

Molecular-Docking Study of Anti-Stress Natural Compounds Against GABA_A Receptor Portends the Novel Approach to Stress Treatment

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ABSTRACT

Introduction: During stress our body responds by activating the nervous system and hormones. The stress response is conveyed by hypothalamic-pituitary-adrenal axis and is responsible for neuroendocrine adaptation of stress response. Hypothalamic-pituitary-adrenal activity is governed by corticotrophin releasing hormone neurons. Increased production of corticotrophin releasing hormone is associated with various stress related metabolic and neurological disorders. The activity of CRH neurons is largely regulated by Gamma-aminobutyric acid (GABA) and is controlled by GABAergic inhibition. To lower down the effect of stress, various synthetic drugs are used which target GABA_A receptor and activates GABA_A activity, producing calming effects but also comes along with various side effects. Hence anti-stress herbs is used as an alternative therapy which helps improving the adrenal system has no side effects and are safe for long term use. **Method:** Our current study is an attempt to analyze the possible mechanism of action of some compounds commonly present in extracts of different anti-stress herbs by using AutoDockVina. **Result:** As a result of our docking studies with GABA_A protein, we have observed that some herbal compounds such as Rosmarinic Acid and Kaempferol are showing comparable binding affinity towards GABA_A receptor, like Lorazepam. Few other compounds sharing common binding site like Lorazepam, indicates that their mechanism of action may also be through activating GABA_A receptor.

INTRODUCTION

Definition of stress is not clear but often described as feeling of being anxious or worried when an individual perceives surrounding demands. Stress has a very wide spectrum of effect on different parts of body in different ways and thus can affect various organs, thoughts, feelings and behavior. If stress is left unchecked, it may ultimately lead to various mental and physical illnesses such as headaches, upset stomach, elevated blood pressure, chest pain, heart disease (Romero *et al.*, 2007).

The hypothalamus in the brain is the in-charge of different stress responses. When a stress response is triggered, hypothalamus sends signals to pituitary gland and the adrenal medulla. The activated hypothalamus stimulates the pituitary gland to produce adrenocorticotropic hormone. It travel through

blood stream to the adrenal cortex and stimulates the adrenal gland to produce the hormone corticosteroid, including cortisol. The cortisol is a stress hormone which enables the body to maintain steady supplies of blood sugar. Adequate and steady blood sugar levels help person to cope with prolonged stressor, and helps body to return to normal (Glover *et al.*, 2010). These stress responses are governed by corticotrophin releasing hormone neurons. The activity of these neurons is largely controlled by robust Gamma-aminobutyric acid (GABA) mediated inhibition. GABA is the most widely distributed inhibitory neurotransmitter in the central nervous system. GABA influences our mood because it reduces high levels of the hormones adrenalin, noradrenalin and dopamine.

Upon neuronal activation, GABA is released from vesicles into the synapse, where it can act on postsynaptic receptors, or diffuse into the extracellular space and activate extra synaptic receptors on postsynaptic neurons (Kalueff *et al.*, 1996; Roozendaal *et al.*, 1997; Nutt *et al.*, 2006). GABA is then removed from the extracellular space by GABA transporters located on presynaptic neurons and glia that take up GABA into cells away

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from postsynaptic GABA receptors (Luján *et al.*, 2005; Mihic *et al.*, 1997). Extracellular levels of GABA are regulated by the balance between GABA release from presynaptic vesicles and GABA uptake by GABA transporters (Sahley *et al.*, 2010). As such, GABA limits the excitability of neuronal activity in all areas of the brain. GABA inhibits the cells from firing, diminishing the anxiety related messages from reaching the cortex (Glover *et al.*, 2010). All the functions of GABA is mediated through its specific receptor; GABA receptor. There are two main types of GABA receptors: fast-acting ionotropic GABA_A and slow acting ionotropic GABA_C (Mihic *et al.*, 1997).

