A novel formulation of veggies with potent anti-migraine activity

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Abstract: Calcitonin gene-related peptide (CGRP) is involved in triggering migraine. Many strategies for antimigraine drug designing have been employed using various CGRP antagonist/ligands but most of them have failed due to their inability to reach target CGRP receptor as they get metabolised before conferring their pharmacological action and they are also toxic to the liver. In the present study, we evaluated the binding of our active ligands present in *real veggies* with the CGRP receptor crystal structure and compared their binding energy and affinity with other reference anti-migraine drugs/ligands present in the market. A high-throughput screening comprising of molecular docking, Absorption, Distribution, Metabolism, Excretion and Toxicity predictions, logP values and % of human oral absorption value led to the identification of two potential compounds present in *Live green real veggies* which could be considered for anti-migraine activity with better binding affinities than the reference drugs used and with liver-protective properties.

Keywords: calcitonin gene-related peptide; CGRP; migraine; veggies; glucosinolate; sulforaphane; indole-3-carbinol; carnitine; *in silico* studies.

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1 Introduction

Migraine is a neurological condition that is characterised by over excitability of certain active protein which leads to inflammatory pain in specific area of the brain. It is three times more common in women than men. Goadsby et al. (2002) states that migraine gets triggered by a brain dysfunction that leads to activation and sensitisation of the trigeminovascular system, particularly trigeminal nociceptive afferents innervating (meninges) and leading to headache.

Migraine attacks essentially include four phases:

- Prodromic phase with premonitory symptoms which precedes by hours to days and leads to mood swings, irritability, depression, fatigue, yawning, excessive sleepiness, craving for certain food stiff muscles, increased urination etc.
- Aura phase, precedes the headache with transient neurological symptoms and signs, classically the patient develops a visual disturbance in the form of flashes of black and white or multicoloured zigzag lines. These develop and expand slowly over 5–20 min and usually last for <60 min. The aura is often followed by intense headache which lasts from 4 h up to three days.
- Headache phase, which is an abnormal sensitivity to pain that develops in many patients.
- Recovery or postdromic phase – Patient recovers from headache, but general malaise and exhaustion persist for variable period of time (Halpern and Silberstein, 2005).

Of all the phases only the headache phase can be treated. Individuals with migraine are more susceptible to 'triggers' that raise risk of migraine attack. These triggers include hormonal fluctuations; environmental stimuli such as weather or bright lights, certain smells, alcohol, certain foods, poor sleep and high stress.

The dysfunction in the central nervous system that results in migraine is associated with release of inflammatory mediators such as calcitonin gene-related peptide (CGRP), substance P, and neurokinin A that mediate vasodilation and mast cell degranulation which further leads to release of pro-inflammatory agents. These pro-inflammatory agents mediate sensitisation and excitation of trigeminal nerves that promote neurogenic inflammation and generation of painful stimuli (Blau and Dexter, 1981). In addition,

CGRP released from the cell body (soma) of trigeminal neurons is thought to act in an autocrine manner to stimulate its own synthesis as well as function in a paracrine manner to stimulate cytokine production in satellite glial cells (O'Conner and Van der Kooy, 1986).

Considering the central role of CGRP receptor in migraine, blocking the physiological effects of CGRP receptor appears to be a logical therapeutic strategy. Shirley (2012) has listed number of CGRP receptor antagonist compounds but they have been shown to be toxic to the liver, a challenge that highlights the difficulty in developing drugs for conditions that affect the brain.

Generally, migraine treatments are aimed at treating headaches and reducing its frequency. There are both specific and non-specific treatments for migraine attacks. Non-specific treatments, such as aspirin, acetaminophen, non-steroidal anti-inflammatory drugs, opioids, and combination analgesics, are used to treat migraine and a wide range of pain disorders (Goadsby et al., 2002). Specific treatments, including ergotamine, dihydroergotamine and the triptans are effective for treating the attacks of primary headaches with neurovascular mechanisms, such as migraine and cluster headache, but not for treating other types of headaches (Lipton et al., 2000).

