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RESEARCH ARTICLE

A Computational approach in identifying the herbal compounds as Lactation inducer

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ABSTRACT:

Lactation disorder (i.e. insufficient lactation) is one of the major problems faced by 60%-80% of the females in this generation during post-pregnancy period. A mother who has insufficient lactation is prescribed drugs to boost the production of prolactin hormone and studies on these drugs reported that they cause some adverse effects for the women as well as infants. Over the past decade the herbal products hold special attention in modern medicine after its efficacy and safety well established in clinical trials, because of its easy availability and standardization procedure. The aim of this research work is to identify the natural bioactive compounds with galactagogue property among the five renowned medicinal plants such as *Sambucus nigra*, *Melissa officinalis*, *Matricaria recutita*, *Urtica dioica*, and *Trigonella foenum-graecum*. In this study the bioactive compounds present in the plants were identified through literature survey and these compounds were screened for its drug likeness properties. Those compounds which satisfy the drug likeness properties were further analysed for its Prolactin inducing activity through computational approaches. The result of this study concluded that the natural bioactive compound holds good inducing activity towards the protein Prolactin when compared with the common prescribed drugs. In future this study could be further designed to highlight the efficiency of prolactin inducing compound towards drug development process.

KEYWORDS: Lactation disorder, Prolactin, Indian Medicinal plant, Bioactive compounds, Drug likeness properties, Docking.

INTRODUCTION:

Every newborn baby should be fed on Breast milk exclusively during first 6 months of life[1]. This milk is the primary source of nutrition for new-borns before they can eat and digest other solid or semi-solid foods by weaning process[2]. By the influence of hormones such as prolactin and oxytocin, the breast milk can be produced after childbirth. The most important hormone responsible for the production of this breast milk is prolactin.

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During the pregnancy period, prolactin prepares the breasts to begin breast milk production. It initiates and stimulates the process of lactation in mammals[3]. Lactation is the process of the secretion of milk by the breast mammary gland. Reportedly, most of post- natal women have an unsuccessful effort of breastfeeding because of insufficient production of breast milk[4]. This can be due to imbalance in hormonal action in breast tissue or inadequate milk ejection due to poor breast-feeding technique. Physiological changes and mental stress of the mother can cause precipitous drop in the level of Prolactin hormone during lactation period[5].

By recent studies, it shows that the problem of insufficient milk production can be improved by galactagogue substances. Galactagogues are substances

that increase the production or flow of milk[6]. It can be synthetic or can be prepared from herbal plants. During lactation period, synthetic drugs like Domperidone, Risperidone, Metoclopramide and Sulpiride are prescribed to boost the production of prolactin but they have some adverse effect like anxiety, breast discomfort, diarrhoea, dry mouth, intestinal gas, nausea, vertigo and restless legs for the women as well as child[7]. By and large, the efficacy of these medicinal herbs is unproven in a clinical or laboratory setting[8]. However, many have enjoyed centuries of use by generations of women, which in itself makes them of interest to ethnobotanical, herbal science and clinical researchers. Nowadays, herbal preparations are known to increase significantly milk production in women and other mammalian species[9]. Some of these kinds of herbs are chosen for this article, namely Sambucus nigra (Black Elderberry), Matricaria recutita (Chamomile), Urtica dioica (Nettle), Trigonella foenum-graecum (Fenugreek), Melissa officinalis (Lemon Balm)[10-12]. These herbs are already known for many years because of their medicinal uses.

Identifying the bioactive compounds of traditional medicinal plants and predicting their pharmacological activities inside the body is the modern goal for using herbs. With an increasing development of a series of new technologies and tools, the structure and function of these complex chemical ingredients found in herbs can be clarified[12]. Virtual screening is a computational method which has designed to search large-scale chemical structures from various chemical databases and for selecting finite number of macromolecules which is likely to be active against a chosen bio-receptor[13]. Molecular docking techniques are used to explore the complementarity relationship at the molecular level of both ligand and receptor. The result of the docking studies can be used to predict the structural features which are essential for binding, as well as for in silico screening analysis in which appropriate binding partners can be recognize[14]. One of the common bindings of this bio-receptor and macromolecule is interaction between enzymes and substrates, hormones and receptors, protein receptors and their directing ligands and so on. Virtual screening by means of molecular docking reflects the ligand-receptor binding process directly[15]. The objective of the present study is to predict the phytochemical from 5 different medicinal species which might act as inducer for the proteins prolactin through Computer-aided drug design.