To lower down the effect of stress, different sedative medications are commonly recommended by clinicians. These sedatives depress the activity of the central nervous system (causing a sense of relaxation, reduced anxiety and tension, sleepiness, and slowed breathing. One of the most commonly prescribed groups of sedatives is the Benzodiazepines which include alprazolam (Xanax), clonazepam (Klonopin), diazepam (Valium), lorazepam (Ativan), triazolam (Halcion), temazepam (Restoril), and chlordiazepoxide (Librium). Benzodiazepines and alcohol produce their calming effects by activating GABA receptor which ultimately inhibit brain activity and thus slow and calm down the body (Kulkarni *et al.*, 1996). However, the use of

these drugs for long period is known to exhibit some severe side effects at physiological and psychological level such as slurred speech, impaired ability to walk around, poor judgment, slowed reflexes, low blood pressure, sexual dysfunction, memory loss. Hence, there is always a need of alternative therapy for stress management (Kinnersley *et al.*, 2000). As per published literature, a large number of herbs are known to help improving the function of adrenal system and reduces the stress too. Additionally, these anti-stress herbs are safe for long term use (Pawar *et al.*, 2012).

Various epidemiological studies have shown that a diet rich in plant derived bioactive dietary constituents has protective effect against stress in human. Many herbs have their own specific components but few compounds are still common in these herbs. Kaempferol, Rosmarinic acid, Caffeic acid and Eugenol are few of the common phytochemicals used in many traditional medicines for stress management (Fujisawa and Murakami, 2016; Lakshmi *et al.*, 2011; Lee *et al.*, 2014; Friedman *et al.*, 2015). These compounds are predominantly present in different well studied antistress herbs such as *Sarcandra glabra*, *Curcuma longa* and *Perilla herba* etc. (Table 1). Numerous preclinical studies have shown their wide range of pharmacological activities and their safety in usage (Calderon *et al.*, 2011; Vladimir *et al.*, 2014; Khalil *et al.*, 2017).

Table 1: Common anti-stress herbs and their important compounds.

Anti-stress Herbs	Genus	Family	Common Name	Important compounds	Reference
<i>Oryzasativa</i>	<i>Oryza</i>	Poaceae	Rice bran	γ -oryzanol, tocopherol, tocotrienols.	Heinemann <i>et al.</i> (2008)
<i>Ginkgo biloba</i>	<i>Ginkgo</i>	Ginkgoaceae	Maidenhair tree	Kaempferol, terpene lactones, ginkgolide, tocopherols	Zahradniková <i>et al.</i> (2007)
<i>Panax ginseng</i>	<i>Panax</i>	Araliaceae	Ginseng (Asian)	Triterpenoid, ginsenosid, panaxtriol, germanium	Heffern (1976)
<i>Eugenia carophyllus</i>	<i>Syzygium</i>	Myrtaceae	Clove	Eugenol, carophylleneoxide, eugenylacetate,	Uddin <i>et al.</i> (2017)
<i>Rhodiolarosea</i>	<i>Rhodiola</i>	Crassulaceae	Golden root	Rosarin, rodiolin, tyrosol, triterpenes	Colitti (2014)
<i>Curcuma longa</i>	<i>Curcuma</i>	Zingiberaceae	Turmeric	Cinnamic acid, eugenol, caffeic acid, capsaicin, gingerol	Gupta <i>et al.</i> (2010)
<i>Perilla herba</i>	<i>Perilla</i>	Lamiaceae	Perilla	Rosmarinic acid, caffeic acid, kaempferol	Ito <i>et al.</i> (2008)
<i>Sarcandra glabra</i>	<i>Sarcandra</i>	Chloranthaceae	Gumok	5-Caffeoylquinic acid, 3-caffeoylquinic acid, caffeic acid, 4-O-b-D-glycopyranosyl rosmarinic acid	He <i>et al.</i> (2009)
<i>Withaniasomnifera</i>	<i>Withania</i>	Solanaceae	Indian ginseng	Withaferin-a, Sitoindoside – IX-H, Sitoindoside – X-Palmitoyl	(Kaur <i>et al.</i> , 2001; Geetha <i>et al.</i> , 2008)
<i>Ficus benghalensis</i>	<i>Ficus</i>	Moraceae	Bargad	Rutin and quercetin	Lotankar <i>et al.</i> (2016)

The presence of these common components in many of these anti-stress herbs indicates towards their most probable role in managing the stress. But the mechanism of action of these compounds as possible anti-stress factor is not yet fully studied. It will be interesting to highlight the possible target and further propose the mechanism of action of these anti-stress herbs. Hence in order to understand it, our study was conducted further by taking the well-studied synthetic drug molecule, as a positive control. As most of the anti-stress synthetic drug targets and activates GABA_A receptor protein, hence in our study, we also docked the common anti-stress herbal components such as Kaempferol, Rosmarinic acid, Caffeic acid and Eugenol with GABA_A receptor and compared the docking results with that of a common synthetic drug Lorazepam.

