins implicated in maternal obesity-related immune dysregulation in progeny have received limited attention.

Network pharmacology has been widely applied by investigators to uncover potential mechanisms of herbal medicines, including compound-target-disease relationships [39]. In this study, enrichment analysis was performed to explore the biological roles of candidate targets via gene ontology terms and pathways, shedding light on how bioactive components of Z. officinale might engage with the relevant pathological conditions.

ADME properties were assessed to determine the drug-likeness of Z. officinale constituents. Lipinski's Rule of Five outlines key physicochemical criteria—molecular weight below 500 Da, logP below 5, fewer than 5 hydrogen bond donors, and fewer than 10 hydrogen bond acceptors—that are especially relevant for orally administered drugs, as they predict aqueous solubility and intestinal permeability [40].



a) b) c) d)

Figure 7. Molecular docking (a) TLR4 with 6-gingerol, (b) TLR4 with 6-shogaol, (c) TNF-α with 6-gingerol, and (d) TNF-α with 6-shogaol

A majority of the phytochemicals from Z. officinale complied with Lipinski's Rule of Five. Their molar refractivity values fell within the desirable range of 40–130. Furthermore, these compounds demonstrated high gastrointestinal absorption potential, and each achieved a bioavailability score greater than 0.3.

The study constructed a therapeutic network focused on immune responses and overlapped it with the predicted target network of Z. officinale bioactive components. Both networks displayed considerable complexity. Their overlap highlighted the precise proteins engaged by the plant's constituents. Specifically, 36 compounds were found to target 47 protein nodes linked to immune dysregulation in offspring exposed to maternal obesity during

gestation. Among these, 20 nodes emerged as particularly central. The principal components, 6-gingerol and 6-shogaol, were predicted to bind TLR4 and TNF-α. Gene Ontology analysis identified key processes—including cellular responses to cytokines, inflammatory pathways, and upregulation of cytokine production—that appear central to the immunomodulatory effects of Z. officinale on the immune profile of children born to obese mothers.

Obesity represents a chronic, low-grade inflammatory condition triggered by excessive caloric intake over time, leading to adipose tissue expansion. This expansion involves adipocyte hypertrophy, hyperplasia, and infiltration by immune cells, resulting in inflammation and remodeling of the extracellular matrix to accommodate growth [2, 4]. Such changes in adipose tissue promote a shift toward metabolic impairment. Normally, adipocytes secrete adipokines like leptin, which maintains energy homeostasis and suppresses appetite, and adiponectin, which supports insulin sensitivity and metabolic balance. In obesity, however, leptin resistance emerges alongside reduced adiponectin levels, prompting Th1 cells to increase IFN-γ secretion and favoring polarization toward pro-inflammatory M1 macrophages. These events escalate release of cytokines such as TNF-α, IL-6, and IL-1β. Conversely, the functions of Th2 and regulatory T cells—which promote anti-inflammatory M2 macrophages and cytokines like IL-10—are suppressed. This cascade is compounded by adipocyte death and persistent pro-inflammatory cytokine release, further aggravating metabolic disturbances [4, 5].

Additionally, maternal obesity impacts placental function. The elevated presence of M1 macrophages leads to heightened placental inflammation [9, 12, 41]. This inflammatory state in the placenta promotes TLR4 activation. Furthermore, TLR4 engagement stimulates MyD88 recruitment, which in turn activates the transcription factor NF-κB through its phosphorylation into p-NF-κB, ultimately driving the synthesis of pro-inflammatory cytokines [41, 42]. Placental inflammation disrupts the intrauterine milieu, thereby elevating the susceptibility to infections in the offspring. This phenomenon is connected to the abundance of immune cells and the altered immune reactions observed in children born to obese mothers. Modifications in hematopoietic stem and progenitor cells lead to fetal reprogramming, as indicated by changes in eosinophil and CD4+ T cell counts, diminished monocyte responsiveness, and reduced dendritic cell numbers in the placentas of infants from obese mothers [19]. Gutvirtz *et al*. observed markedly higher rates of respiratory tract infections, along with ear, nose, throat (ENT), and eye disorders, among infants delivered by obese mothers compared to those from normal-weight mothers [14]. Parsons *et al*. found notable differences in the duration of treatment required for respiratory infections in children of obese mothers versus those of normal-weight mothers [15]. Similarly, Cameron *et al*. reported an association between maternal obesity and increased hospitalization rates in offspring during their first five years, primarily due to infectious diseases [7].