MATERIALS AND METHODS:

Examining drug likeness properties and bioactivity of the phytochemical compounds:

Through literature study, 123 phytochemicals compounds were collected from the medicinal plants. By using PubChem databases (http://pubchem.ncbi.nlm.nih.gov/), two dimensional structures of the compounds were predicted[16]. PubChem is a database of chemical molecules and their activities against biological assays. In Molinspiration, using the SMILES notation from PubChem, the collected compounds could be examined whether it can be a drug or not. Molinspiration (https://www.molinspiration.com). If the compounds excite the Lipinski's rules of five and then the physical property of the drug is satisfied to be a drug. Lipinski's rule describes the pharmacokinetic drug properties with four principles: First postulate – molecular weight ≤ 500, second postulate - partition coefficient (logP) ≤ 5; third postulate - number of hydrogen bond donors (HBD) ≤ 5; fourth postulate - number of hydrogen bond acceptors (HBA) ≤ 10[17]. It also stated that more than two rules must not be violated by the compound, if so then the compounds fail to satisfy being an oral drug. Molinspiration itself predicts the drugs bioactivity like hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size, flexibility and pharmacophoric features like bioavailability, transport properties, affinity to proteins, reactivity, toxicity and metabolic stability[18].

Preparation of Protein:

The protein prolactin (PDB ID: 1BP3) was found to play a major role in the production of milk[19,20]. The 3D structure of protein was retrieved from the protein structural database-Protein Data Bank (PDB) (www.rcsb.org) which is an open access archive, storing all information biological molecules such as proteins, nucleic acids, and complex assemblies. The active sites of small molecules which has to be the ligand-receptor binding sites, present in the protein were predicted by using CASTp (http://sts.bioe.uic.edu/castp)[21]. Computed Atlas of Surface Topography of proteins server aims to provide an online resource for locating, delineating and a detailed characterization of topographic features by measuring concave surface regions on three-dimensional structures of proteins.

Molecular Docking analysis:

The energy minimization of both molecules was carried out with different tools. The sdf (Spatial Data File) format of the selected compounds was changed into pdbqt file using OpenBabel[22], (http:openbabel.org). As for the protein, the energy minimization i.e., the removal of water molecule and addition of hydrogen molecules to the three dimensional structures of the macromolecule was carried using PyMOL–*software*[23]. The predict the three dimensional bio-molecular interactions between the protein and the ligands, molecular docking is done. Docking studies can be performed by using PyRx AutoDock Vina software (http://pyrx.sourceforge.net/)[24].

The best docking pose were analysed using PyMOL which can be used to visualize molecular interactions between protein and ligands three dimensionally. The bonding between the ligands and the protein can be clearly observed and predict the distance of hydrogen bond formed[25]. The predicted distance reveals the stability of binding interaction between small molecule and protein.

RESULTS AND DISCUSSION:

Preparation of phytochemical compounds and protein:

The two dimensional Structures of 123 phytochemical compounds were downloaded in the .sdf file format from the NCBI-PubChem Database. The physicochemical properties of all the compounds satisfy the criteria of Lipinski's rule of five were further tested for its bioactive score and the result revealed that the out of 123, only 69 compounds satisfied both drug likeness and bioactivity score which could be analysed for oral drug activity (Table 1). The three dimensional crystal structure of the Growth Hormone-Prolactin receptor complex with PDB ID: 1BP3 determined by X-Ray crystallography at a resolution of 2.90 Å with Chain A – 191 amino acids and Chain B - 211 amino acids were retrieved from the Protein Data Bank. A total of 15 binding sites were assessed in the structure (1BP3) through CASTp software with ideal parameters. The pockets were predicted in both Chain A (38-Lys, 39-Glu, 41-Lys, 67-Thr, 171-Asp, 175-Thr) as well as Chain B (202-Leu, 204-Pro, 205-Gly, 206-Lys, 207-Pro, 208-Glu, 209-Ile, 291-Asp, 292-Glu, 293-Leu, 294-Tys, 339-Trp) with an area of 203.047 Å and volume 280.23 Å occupied in the structure.