Some of these therapies used to relieve the pain may be overused or abused, and they are also associated with a lot of side effects, leading to further deterioration of the health condition. Medications such as acetaminophen, ibuprofen, or aspirin are often helpful for mild migraine, however, taking these medicines more than 3 days a week may lead to rebound headaches (Gilmore and Michael, 2011). Taking too much acetaminophen can damage your liver and too much ibuprofen or aspirin can irritate your stomach (Ebell, 2006). Other strong migraine medications includes Triptans (Loder, 2010), Ergots (Gilmore and Michael, 2011) and topiramate (Pringsheim et al., 2012).

We propose the use of *Live green real veggies* as an antagonist to CGRP receptor thus having the potential to prevent migraine with its additional benefit of liver detoxification activity (unpublished data).We have used Triptan and Acetominophen as a reference drugs for our study as they are known to act by blocking the CGRP receptor (Durham, 2006).

2 Materials and methods

2.1 Preparation of receptor

The three-dimensional structure of a class-B GPCR receptor (PDB ID: 3N7S) was retrieved from the Protein Data Bank (www.rcsb.org).

PDB file of class-B GPCR receptor (PDB ID: 3N7S) has four chains A, B, C and D. It consists of heterodimer (CGRP type 1 receptor and Receptor activity-modifying protein 1). The chain A (94 amino acid residues) was used for docking study. The proteins were pre-processed separately by deleting the substrate cofactor as well as the crystallographically observed water molecules (water without H bonds), correcting the mistakes in PDB file, optimising hydrogen bonds. After assigning charge and protonation state finally energy minimisation was done using OPLS2005 force field.

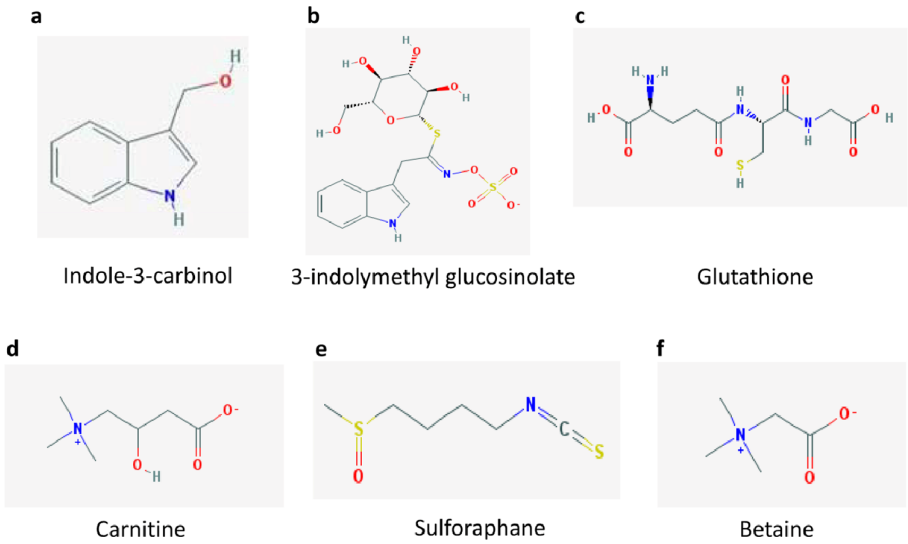
2.2 Compounds used for study

In this study, we used the active compounds of the vegetables used in the composition of the product *real veggies* (Table 1). The main active ingredients in *real veggies* are Glutathione, Indole-3-carbinol, Glucosinolate, Sulforaphane, Betaine and Carnitine. The 2D structures of all these active ingredients presents in *real veggies* were shown in Figure 1.

Table 1 List of active ingredients of *Live Green – Real Veggies*

S. No.	*Organically certified ingredients*	*Actives*
1	Cabbage	Indole-3-carbinol
		Glucosinolate
		Glutathione
		Sulforaphane
2	Broccoli	Glutathione
		Sulforaphane
		Glucosinolate
3	Beetroot	Betaine
4	Parsley	Glutathione
5	Spinach	Betaine
6	Carrot	Carnitine

Figure 1 2D structure of active ingredients of Live green – *real veggies* which shows anti – migraine activity (see online version for colours)



2.3 Ligands preparation

The structure of the active compound of the vegetables used in the composition of the product *real veggie*s was retrieved from PubChem compound database of NCBI

(National Center for Biotechnology Information) (http://pubchem.ncbi.nlm.nih.gov). Further molecular mechanics energy was applied to them and it was then subjected to single step minimisation using steepest descent method for 500 steps at RMS gradient of 0.01.