Few in vitro investigations have examined the interactions of 6-gingerol and 6-shogaol with TLR4. Park *et al*. showed that 6-shogaol can regulate the expression of TLR-dependent immune genes triggered by microbial challenges [43]. In human dendritic cells, Pazmandi *et al*. demonstrated that both 6-gingerol and 6-shogaol suppress TLR-mediated signaling, though only 6-shogaol diminishes dendritic cell immunogenicity via activation of AMPK and Nrf2 pathways [44].

Other in vitro and in vivo research has revealed that 6-shogaol provides intestinal protection involving TNF-α. Additionally, 6-gingerol has been found to lower TNF-α and IL-6 concentrations in the serum, adipose tissue, and liver of obese mice with metabolic syndrome [21]. Further evidence suggests that ginger inhibits the TLR4/NF-κB pathway in murine models of non-alcoholic steatohepatitis [45]. Beyond 6-shogaol, compounds like zingerone and 8-shogaol have shown promise in managing inflammation-associated conditions, including lupus, psoriasis, and inflammatory bowel disease [38, 46-48].

Clinical studies have also documented ginger's ability to decrease inflammatory markers in disorders such as type 2 diabetes, hypertension, dyslipidemia, and multiple sclerosis [49-52]. These reductions in markers like hs-CRP, IL-6, and TNF-α were linked to improvements in parameters such as blood pressure, glucose control, and lipid profiles [50-52].

The long-term safety profile of Zingiber officinale for managing obesity in pregnant women remains undetermined. Establishing its safety for extended use is essential to ensure appropriate application in maternal obesity treatment. Ginger is typically considered safe at doses up to 4 g per day in the general population, although higher amounts may exacerbate issues like gingival irritation, bleeding, or heartburn [53]. A large cohort study in Norway involving over 60,000 pregnant women using ginger reported no elevated risks of congenital anomalies or low birth weight [54]. Short-term trials administering 1–2 g daily for pregnancy-related nausea and vomiting in the first trimester have likewise been deemed safe [55, 56].

This study has certain limitations. The database of Z. officinale phytochemicals was incomplete, excluding some compounds present in the plant. Moreover, it evaluated constituents individually rather than considering potential synergistic or antagonistic interactions among them. Additional molecular dynamics simulations are needed, alongside in vitro, in vivo, and clinical investigations, to substantiate the in silico predictions.

In summary, the current investigation reveals the promise of Z. officinale-derived bioactives, especially 6-gingerol and 6-shogaol, in addressing immune imbalances linked to maternal obesity in progeny. Through computational approaches, these compounds displayed strong binding to pivotal inflammatory targets (e.g., TLR4, NF-κB, TNF-α), implying their capacity to disrupt pro-inflammatory cascades driven by maternal obesity. Notably, 6-shogaol emerged as the superior binder to TLR4, highlighting its relevance since TLR4-driven inflammation is central to metabolic disturbances—yet its role in developmental immunology is underexplored.

The findings underscore the value of phytochemicals in preventive neonatal nutrition and open avenues for maternal dietary strategies to interrupt the intergenerational transmission of obesity-associated immune issues. Rigorous experimental confirmation is required to verify in vivo efficacy and evaluate safety and suitability for neonatal contexts.

Conclusion

In conclusion, 36 active constituents from Zingiber officinale were identified that influence major proteins (e.g., TLR4, NF-κB, TNF-α) involved in inflammatory and cytokine signaling pathways, with 6-shogaol demonstrating the highest affinity for TLR4. Nonetheless, the multi-component nature of Z. officinale requires further characterization to support its safe, long-term therapeutic use.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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