Table 1. Bioactivity properties given by Molinspiration for 69 phytochemical compounds that passed Lipinski's rule of five

Compounds	GPCR	ICM	KI	NRL	PI	EI	Compounds	GPCR	ICM	KI	NRL	PI	EI
5-Fluorouracil	-2.6	-1.95	-2.62	-3.04	-3.15	-1.56	α-Pinene	-0.48	-0.43	-1.5	-0.62	-0.85	-0.34
Bornyl Acetate	-0.32	-0.33	-1.33	-0.59	-0.44	-0.12	Eucalyptol	-0.93	0.01	-1.6	-1.07	-0.9	-0.15
Camphene	-1.02	-0.55	-1.85	-1.15	-1.4	-0.82	Pyrrolizidine	-2.49	-2.18	-3.31	-3.41	-2.88	-2.79
Hydroxyethanoyl	-3.8	-3.77	-3.82	-3.81	-3.8	-3.78	Mesityl Oxide	-3.72	-3.56	-3.88	-3.3	-3.56	-3.09
Perillyl Alcohol	-0.61	0.04	-1.31	0.03	-0.93	0.14	Ascorbic Acid	-0.53	-0.24	-1.09	-1.01	-0.81	0.2
α-Phellandrene	-1	-0.4	-1.4	-0.32	-1.38	-0.15	Flavylium	-0.61	-0.3	-0.57	-0.65	-0.75	-0.38
Ocimene	-0.98	-0.08	-1.26	-0.49	-1.24	0.06	Carvacrol	-1.02	-0.51	-1.15	-0.7	-1.25	-0.56
Ethyl Decanoate	-0.6	-0.21	-0.93	-0.57	-0.62	-0.23	Choline Chloride	-2.64	-2.21	-3.63	-3.89	-3.66	-2.18
Beta-Pinene	-0.53	-0.32	-1.45	-0.5	-0.8	-0.34	Citronellol	-0.81	-0.24	-1.16	-0.61	-0.83	-0.12
Myrcene	-1.11	-0.33	-1.51	-0.45	-1.31	-0.07	Hotrienol	-0.76	0.08	-1.39	0.11	-1.1	0.03
Hydroxycitronellol	-0.47	-0.1	-0.81	-0.23	-0.49	-0.1	Umbelliferone	-1.22	-0.72	-1.3	-0.92	-1.3	-0.35
Limonene	-0.91	-0.27	-2.01	-0.34	-1.38	-0.21	Coumarin	-1.44	-0.86	-1.57	-1.42	-1.43	-0.58
Linalool	-0.73	0.07	-1.26	-0.06	-0.94	0.07	Furfural	-3.76	-3.78	-3.86	-3.81	-3.92	-3.8
Menthol	-0.76	-0.3	-1.36	-0.6	-0.67	-0.22	γ -Terpinene	-0.9	-0.24	-1.37	-0.33	-1.55	-0.07
Rose Oxide	-0.77	-0.05	-1.38	-0.7	-0.87	0.03	Herniarin	-1.23	-0.84	-1.28	-1.06	-1.28	-0.47
α-Terpineol	-0.51	0.15	-1.45	-0.02	-0.78	0.14	P-Cimene	-1.18	-0.61	-1.4	-1.21	-1.42	-0.78
β – Phellandrene	-0.99	-0.48	-1.55	-0.28	-1.31	-0.27	Isobutylamide	-3.68	-3.82	-3.75	-3.73	-3.53	-3.67
Thiobarbituric Acid	-2.93	-2.18	-3.08	-3.64	-2.83	-2.07	Scopoletin-7-Glucoside	-0.16	-0.26	-0.26	-0.14	-0.16	0.29
Borneol	-0.47	-0.51	-1.57	-0.84	-0.8	-0.23	Choline	-2.64	-2.21	-3.63	-3.89	-3.66	-2.18
Chamazulene	-0.33	-0.34	-0.67	-0.4	-0.72	0.04	Angelic Acid	-3.45	-3.08	-3.79	-2.35	-3.58	-2.48
3-Carene	-1.29	-0.79	-1.51	-1.28	-1.28	-0.53	Sulcatone	-2.45	-1.6	-3.31	-1.99	-2.48	-1.3
Anethole	-1.23	-0.69	-1.31	-0.94	-1.46	-0.73	Carane	-1.32	-0.89	-1.52	-1.59	-0.92	-0.78
Calycosin	-0.25	-0.65	-0.08	0.06	-0.78	0.01	Eugenol	-0.86	-0.36	-1.14	-0.78	-1.29	-0.41
Carvone	-1.23	-0.3	-2.51	-0.54	-1.21	-0.45	γ -Gurjunene	-0.48	-0.04	-1.07	-0.08	-0.62	0.1
Daidzein	-0.3	-0.64	-0.2	0.04	-0.83	0.02	Myrtenal	-0.32	-0.27	-1.16	-0.36	-0.42	-0.02
Formononetin	-0.3	-0.69	-0.19	0.05	-0.8	-0.02	Pinocarvone	-0.77	-0.58	-2.06	-0.65	-0.67	-0.38
Gentianine	-0.53	0.02	-0.58	-0.81	-0.92	-0.09	Pulegone	-1.33	-0.81	-2.13	-0.86	-1.09	-0.48
Irilone	-0.2	-0.67	-0.16	0.07	-0.7	0.02	Sylvestrene	-0.94	-0.33	-1.99	-0.39	-1.42	-0.21
Tricine	-0.49	-0.21	-1.06	-0.99	-0.32	-0.06	Citronellal	-0.83	-0.08	-1.3	-0.61	-0.5	-0.03
Trimethylamine	-3.71	-3.69	-3.74	-3.82	-3.76	-3.75	(Z)-B-Ocimene	-0.98	-0.08	-1.26	-0.49	-1.24	0.06
Betaine Hydrochloride	-2.5	-1.83	-3.61	-3.68	-3.21	-1.84	Quararibea Lactone	-0.49	0.03	-1.36	-0.72	-0.13	0.14
P-Cymene	-1.18	-0.61	-1.4	-1.21	-1.42	-0.78	Caffeic Acid	-0.48	-0.23	-0.81	-0.1	-0.79	-0.09
Artemisia	-1.14	-0.23	-1.88	-0.43	-0.94	0	Cinerone	-0.8	-0.41	-1.93	-0.7	-0.94	0