2.4 Docking methods

We used two docking methods namely Hex (Pringsheim, 2012) and GLIDE (Durham, 2006) for studying the interactions of all active ingredients with CGRP receptor. The basic aim of docking procedures was to identify the correct conformation of ligands in the binding pocket of a protein and to predict the best affinity between the ligand and the protein. The results were selected on the basis of docking scores as well as the interaction of active site of the CGRP receptor with ligands.

2.5 2D diagram preparation

For studying hydrogen bonds as well as the hydrophobic interactions of all docking results we drew two-dimensional diagrams. The two-dimensional structure showing the hydrogen bond and hydrophobic interactions were produced by Ligand Interaction tool of *Schrodinger (Schrodinger Inc. USA)*.

2.6 Absorption, distribution, metabolism, excretion and toxicity (ADME/T) properties prediction

ADME/T properties of all the active ingredients were predicted by using ACD Lab tool (ACD/ADME-Tox, version 12.01) and QikProp tool of Schrodinger (Ritchie and Kemp, 2000). QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. With the help of these tools, we determined the physiochemical properties of compounds such as LogP, pKa, pKb and also the blood brain barrier properties of active ingredients presents in *real veggies*.

3 Results and discussions

The importance of natural inhibitors against various diseases is well known. So, it was important to explore the mode of interaction of these inhibitors against our target protein. For the present study, we selected the active ingredients of real veggies which are promising inhibitors against CGRP receptor on the basis of the docking score as well as the interacting residues present in active or binding side of these proteins. We used two docking methods, Glide and Hex for evaluation of interaction of receptors and individual active compounds presents in real veggies. The comparative studies of two docking results are shown in Table 2. Among all the natural inhibitors docked within the active site of the CGRP receptor, Glucosinolate and Sulforaphane was found to bind with the best efficacy to CGRP receptor, Glutathione and Carnitine were also effective. All the comparisons were made on the basis of dock scores obtained from Hex and Glide software. These ingredients showed better docking than reference drugs. Glucosinolate and Sulforaphane formed five and two hydrogen bonds with CGRP receptor, respectively

(Figure 2(a) and (b)). Interaction diagram (two-dimensional) of glutathione and carnitine with CGRP receptor also depicts four and two H bond interactions, respectively (Figure 2(c) and (d)). Some amino acid residues were found to play an important role in the binding of inhibitors within the active site of CGRP receptor. In general, ARG 119, TRP121, ASP 70, THR 122, TYR 125 and GLY71 of CGRP receptor were involved in making hydrophobic contacts with active ingredients of *Live green real veggies*, and they are presumed to be key players. In silico pharmacokinetics study was done with QikProp and ACD Lab tools for all the six ingredients present in the *Live green real veggies* (Table 3) and they were evaluated for their levels of absorption. Indole-3-carbinol showed the highest oral absorption (>95%) followed by Sulforaphane which showed moderate level of oral absorption (>67%) (Table 3). Egner et al. (2011), reported that sulforaphane from broccoli has a bioavailability of 70% which matched with our Qikprop results. In addition to its oral absorption capacity, it has 100% passive absorption value and has the capacity of crossing the blood brain barrier thus can act directly on CNS inhibiting the activation of CGRP receptor signalling. Other ingredients presents in real veggies showed low levels of oral absorption. In particular, poor aqueous solubility and slow dissolution rate could have lead to their poor oral absorption. All the ingredients except Indole-3-carbinol and sulforaphane showed low probability of crossing the blood brain barrier.