MATERIAL AND METHODS

In order to evaluate the affinity of different anti-stress herbal components towards GABA_A, mainly four compounds

namely, Eugenol (ID 3314), Rosmarinic-acid (ID 5281792), Kaempferol (ID 5280863) and Caffeic-acid (ID 689043) were selected. Lorazepam (ID 3958) a synthetic drug molecule was also used as positive control for the docking study.

Protein sequence homology search

BLAST (Basic Local Alignment Search Tool) is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences (Kloet *et al.*, 2003). GABA_A receptor sequence of Homo sapiens (ID 119619829) was run on pBLAST against non-redundant protein database with default parameters. The sequences of the target protein and those of the orthologs found were extracted from the NCBI Protein database.

Evolutionary analysis of sequence

For evolutionary analysis of each amino acid, the FASTA format of the GABA_A protein sequences from all the above

selected organisms was used under the option of Estimate Position By Position rates in MEGA software version 7 (Pennsylvania State University, License—Proprietary freeware) (Kumar *et al.*, 2016). Mean (relative) evolutionary rate were shown for each site next to the site number. These rates are scaled such that the average evolutionary rate across all sites is 1. This means that sites showing a rate < 1 are evolving slower than average, and those with a rate > 1 are evolving faster than average. These relative rate were estimated under the Jones-Taylor-Thornton model (+G) (Jones *et al.*, 1992). A discrete Gamma (+G) distribution was used to model evolutionary rate differences among sites (5 categories). All positions containing gaps and missing data were eliminated. There were a total of 491 positions in the final dataset.

Sequence aignificance

As domains play an influential role in deciding protein structure and function. Hence, in order to identify the domains, GABAa sequence was run on Interpro, online software of EMBL-EBI.

Computational docking

In order to evaluate the affinity of different ligands (synthetic as well as different components of anti-stress herbs) towards Gamma-Aminobutyric Acid Receptor A (GABAa), computational docking was performed using Autodockvina 1.1.2 (Trott and Olson, 2010; Seeliger and Groot, 2010). The files for all these ligands were downloaded from NCBI-PubChem. As the GABAa receptor protein is active in homopentamer state, not in monomeric form, hence, the crystal structure of human GABAaR-beta3 homopentamer was downloaded from PDB database (PDB ID 4COF) (Figure 1). Active site prediction was done using published literature (Miller *et al.*, 2014) and Uniprot. Before docking, protein structure was prepared by adding hydrogen and removing water molecules.

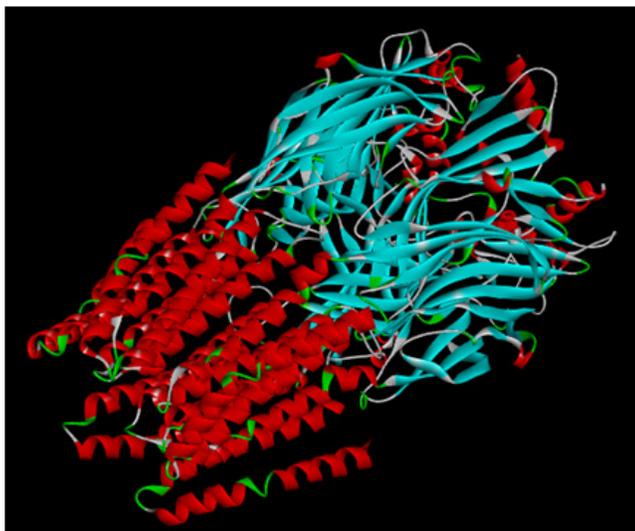


Fig. 1: Crystal structure of human GABAaR-beta3 homopentamer obtained from PDB database (PDB ID 4COF).

