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Studies on the Molecular Interaction of Colchicine with Antioxidant Signaling Molecules and Identification Antidiabetic Activity: Evidences through *In-silico* Analysis

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Abstract

Colchicine, a naturally occurring alkaloid, has garnered attention for its potential anti-diabetic properties. This study delves into the molecular interactions between colchicine and antioxidant signaling molecules, aiming to uncover its therapeutic potential in managing diabetes. The primary aim of this research is to investigate the intricate molecular interactions between colchicine and key antioxidant signaling molecules. Additionally, through in-silico analysis, the study seeks to identify the antidiabetic activity of colchine. In this study, molecular docking simulations were employed to explore the binding affinities and interactions of colchicine with antioxidant signaling molecules like superoxide dismutase, catalase, glutathione peroxidase, Peroxiredoxin and Hemeoxygenase. The computational analysis was carried out using state-of-the-art software tools, allowing for a comprehensive assessment of potential binding energies. Furthermore, an in-silico analysis was conducted to predict colchicine's ability to modulate key pathways related to diabetes. The findings reveal that colchicine exhibits strong binding affinities with antioxidant enzymes, suggesting its potential as an antioxidant agent. This study provides valuable insight into the molecular interactions between colchicine and antioxidant signaling molecules. The promising binding affinities and potential antidiabetic activity identified through in-silico analysis highlight colchicine as a candidate for further investigation as a therapeutic agent for diabetes. Further in vitro and in vivo experiments are warranted to validate these in-silico finding and unlock the full potential of colchicine in diabetes management.

Keywords: *Antioxidant, Health and well-being, Novel Method, Antidiabetic, Binding Site, Colchicine, Novelty, Public Health, Therapeutics.*

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Introduction

The survey showed that over 451 million people were affected by diabetes and it's one of the main causes of mortality in the patients. A combination of inadequate insulin secretory responses and malfunctioning beta cells, diabetes mellitus is a disorder of metabolism that primarily affects the endocrine gland and is characterized by hyperglycemia and intolerance to glucose. In both enzymatic and non-enzymatic reactions result in the continual production of 10000 to 20000 free radicals in the average person. By the process of overly glycating protein, degrading glycated proteins and oxidizing glucose, free radicals are produced in diabetes [1]. Both extracellular and intracellular triggers may initiate a cycle of these extremely reactive molecules. Insulin(hormone), tumor necrosis factor, and growth factors like epithelial growth factor and platelet-derived growth factor on the plasma membrane receptor act as external factor in eliciting reactive species. Mitochondrial electron transport, Nitric oxide synthase and nicotinamide adenine dinucleotide phosphate oxidase are intracellular triggers that cause the synthesis of reactive species. Additionally, different enzymes, such as xanthine oxidase, glucose oxidase, monoamine oxidase and lipoxygenase can also produce reactive species [2-5]. Reactive oxygen species are not directly generated by insulin nonetheless insulin impacts on cellular metabolism and other signaling pathways can indirectly affect ROS generation. In the cells, oxidative phosphorylation takes place, even though it is crucial for the production of energy, it can also result in the production of ROS as an outcome of mitochondrial respiration. Increased production of ROS can result from excessive glucose metabolism, which is frequently accompanied with insulin resistance or hyperglycemia [6]. In context with this, cells have developed a balanced system to neutralize excess ROS. This system is called an antioxidant system, and it consists of both enzymatic antioxidants like superoxide dismutase (SOD) [7], glutathione peroxidases (GPxs), Nrf-2 [8], Peroxiredoxin and hemeoxygenase, as well as non-enzymatic antioxidants that reduce oxidative state collectively [9]. In this *In-silico* study, molecular interaction of colchicine with the antioxidant molecules in the elimination of ROS are primarily the area of concern.

Materials and Methods

Preparation of Ligand

The Colchicine (CID: 6167) [10] 3D chemical structure was downloaded from PubChem database. The chemical structure was downloaded in SDF file format, then it is converted into PDB file format by using the online translator. Ultimately the ligand format was changed by using Auto Dock Tool for the further analysis.

