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In silico Analysis of Immunomodulatory Potential of Glucomoringin in Chicken

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ABSTRACT

Ethano-veterinary practices are followed for treatment of different health conditions in India as well as in other parts of the world. Many reports have shown that inclusion of herbs in poultry diet can lead to improved health condition of birds. Several plants are reported to possess immunomodulatory activity. However there is paucity of studies proving molecular mechanism responsible for immunomodulatory potential of plants. Cytokines play crucial role in modulation of immune response. Herbal plants exhibit their therapeutic potential due to the presence of various phytoconstituents that may cause immunomodulation through directly interacting with cytokines. Moringa oleifera (drum stick) of family Moringaceae is one of the well-known medicinal plants. It can act as cardiac stimulants, possess antitumor, immunomodulatory, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic properties. Glucosinolates are secondary plant compounds typically found in members of the Brassicaceae and a few other plant families like Moringaceae. Thus the present study was carried out to explore immunomodulatory potential of one of the phytoconstituents glucomoringin of Moringa oleifera Lam. in chicken by conducting in silico interaction studies with six different cytokines of Gallus gallus. The protein sequence of cytokines genes were retrieved from NCBI and 3D structures were predicted through Swiss Model tool. The 3D structure of the phytochemical was retrieved from pubchem. Docking studies were performed by using PatchDock server between glucomoringin and receptors (IL-1β, IL-2, IL-4, IL-5, IL-10 and Interferon gamma) and the results were analyzed. Glucomoringin showed maximum score with IL-1β followed by IL-10. Thus it could be predicted from the in silico analysis conducted that binding of glucomoringin with cytokines may be responsible for its active role for its immunomodulatory potential. However there is a need to further explore its immunomodulatory potential through suitable in vitro and in vivo analyses to confirm these findings.

Key words: *Immunomodulation; Moringa oleifera; Glucomoringin; Gallus gallus; Molecular docking; Cytokines.*

INTRODUCTION

Medicinal plants are used to treat illness and diseases for thousands of years. They have gained economical importance because of their application in pharmaceutical, cosmetic, perfumery and food industries.

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There is renewed interest in herbal systems of medicine for curing many diseases due to their high efficacy, safety and least side effects[1]. Crucifers are characterized by the presence of a group of secondary compounds called glucosinolates. Several other plant families as Capparidaceae, Moringaceae, Resedaceae, Stegnospermaceae, and Tovariaceaehave been found to possess glucosinolates. Glucosinolates are glucose and sulfur-containing organic anions and reported to exhibit anti-cancer and anti-inflammatory properties[14]. Glucosinolates containing plants have always made major contributions to the diets of humans and farm animals that include condiments and relishes, traditional leafy vegetables and crops used as animal feedstuffs[4].

Cytokines are small glycoproteins produced by a number of cell types, predominantly leukocytes that regulate immunity, inflammation and hematopoiesis. They regulate a number of physiological and pathological functions including innate immunity, acquired immunity and a plethora of inflammatory responses. These molecules can exhibit synergistic, antagonistic and pleiotropic activities. IL-1β plays an important role in both innate and adaptive immunity and is a crucial mediator of the host inflammatory response in natural immunity[12]. IL-2 has essential roles in key functions of the immune system, tolerance and immunity, primarily via its direct effects on T cells. In the thymus, where T cells mature, it prevents autoimmune diseases by promoting the differentiation of certain immature T cells into regulatory T cells, which suppress other T cells that are otherwise primed to attack normal healthy cells in the body. Interleukin -2 also promotes the differentiation of T cells into effector T cells and into memory T cells when the initial T cell is also stimulated by an antigen, thus helping the body fight off infections[11]. Interleukin-4 has many biological roles, including the stimulation of activated B-cell and T-cell proliferation and the differentiation of B cells into plasma cells. It is a key regulator in humoral and adaptive immunity. IL-4 induces B-cell class switching to IgE, and up-regulates MHC class II production. IL-4 decreases the production of Th1 cells, macrophages, IFN-gamma, and dendritic cell IL-12[10]. Through binding to the interleukin-5 receptor, interleukin-5 stimulates B cell growth and increases immunoglobulin secretion, primarily IgA. It is also a key mediator in eosinophil activation. IL-10 is a cytokine with multiple, pleiotropic, effects in immunoregulation and inflammation. It also enhances B cell survival, proliferation, and antibody production. IL-10 can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway. IFN-γ or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral, some bacterial and protozoal infections. IFN-γ is an important activator of macrophages and inducer of Class II major histocompatibility complex (MHC) molecule expression[16].