Structural Visualization

For visualizing the result of docking Schrödinger PyMOL v1.8.6.0, molecular visualization software (Seeliger

and Groot, 2010) that can produce high-quality 3D images of small molecules and biological macromolecules was used.

RESULT AND DISCUSSION

In order to find close relative of the query human GABAa protein, pBLAST was run and from the similarity search result, primates with at least 98% similarity and high E value (that is zero), 7 closest relatives namely Pan paniscus, Pongoabelii, Macacacnemestrina, Cebuscapucinus imitator, Saimiriboliviensis, Propithecuscoquereli and Callithrixjacchus were selected. For the purpose of studying the evolutionary rates of the protein on site by site basis, sequences of all the selected organisms were ran on MEGA software. As a result, across the entire length of GABAa protein sequence (493 amino acids), total 5 separate gamma parameters (between 0.1 to 1.0) were suggested, for each amino acid (AA) residue. Taking the highest gamma parameters for each site, a graph was prepared and on comparison with average gamma value (0.206), those AA sites were identified which had, highest and lowest rates of evolution. As a result the AAs stretch from 24 to 384 was found to be below the average value and so comparatively conserved (Figure 2). Similarly, Interpro search showed 2 domain that lie in the conserved region of the protein i.e. 68-277 (Neurotransmitter-gated ion-channel ligand-binding domain (IPR006202)) and 276-481 (Neurotransmitter-gated ion channel transmembrane-domain (IPR006029)). So any ligand binding in these conserved regions could be considered a better ligand since chances of mutation would be comparatively less in this region.

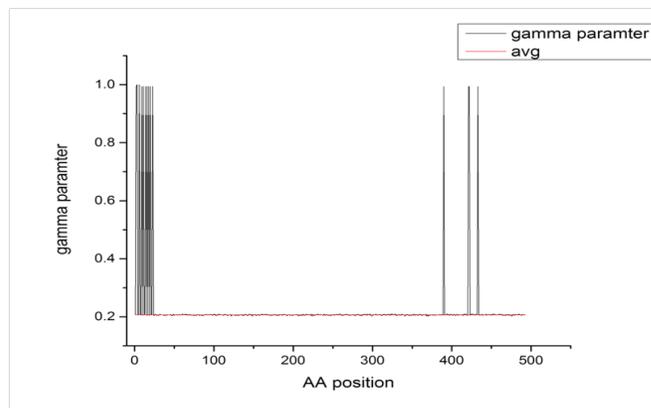


Fig. 2: Graph showing the gamma parameter for each amino acid of GABAa receptor. Average Gamma parameter is 0.206 and showing AA positions that have lower and higher evolutionary rate in the protein sequence.

Further, *in-silico* docking was done to produce many conformation of protein-ligand complex, among which at least one native-like conformation was selected and its binding affinity was checked. Docking is important to determine the properties associated with protein-ligand interaction such as binding energy and hydrophobicity as well. Binding affinity of all the selected natural compounds was compared with that of Lorazepam drug.

In our analysis, all those natural compounds which exhibited almost similar binding affinity like Lorazepam, was suggested to bind to GABAa receptor. Further the conformation

with highest binding affinity was considered to be the most suitable docking pose. As shown in Table 2, docking score of all ligand was found ranging between -6.9 to -9.2 kcal/mol. It was observed that out of all the natural compounds, Kaempferol and Rosmarinic acid showed the binding affinity close to Lorazepam (-9.2 and -8.8 kcal/mol respectively), however the other two compounds like Eugenol and Caffeic acid showed relatively low affinity towards the GABA_A receptor.

Table 2: Comparative docking study of the different ligands to the target (GABA_A receptor protein).

Compound	Affinity (Kcal/mol)	Position
Lorazepam	-9.1	TYR (157,205), THR (202)
Kaempferol	-9.2	SER (156)
Rosmarinic acid	-8.8	THR (176), ASN (41), GLU (155), TYR (97), ARG (180)
Eugenol	-6.9	GLN (64), THR (202), TYR (205)
Caffeic-acid	-7.2	TYR (62), ASP (43), ARG (180), GLN (64), THR (202), TYR (205)

Further binding position and number of bonds of these ligand-protein complexes were checked using PyMol. The GABA_A protein is 493 AAs long and with a binding affinity of -9.1 kcal/mol, Lorazepam form 3 hydrogen bonds inside the ligand binding domain of receptor protein at TYR (157,205) and THR (202) positions (Table 2). It binds with hydroxyl group present in the drug structure as shown in Figure 3a.