Preparation of Receptors

Three-dimensional coordinates of superoxide dismutase (P08294) [11], Nuclear factor erythroid 2-related factor (Q16236) [12], Glutathione peroxidase (P07203) [13], Peroxiredoxin (Q13162) [14] and Hemeoxygenase (P09601) [15] was retrieved from Uniprot Protein Data Bank and colchicine 3D chemical structure were obtained from PubChem database the structure was downloaded in SDF format and then converted into PDF format by using online translators. The auto dock tool is used to create the receptor molecule, after which the protein molecules are given kollman charges and the missing atoms and polar hydrogen are inserted. And finally, the file format was changed to PDBQT format for further analysis.

Active Site Identification

Utilising the CSATp server, the binding site identification was completed. This server can identify the atoms that line pockets, pocket apertures and concealed cavities, as well as the quantity and location of pockets and cavities, as well as the placement and width of mouth openings.

Docking

The docking of the obtained molecules was carried out by using Auto Dock Tools. The Auto Grid strategy then produced the three-dimensional grid boxes to assess the binding energies on the coordinates of macromolecules. Using Auto Grid, grid maps representing the whole ligand at the actual docking target site were generated. The complete ligand was lodged in the binding site, which was eventually surrounded by cubic grids. Then, the graphical user interface of Auto Dock, version 4.2.6, provided by MGL Tools was used to design the Auto Dock atom kinds. One of the most effective docking techniques readily available in Auto Dock, the Lamarckian genetic algorithm, was used [16]. The binding free energy and the ideal fit of a ligand conformation in the macromolecular structure were calculated and assessed using Auto Dock. This can be beneficial for understanding the nature of the binding as well as for creating more effective drug possibilities.

Results

Interaction of Superoxide Dismutase with Colchicine

The result highlighted the VAL-5, CYS-6, VAL-7, LYS-9, GLY-10, ASP-11, GLY-51, ASN-53, THR-54, GLY-56, CYS-57, CYS-146, GLY-147, VAL-148. Figure 1A & B represent the binding pocket in superoxide dismutase. Our result demonstrated that colchicine exhibited significant binding affinities towards superoxide dismutase with a binding energy of -8.46 Kcal/Mol was obtained (Table 1). The superoxide dismutase establishing a three-hydrogen bond interaction involves the participation of VAL7, ASN-53, and VAL148, and results in the creation of a binding pocket that includes VAL-5, CYS-6, VAL-7, GLY-51, ASN-53, LYS-146, VAL-148, GLY-150 (Figure 1A & B).

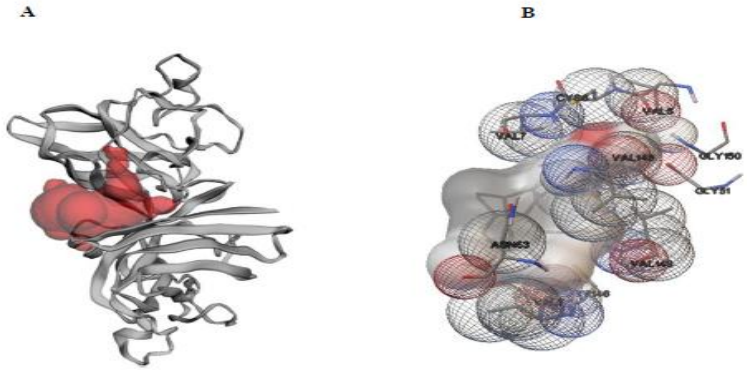

Figure 1. Binding interaction of Superoxide dismutase dismutase with colchicine. **A**-Delineates the binding pocket of superoxide dismutase, providing a visual depiction of the molecular environment in which enzymatic reactions occurs. **B**-Visual representation of the molecular interactions between colchicine and superoxide dismutase, elucidating the amino acid residues pivotal to the binding site.