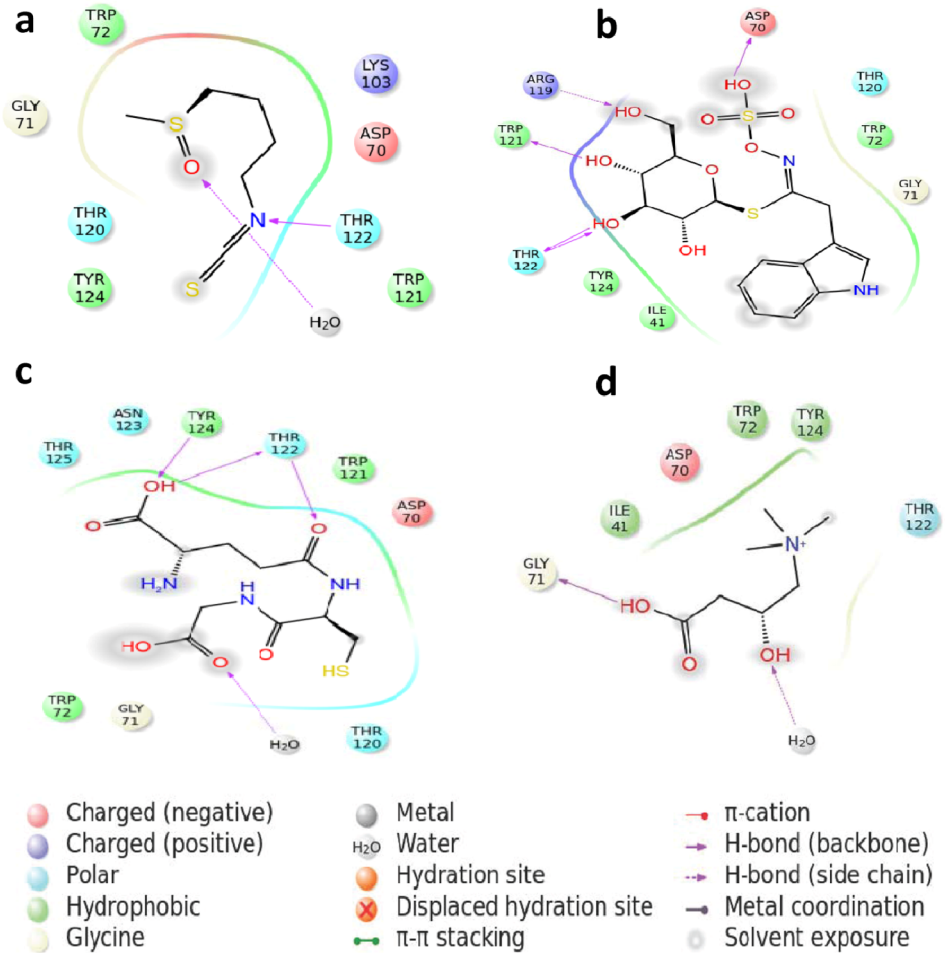
Table 2 Docking scores of Hex docking tool and Glide docking tool

S. No.	*Ligand*	*Etotal using HEX*	*Glide score*
1	Indole-3-carbinol	–170.97	–3.79
2	Glucosinolate	**–281.86**	**–6.56**
3	Sulforaphane	**–215.82**	–2.04
4	Betaine	–149.14	–3.12
5	Glutathione	**–225.88**	**–7.1**
6	Carnitine	**–200.32**	**–5.71**
7	Acetominophen (Reference drug)	–211.47	–4.63
8	Triptan (Reference drug)	–145.58	0.09

Table 3 Qikprop and ACD lab results for *Live green real veggies* ingredients

		Qikprop results			*ACD lab results*		
Ligand name	*Mol. wt.*	*Log Po/W*	*BBB absorption*	*% of human oral absorption*	*log P*	*Extent of brain penetration*	*% of passive absorption*
Glucosinolate	448.5	–0.8	–3.3	0.4	–1.7	LogPB: –2	Poor
Glutathione	307.3	–0.3	–3.3	25.3	–4.9	LogPB: –2	Poor
Sulforaphane	177.2	0.7	0.2	67.9	1.8	LogPB: 0	100%
Indole-3-carbinol	147.2	1.7	–0.2	95.1	1.6	LogPB: 0	100%
Carnitine	161.2	4.57	–2.2	52.7	–4.6	LogPB: –2	Poor
Betaine	117.1	–	–	–	–4.42	LogPB: –2	34%

Figure 2 The docking pattern of Real Veggies active ingredients with CGRP receptor from Glide dock outputs: (a) sulforaphane makes two hydrogen bonds with CGRP receptor; (b) glucosinolate makes five hydrogen bonds with CGRP receptor; (c) glutathione makes four hydrogen bonds with CGRP receptor; (d) carnitine makes two hydrogen bonds interactions with CGRP receptor. The hydrophobic, polar and positively charged residues are coloured green, blue and violet, respectively (see online version for colours)