Ketone													
Sabinene	-1.15	-0.33	-1.79	-0.69	-0.78	-0.6	Cis-Myrtanol	-0.14	-0.16	-0.78	-0.45	-0.22	-0.02
Thymol	-1.05	-0.53	-1.29	-0.78	-1.34	-0.57							

Note: GPCR - G protein-coupled receptors; ICM-Ion channel modulator; KI -Kinase inhibitor; NRL-Nuclear receptor ligand; PI-Protease inhibitor; EI-Enzyme inhibitor

Molecular Docking Interaction:

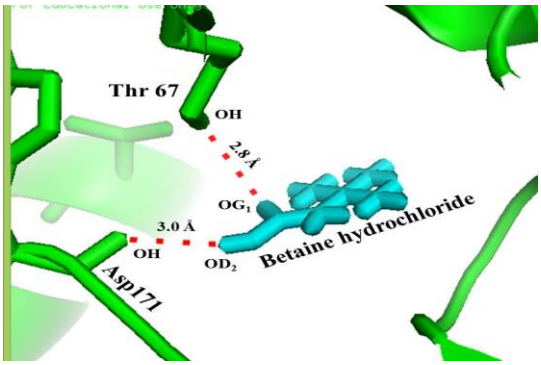
Molecular docking was carried out for 69 compounds, 4 drugs and the protein prolactin receptor using PyRx AutoDock Vina. On analysing the docking result it was predicted that out of 69 compounds, 50 compounds interacted with the protein receptor.

- The Betaine hydrochloride compounds docked with the protein 1BP3 exhibited the least binding interaction of -8.5 kcal/mol
- The 6 compounds - Irilone, Formononetin, Daidzein, Gamma-Gurjunene, Chamazulene and Calycosin docked with the protein 1BP3 exhibited the good binding interaction which ranges between 7-6 kcal/mol with the receptor.
- The binding interaction of the 13 compounds - α-Terpineol, Umbelliferone, Carane, Coumarin, Sabinene, 3-Carene, Bornyl Acetate, Caffeic Acid, Gentianine, Carvone, Carvacrol, Sylvestrene and Tricine ranges between 6-5 kcal/mol with the receptor..
- Further 30 compounds - Thymol, Beta-Phellandrene, α-Phellandrene, Gamma-Terpinene, Menthol, Borneol, Pulegone, P-Cymene, Limonene, Trimethylamine, Linalool, Eugenol, Pinocarvone, Perillyl Alcohol, Rose Oxide, Citronellol, Cinerone, Anethole, 5-Fluorouracil, Myrtenal, Ethyl Decanoate, Cis-Myrtanol, Beta-Pinene, Ocimene, Quararibea Lactone, Myrcene, Citronellal, Sulcatone, Thiobarbituric Acid and Mesityl Oxide showed the binding energy less than -4kcal/mol with the receptor..
- The remaining 19 compounds- Camphene, Isobutylamide, Pyrrolizidine, α-Pinene, Eucalyptol, (Z)-β-ocimene, Ascorbic acid, Flavylium, Choline chloride, Hotrienol, Hydroxycitronellol, Angelic acid, Artemisia ketone, Choline, Furfural, Herniarin, P-Cimene, Scopoletin-7-glucoside and Hydroxyethanoyl does not exhibit any binding interaction with the receptor.
- Drugs exhibited the good binding interaction with protein 1BP3: Domperidone (-7.8 kcal/mol), Risperidone (-8.3 kcal/mol), Metoclopramide (-7.3 kcal/mol) and Sulpiride (-7.1 kcal/mol).