Table 1. Binding Interaction of Superoxide Dismutase Dismutase with Colchicine

Target molecules	Compound	Binding energy	Amino acid interacted	Bond formed
Superoxide Dismutase	Colchicine	-8.46	VAL-5, CYS-6, VAL-7, GLY-51, ASN-53, LYS-146, VAL-148, GLY-150	3-hydrogen bond ASN-53, VAL148, VAL7

Interaction of Nuclear Factor erythroid 2-related Factor with Colchicine

The outcome emphasized that in nuclear factor erythroid 2-related factor, the binding pocket comprises ARG-326, TYR-334, GLY-364, LEU-365, ALA-366, GLY-367, CYS-368, VAL-369, VAL-370, GLY-371, GLY-372, ARG-380, ASP-389, ASN-414, ARG-415, ILE-416, GLY-417, VAL-418, GLY-419, VAL-420, VAL-421, ASP-422, GLY-423, HIS-424, SER-431, GLY-433, HIS-436, GLU-444, PRO-445, GLU-446, ILE-461, GLY-462, VAL-463, GLY-464, VAL-465, ALA-466, VAL-467, LEU-468, ASN-469, ARG-470, PHE-478, ARG-486, GLY-509, ALA-510, GLY-511, VAL-512, CYS-513, VAL-514, LEU-515, HIS-516, ASN-517, CYS-518, TYR-520, ARG-536, ASP-538, ALA-556, LEU-557, GLY-558, ILE-559, THR-560, VAL-561, HIS-562, GLN-563, GLY-564, ARG-565, ASP-585, PRO-586, ASP-587, SER-602, GLY-603, VAL-604, GLY-605, VAL-606, ALA-607, VAL-608, chain B has one more MET-610 and the same binding pocket as chain A and P chain binding amino acids such as ASP-77, GLU-79, THR-80, GLU-82, LEU-84 (Figure 2A). Our finding indicate that colchicine displayed noteworthy binding affinity with nuclear factor erythroid 2-related factor, resulting in a binding energy of -8.95 Kcal/Mol (Table 2). As for the nuclear factor erythroid 2-related factor has highest affinity for colchicine with binding centre comprises the amino acid residues ALA-366, GLY-367, VAL-418, GLY-419, VAL-420, VAL-467, CYS-513, VAL514, VAL-561, ALA-607, VAL-606 (Figure 2B).

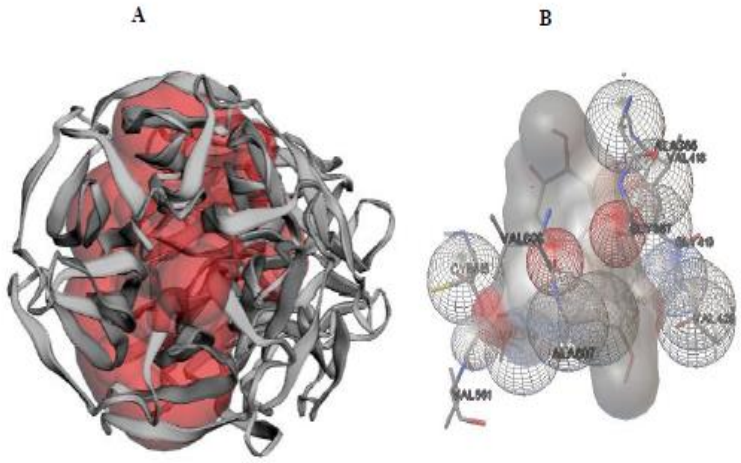

Figure 2. Binding nuclear factor erythroid 2-related factor erythroid 2 (Nrf2) with colchicine. **A**- The binding pocket of nuclear factor erythroid 2-related factor, providing a visual insight into the molecular context where enzymatic reactions occur. **B**-Depicting the molecular interaction between colchicine and nuclear factor erythroid 2-related factor, this figure reveals the essential amino acid residues within the binding site, shedding light on their involvement in the interaction.