Kaempferol also bind to GABA_A protein with almost similar affinity (-9.2 kcal/mol) like Lorazepam. It binds to the receptor protein by one hydrogen bond at SER (156) (Figure 3b). Though this position is different from the position where Lorazepam binds to the receptor, however the SER at 156 position is also located in the conserved ligand binding domain of GABA_A receptor. This may be the reason that the binding affinity of Kaempferol was comparable to that of Lorazepam. Further, it is known that Kaempferol is a flavanoid having a prenyl group that might interact with human GABA_A protein through additional hydrophobic and anionic characteristic moieties (Jung *et al.*, 2012). The presence of a 4'-hydroxyl group on the Kaempferol can also increase the affinity and inhibitory potency against GABA_A via interaction with SER (156) at the active site.

Next to Kaempferol, Rosmarinic acid was another important compound in our study. It was found that Rosmarinic acid with binding affinity of -8.8 kcal/mol, formed 5 Hydrogen-bond with the GABA_A receptor and AA residue conscientious for its binding interactions were THR (176), ASN (41), GLU (155), TYR (97) and ARG (180) (Figure 3c). Out of all these five AAs, except ASN (41), rest all the AAs were present in conserved ligand binding domain of GABA_A receptor protein. Since Rosmarinic acid has only one double bond between the two aromatic rings, it has a flexible structure (Dileep *et al.*, 2011), which may help in binding to different positions of the receptor protein. Further there are eight oxygen atoms which can act as hydrogen bond acceptors/donors in rosmarinic acid important for interaction with other proteins.

While comparing the binding positions of Kaempferol

and Rosmarinic acid, it is interesting to note that, in case of Kaempferol the bond was formed in the ligand binding domain while as for Rosmarinic acid one bond ASN (41) was not formed inside the conserved ligand binding domain region of the GABA_A protein which was further reflected in terms of low binding affinity for Rosmarinic acid.

Next to Kaempferol and Rosmarinic acid, Caffeic acid showed the binding affinity of -7.2 kcal/mol. It was found to form 6 Hydrogen-bond with receptor protein at TYR (62), ASP (43), ARG (180), GLN (64), THR (202), TYR (205) AA residues (Figure 3d). Out of all the 6 positions, only three AA reside in ligand binding domain of receptor, remaining three (TYR (62), ASP (43) and GLN (64)) was not the part of conserved binding domain. This may be the reason that the overall binding affinity for Caffeic acid was less than Lorazepam, Kaempferol and Rosmarinic acid as well. Similar to Caffeic acid, Eugenol also showed lower binding affinity (-6.9 kcal/mol) towards GABA_A receptor protein. It formed 3 Hydrogen-bond at GLN (64), THR (202), TYR (205) (Figure 3e) and thus have common binding partner like Caffeic acid. All three compounds, Lorazepam, Eugenol and Caffeic acid interact with the GABA_A receptor protein at two common position that is THR (202), TYR (205). However there was significant difference in the binding affinity of these three ligands. It may be due to the structural differences amongst them.

So after analyzing all the results of our selected ligands, the study suggested that Rosmarinic acid and Kaempferol (with a binding affinity of -8.8 and -9.2 kcal/mol respectively) binds at conserved ligand binding domain of GABA receptor and could be use as substitute for Lorazepam drug. While the other two ligands, Eugenol and Caffeic-acid showed relatively low binding affinity towards the protein receptor and were not considered further.

CONCLUSION

The protein-ligand interaction plays a significant role in drug design. Our study shows that all the studied herbal compounds are binding to GABA_A receptor at its conserved sequence region from 24 to 384 and at Neurotransmitter-gated ion-channel ligand-binding domain. Additionally, the binding affinity of Rosmarinic Acid and Kaempferol (-8.8 and -9.2 kcal/mol, respectively) with receptor GABA_A is highly comparable to the binding affinity of drug Lorazepam (-9.1 kcal/mol). So, it can be proposed that extracts of some herbs such as *Sarcandra glabra*, *Perilla herba*, *Ginkgo biloba* are almost equally good anti-stress factors as Lorazepam and can be used as a substitute for the synthetic drug. However, a complete analysis of these compounds for their absorption, distribution, metabolism, excretion/toxicity properties is need to be studied further by different *in-vitro* and *in-vivo* assays.