Glucosinolates are secondary plant compounds typically found in members of the Brassicaceae and a few other plant families. Usually each plant species contains a specific subset of the about 130 different glucosinolates identified to date. However, intraspecific variation in glucosinolate profiles is commonly found. Benzyl isothiocyanate, a hydrolysis product of benzyl glucosinolate, is one of the most potent anticancer agents for breast, lung liver and colon cancers[13]. Based on nuclear magnetic resonance (NMR) and mass spectrometry analyses of the intact glucosinolate as well as of the products formed after enzymatic conversion by sulfatase or myrosinase, this compound was identified as 4-α-rhamnosyloxy benzyl glucosinolate (glucomoringin). So far, glucomoringin had only been reported as the main glucosinolate of Moringa species (Moringaceae) which are tropical tree species[13]. The isothiocyanate that is formed after conversion of glucomoringin is a potent antimicrobial, antitumor and anti-inflammatory agent[9,8]. Keeping in view of above the study was conducted to explore immunomodulatory potential of glucomoringin of *Moringa oleifera* Lam. in

chicken by conducting *in silico* molecular interaction studies with different cytokines *viz*., IL-1β, IL-2, IL-4, IL-5, IL-10, and Interferon gamma of *Gallus gallus*.

MATERIAL AND METHODS

Ligand Preparation

Three-dimensional (3D) structure of glucomoringin, the phytoconstituents of *Moringa oleifera* that was chosen in the present study, was retrieved from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format and then it was converted into pdb format by using Open Babel, a software package used to interconvert the format of the input file for further studies (Table 1).

Receptors Preparation

The sequences of the target proteins FASTA format were retrieved from the NCBI data bank. The FASTA sequence converted to 3D structure by SWISS Model. A typical amino acid structure file consists of heavy atoms, water molecules, cofactors, metal ions and it can be in the polymeric form. The structure generally has no information about bond orders, topologies, or formal atomic charges. So, the raw PDB structure should be prepared in a suitable manner for docking studies. AutoDock Tools were used to prepare the final 3D protein structure for docking. During protein preparation, water molecules and peptide substrate were deleted and hydrogen atoms for generation of 3D structures of respective compound were added.

Automated Molecular Docking

PatchDock server (https://bioinfo3d.cs.tau.ac.il/PatchDock/) was used for carrying out molecular docking experiments. It is a molecular docking tool, which is used to find docking transformations that produce good molecular shape complementarities based on shape complementarily principles. The input files include the receptor protein and ligand in PDB format. PatchDock server provides an URL which gives the top 20 solutions in a table and after the fast transformational search the best geometric fit obtained the highest scores, while the low scores exhibited poor matches. Best negative values of ACE indicate more binding affinity between the ligand and receptor.

RESULTS AND DISCUSSION

The molecular docking experiments revealed the interaction patterns of ligands with the chosen cytokines. The results were obtained in the form of scores and ACE (atomic contact energies) of the docked complexes (Table 2). Glucomoringin showed maximum score with IL-Iβ followed by IL-10, Interferon gamma, IL-2 and IL-4. Figure 1 shows the graphical representation of the score and ACE of docked complexes. The docked complexes were analyzed on PyMol molecular viewer. Figure 2 indicates the interaction of glucomoringin with all studied cytokines.

Numerous studies have been conducted on the different plant parts of *Moringa oleifera*, as it is considered as an important medicinal plant. Various preparations of the different plant parts are also reported to possess immunomodulatory activities[14,5]. *Moringa oleifera*, the drumstick tree, produces unique glucosinolates **(**GS) but little is known about GS variation within *Moringa oleifera*[6]. 4-α-L-rhamnopyranosyloxy benzyl isothiocyanate (4RBITC), the isothiocyanate created by hydrolysis of "glucomoringin" (4RBGS or 4-α-L-rhamnopyranosyloxy benzyl glucosinolate) from *Moringa oleifera* is a potent and selective antibiotic[9,7]. Glucomoringin isothiocyanate is also reported to exhibit potent anti-inflammatory activity in mouse models[2,17,8]. In the present study, glucomoringin showed significant interaction with IL-10 which is an anti-inflammatory mediator. Glucomoringin displayed significant binding with the various cytokines which signifies its active involvement in immunomodulation through cytokines mediated signaling pathways.