Table 2. Binding Nuclear Factor erythroid 2-related Factor erythroid 2 (Nrf2) with Colchicine

Target molecules	Compound	Binding energy	Amino acid interacted	Bond formed
nuclear factor erythroid 2-related factor	Colchicine	-8.95	ALA-366, GLY-367, VAL-418, GLY-419, VAL-420, VAL-467, CYS-513, VAL514, VAL-561, ALA-607, VAL-606	No hydrogen bond

The result showed that the binding sites for glutathione peroxidase are PRO-132, ALA-133, PRO-134, ASP-137, ALA-138, THR-139, ALA-140, LEU-141, MET-142, THR-143, ARG-155, ASN-156, ASP-157, ALA-159, GLU-163, ARG-170, SER-178, ARG-179, ARG-180 (Figure 3A). Our findings illustrate that colchicine displayed notable binding affinity to glutathione peroxidase, yielding a binding energy of -6.66 Kcal/Mol (Table 3). Glutathione peroxidase, binding centre comprises the amino acid residues ALA-133, PRO-134, ALA-138, THR-139, LEU-141, ARG-155, ASP157 (Figure 3B).

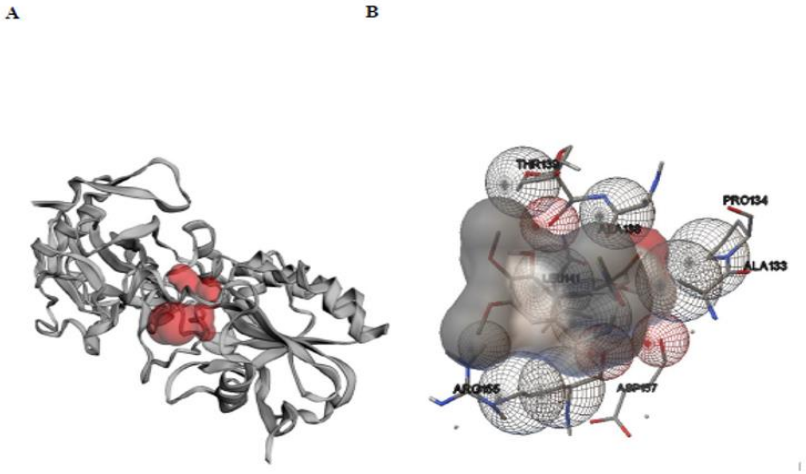

Figure 3. Binding interaction of glutathione peroxidase with colchicine. **A**- Visual depiction of the molecular milieu where enzymatic reaction occurs. **B**-the molecular interactions between colchicine and glutathione peroxidase, elucidating the pivotal amino acid residues within the binding site.

Table 3. Binding Interaction of Glutathione Peroxidase with Colchicine

Target molecules	Compound	Binding energy	Amino acid interacted	Bond formed
Glutathione peroxidases	Colchicine	-6.66	ALA-133, PRO-134, ALA-138, THR-139, LEU-141, ARG-155, ASP157	1hydrogen bond ALA-138

Interaction of Peroxiredoxin with Colchicine

The result revealed that the binding sites for peroxiredoxin are TYR-79, PHE-80, GLN-81, SER-82, MET-83, PRO-84, PRO-86, TYR87, TRP-88, GLU-89, GLY-90, THR-91, GLU-100, GLN-153, SER-178, ASP-179, LEU-180, THR-181, HIS-182, GLN-183, ILE-184, LYS-186, ASP-187, TYR-188, GLY-189, VAL-190, TYR-191, LEU-192, GLU-193, ASP-194, LEU-199, PHE-203, ILE-210, LEU-211, ARG-212, GLN-213, ILE-214, LEU-216,

ASN-217, ASP-218,LEU-219 (Figure 4A). Our findings clearly indicate that colchicine displayed notable binding affinity with peroxiredoxin, resulting in a binding energy of 6.78 Kcal/Mol (Table 4). Peroxiredoxin, binding centre comprises the amino acid residues Chain A- TYR-79, MET-83, LEU-211, ILE-214 and Chain B- TYR-87, LEU-101, GLU-100 LYS-102, THR-104, ASP-105 (Figure 4B&C).