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CONFLICT OF INTEREST

There are no conflicts of interests.

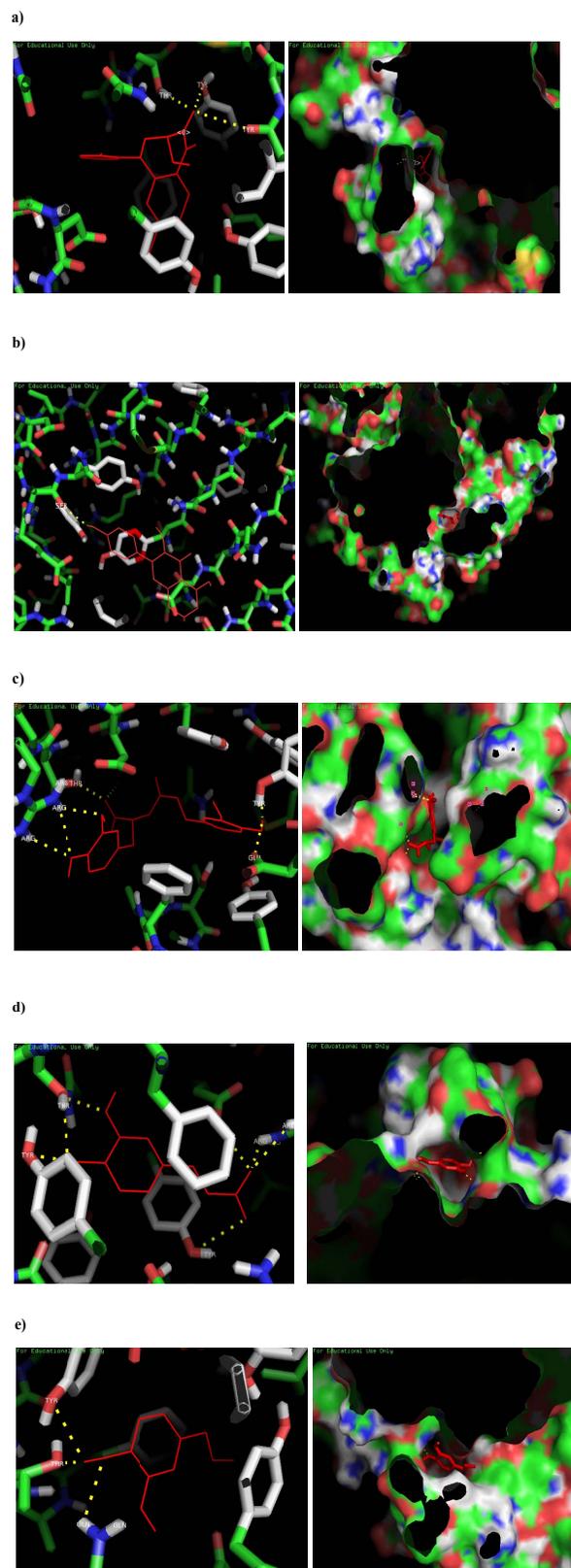


Fig. 3: The figure show overall binding interaction of GABA α protein receptor amino acids with different ligands. The 3D stick view (on left) and surface view (on right) was created using PyMol v1.8.6.0. The hydrogen bonds are indicated by yellow dash line and ligand is shown in red color. (a) Lorazepam forming 3 hydrogen bond with a binding affinity of -9.1 kcal/mol. (b) Kaempferol forming 1 hydrogen bond with an affinity of -9.2 kcal/mol. (c) Rosmarinic acid forming 7 hydrogen bond with an affinity of -8.8 kcal/mol. (d) Caffeic acid forming 6 hydrogen bond with a binding affinity of -7.2 kcal/mol. (e) Eugenol forming 3 hydrogen bond with a binding affinity of -6.9 kcal/mol.

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