Table 1- Properties and three-dimensional (3D) structure of glucomoringin

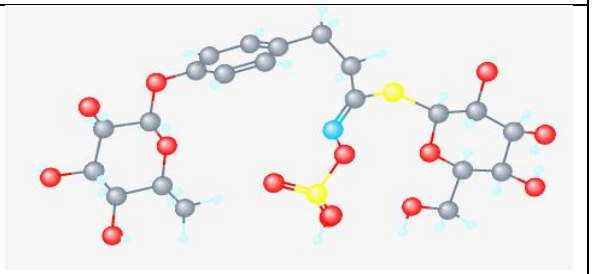
Compound name:	Glucomoringin
PubChem CID:	102222710
Molecular Formula:	$C_{21}H_{31}NO_{14}S_2$
Molecular Weight:	585.592 g/mol
3D Structure:	
Hydrogen Bond Dnor Count:	8
Hydrogen Bond Acceptor Count:	16
XLogP3-AA:	-1.6

Table 2- The scores and atomic contact energy (ACE) of docked complexes

Receptor	Glucomoringin	
	Score	**ACE**
Interleukin-1 β	6256	-367.18
Interleukin-2	5366	-215.00
Interleukin-4	5190	-212.82
Interleukin-5	4908	-369.76
Interleukin-10	5480	-344.75
Interferon gamma	5438	-169.12

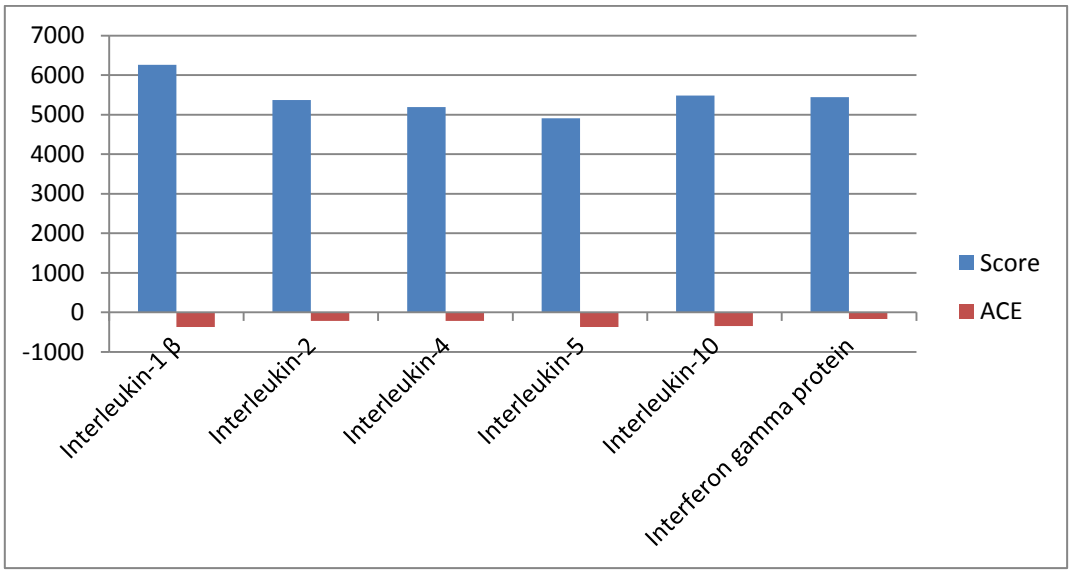


Fig. 1 – Graph representing molecular docking scores and ACE of glucomoringin with the cytokines