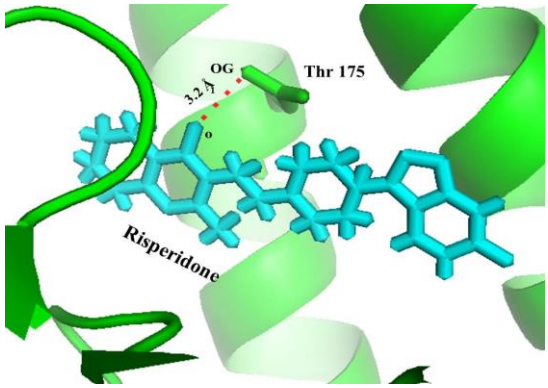
Visualisation of three dimensional docking interactions:

The compound Betaine hydrochloride found to exhibits the better binding interaction (-8.5 kcal/mol) by forming 2 hydrogen bonds conformation with the protein (1BP3) when compared with the binding interaction of the recommended Drug Risperidone -8.3 kcal/mol forming only one hydrogen bond (Table 2)

Table 2: Docking interactions between Betaine hydrochloride (Natural Compounds), Drug (Risperidone) and Protein 1BP3



Docking interactions between Betaine hydrochloride (Natural Compounds) and Protein 1BP3



Docking interactions between Drug (Risperidone) and Protein 1BP3

- **Binding affinity between protein and betaine hydrochloride:** The atom OH presents in the amino acid Threonine 67 formed two hydrogen bonds with OG_1atom of Betaine hydrochloride by the bond length of 2.8 Å. The amino acid Aspartic acid 171 formed hydrogen bond between OH atom and OD_2 atom of the ligand with a bond length of 3.0 Å. All the three binding interaction were observed in the helical structure of the protein which confirm the strong inhibitory activity and the stability of the compound.
- **Binding affinity between protein and risperidone:** The amino acid Threonine 175 formed only one hydrogen bond between OG_1 atom of the protein and O atom of the prescribed drug risperidone with a bond length of 3.2 Å.

The predicted bioactive compound Betaine hydrochloride is found in three medicinal plant of this study (*Melissa officinalis*, *Matricaria recutita* and *Trigonella foenum-graecum*). The Betaine hydrochloride is already used to treat anaemia, atherosclerosis, gallstones, hypokalemia, hay fever, yeast infections, food allergies, inner ear infections and thyroid disorders[26-29]. The Betaine hydrochloride also used to protect the liver of the humans[30]. So the Betaine hydrochloride predicted from this *insilico* study can be providing a strong recommendation for the compound to act as the prolactin inducer.

CONCLUSION:

In this research work, 123 phytochemical compounds were retrieved from the 5 medicinal plants (*Sambucus nigra*, *Matricaria recutita*, *Urtica dioica*, *Trigonella foenum-graecum* and *Melissa officinalis)* were screened for the inducing activity towards the protein prolactin through *in Silico* tools. Out of 123 compounds, only 69 compounds pass the screening test of drug likeness properties and these natural phytochemicals can be further evaluated for the docking process. From the result of docking studies, it was clearly predicted that the natural compound exhibits the good binding affinity as that of standard lactation inducer drugs and it could be concluded that the compound Betaine hydrochloride may act as a good inducer for protein prolactin. The result of the current study highly suggests the compound Betaine hydrochloride might be promoted as a potential lead molecule for the designing and synthesis of the prolactin inducing drug through *in vitro* and *in vivo* experimental studies.

CONFLICT OF INTEREST:

The authors declare they have no competing interests

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