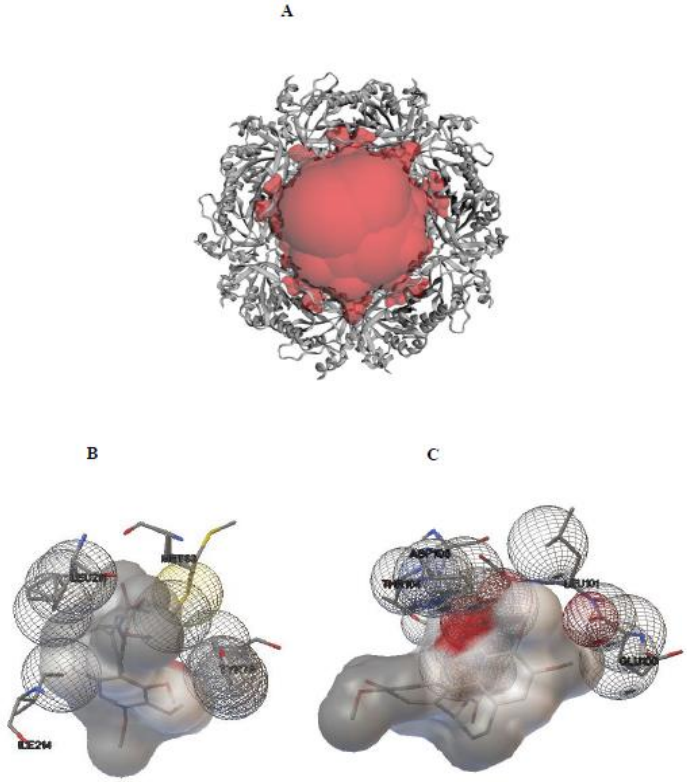

Figure 4. **A**- Binding site of peroxiredoxin. **B**-Visual representation of molecular interaction between colchicine and peroxiredoxin chain A. **C**-Visual representation of molecular interaction between colchicine and peroxiredoxin chain B.

Table 4: Binding Interaction of Peroxiredoxin with Colchicine

Target molecules	Compound	Binding energy	Amino acid interacted	Bond formed
Peroxiredoxin	Colchicine	-6.78	Chain A- TYR-79, MET-83, LEU-211, ILE-214 Chain B- TYR-87, LEU-101, GLU-100 LYS-102, THR-104, ASP-105	No hydrogen bond

Interaction of Hemeoxygenase with Colchicine

The result revealed that the binding sites for hemeoxygenase are A chain ALA-130, LYS-133, GLU-136, ARG-137, TYR-140, GLU-145, LEU-148, LYS-214, GLU-217, ARG-218, GLU-221, GLU-222, LYS-225, GLU-228, TYR-229, GLN-232, B chain are 137-ARG, TYR-140, ILE-141, ASN-144, GLU-145, GLU-147, LEU-148, ASN-212, LYS-214, THR-215, ARG-218, GLU-221, GLU-222, LYS-225 (Figure 5A). The outcome of our study revealed that colchicine displayed notable binding affinity with hemeoxygenase, achieving a binding energy of -6.21 Kcal/Mol (Table 5). Hemeoxygenase, binding centre comprises the amino acid residues LYS-133,

ARG-137, LYS-214, ARG-218, GLU-221, LYS-225, TYR-229 (Figure 5B).

The binding energies for these interactions were notably favourable, suggesting strong binding capacities [11]. In the investigation of molecular interactions, colchicine displayed robust binding affinities with key antioxidant signaling molecules, including superoxide dismutase (SOD) [7], glutathione peroxidases (GPxs), Nrf-2 [8], Peroxiredoxin and hemeoxygenase. these interactions imply that colchicine may function as an effective antioxidant, thereby attenuating oxidative stress, a hallmark of diabetes pathogenesis. The result reveals a promising connection between the antioxidant properties of colchicine and its potential to modulate diabetic activity.