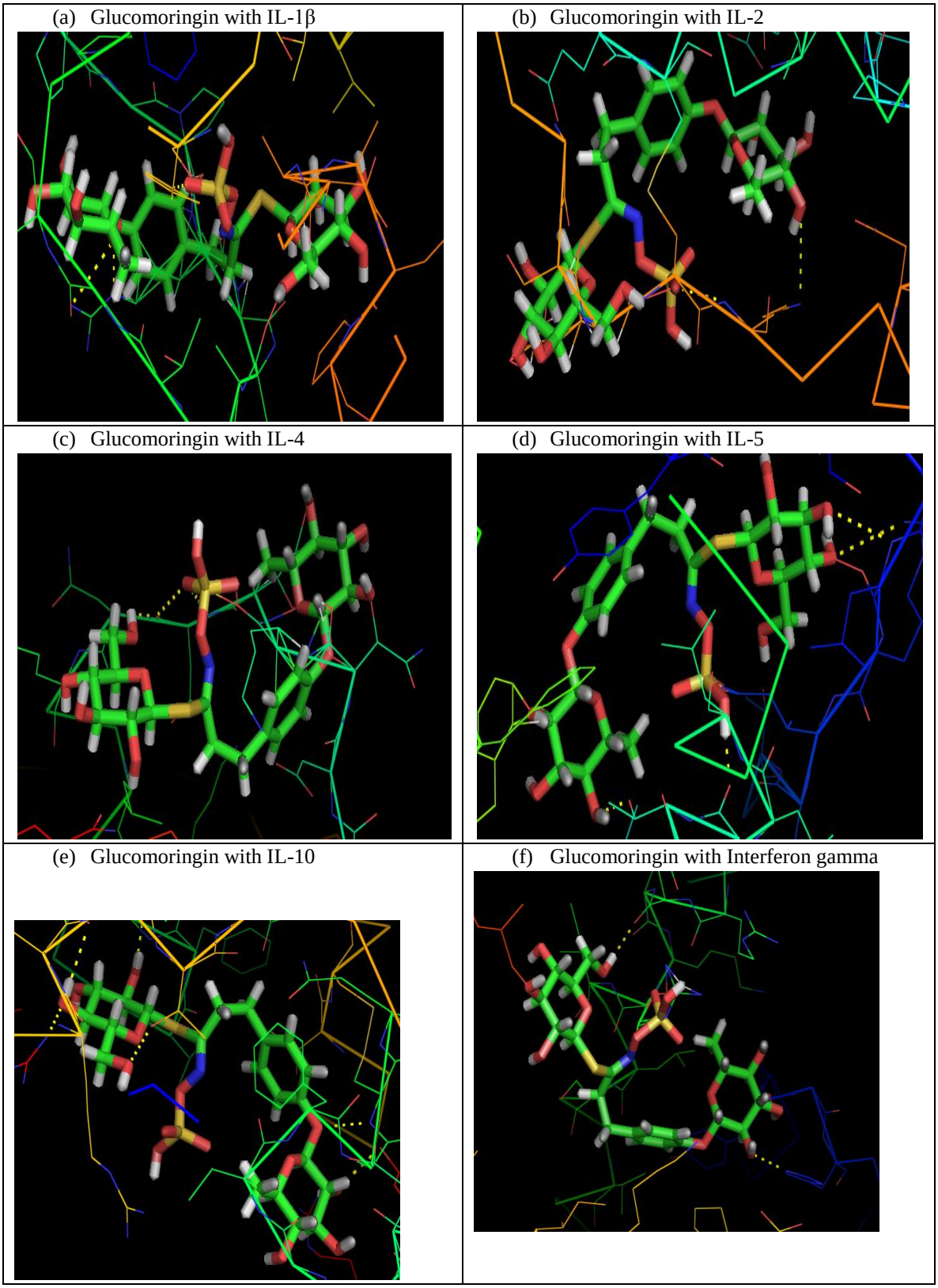


Fig.2- Molecular docking interactions of Glucomoringin with various chicken cytokines

CONCLUSION

In the present study glucomoringin showed overall good scores with various receptors studied which is indicative of active and significant role of it in immunomodulatory properties of the plant. Such *in silico* studies could be helpful in identifying direct involvement of specific phytoconstituent and their biological targets there by in delineating the molecular pathways involved and provide

a scientific rationale behind the therapeutic potential of the phytoconstituent in particular. Outcome of such *in silico* analysis could be used for development of natural immunomodulatory preparations for poultry. However, elaborate *in vitro*/ *in vivo* studies are needed to confirm these findings.

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