4 Conclusion

The *in silico* molecular docking study and pharmacokinetics study reveal that the active ingredients of real veggies showed favourable interaction with CGRP receptor with b docking score, hydrogen bonding and ligand–protein interaction than reference drugs. From the ADME-Toxicity prediction it revealed that the docked compounds are in the acceptable range of various pharmacological parameters and they have better behaviour for health effects when compared with reference drugs. They also showed

higher human oral absorption ranging from >67% (Sulforaphane) to >95% (Indole-3-carbinol) and has the capacity of crossing the blood brain barrier thus can act directly on CNS and can inhibit the activation of CGRP receptor signalling. This study provides strong evidence that active ingredients present in *Live green real veggies* can act as an antagonist to CGRP receptor thus having the potential to prevent migraine with its additional benefit of liver detoxification activity. Thus, we conclude that *Real Veggies* can be a potent formulation to act as an effective dietary supplement for migraine patients.

References

Blau, J.N. and Dexter, S.L. (1981) 'The site of pain origin during migraine attacks', *Cephalalgia: An International Journal of Headache*, Vol. 1, No. 3, pp.143–147.

Durham, P.L. (2006) 'Calcitonin Gene-Related Peptide (CGRP) and migraine', *Headache*, Vol. 46, Suppl. 1, pp.S3–S8.

Ebell, M.H. (2006) 'Diagnosis of migraine headache', *Am. Fam. Physician*, Vol. 74, No. 12, pp.2087–2088.

Egner, P.A., Chen, J.G., Wang, J.B., Wu, Y., Sun, Y., Lu, J.H., Zhu, J., Zhang, Y.H., Chen, Y.S., Friesen, M.D., Jacobson, L.P., Muñoz, A., Ng, D., Qian, G.S., Zhu, Y.R., Chen, T.Y., Botting, N.P., Zhang, Q., Fahey, J.W., Talalay, P., Groopman, J.D. and Kensler, T.W. (2011) 'Bioavailability of sulforaphane from two broccoli sprout beverages: results of a short term, cross-over clinical trial in Qidong, China', *Cancer Prev. Res. (Phila)*, Vol. 4, No. 3, pp.384–395.

Gilmore, B. and Michael, M. (2011) 'Treatment of acute migraine headache', *Am. Fam. Physician*, Vol. 83, pp.271–280.

Goadsby, P.J., Lipton, R.B. and Ferrari, M.D. (2002) 'Migraine – current understanding and treatment', *N. Engl. J. Med.*, Vol. 346, pp.257–270.

Halpern, A.L. and. Silberstein, S.D. (2005) *The Migraine Attack – A Clinical Description*, Imitators of Epilepsy, 2nd ed.

Lipton, R.B., Stewart, W.F., Cady, R., Hall, C., O'Quinn, S., Khun, T. and Gutterman, D. (2000) 'Sumatriptan for the range of headaches in migraine sufferers: results of the spectrum study', *Headache*, Vol. 40, pp.783–791.

Loder, E. (2010) 'Triptan therapy in migraine', *N. Engl. J. Med.*, Vol. 363, No. 1, pp.63–70.

O'Conner, T. and Van der Kooy, D. (1986) 'Pattern of intracranial and extracranial projections of trigeminal ganglion cells', *J. Neurosci*., Vol. 6, pp.2200–2207.

Pringsheim, T. (2012) 'Systematic review: medications for migraine prophylaxis – Section II', *Can. J. Neurol. Sci.*, Vol. 39, Suppl. 2, pp.S8–S28.

Pringsheim, T., Davenport, W., Mackie, G., Worthington, I., Aubé, M., Christie, S.N., Gladstone, J. and Becker, W.J. and Canadian Headache Society Prophylactic Guidelines Development Group (2012) 'Canadian Headache Society guideline for migraine prophylaxis', *Can. J. Neurol. Sci*., Vol. 39, No. 2, Suppl. 2, pp.S1–59.

Ritchie, D.W. and Kemp, G.J. (2000) 'Protein docking using spherical polar Fourier correlations', *Proteins*, Vol. 39, No. 2, pp.178–194.

Shirley, S.W. (2012) 'Health and wellness', *The Wall Street Journal*.