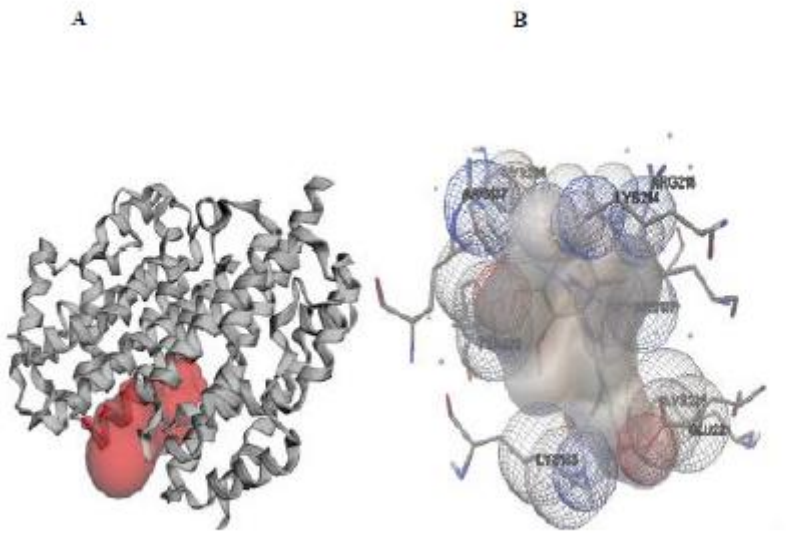

Figure 5. Binding site of Hemeoxygenase with colchicine. **A**-Visual representation of the molecular environment where enzymatic reaction take place. **B**-Visual representation of the molecular interaction between colchicine and heme oxygenase

Table 5. Binding Interaction of Heme oxygenase with Colchicine

Target molecules	Compound	Binding energy	Amino acid interacted	Bond formed
HemeOxygenase	Colchicine	-6.21	LYS-133, ARG-137, LYS-214, ARG-218, GLU-221, LYS-225, TYR-229	1hydrogen bond TRY-229

Discussion

In this study, we employed molecular docking techniques using the software Auto Dock version 4.2.6 to investigate the potential molecular interaction between colchicine, a naturally occurring alkaloid with known anti-inflammatory properties, and various antioxidant signalling molecules implicated in the pathogenesis of diabetes. Our *in-silico* analysis aimed to shed light on the possible antidiabetic activity of colchicine through its interaction with these targets. The 5 important antioxidant molecules such as superoxide dismutase, nuclear factor erythroid 2-related factor**,** Glutathione peroxidase, Peroxiredoxin and Hemeoxygenase were selected as the target protein and subjected to molecular docking.

Smith and Younus et al., study showed that the strong binding affinity between colchicine and SOD may play a role in enhancing the

antioxidant defence system [17,18]. *In-silico* experiment also supported that the superoxide dismutase exhibits the high binding affinity for colchicine, establishing a single hydrogen bond interaction. Lei et al., study uncovered the distinct role played by glutathione peroxidase and superoxide dismutase, two crucial intracellular antioxidant enzymes, in the regulation of insulin secretion but also emphasize the context-dependent effects of their mimics concerning ROS concentration or redox balance. This intricate interplay underscores both the essential need for and the potential benefits of judiciously applying different antioxidant enzyme mimics in the treatment of disorders related to insulin regulation. The absence of Glutathione peroxidase-1 exacerbated atherosclerosis in diabetic Apo E-knockout mice [19]. The favourable binding affinity of colchicine may activate Nrf2, a master regulator of antioxidant defence. The activation of Nrf2 could lead to increased expression of antioxidant enzymes and enhanced cellular defence against oxidative damage [20, 21]. Stancill et al. hypothesised that peroxiredoxins perform at least two distinct physiological roles within β-cells. Firstly, they serve as protectors of β-cells by neutralizing hydrogen peroxide when it attains cytotoxic concentration, guarding against oxidative harm. Secondly, they assume a pivotal role in glucose-stimulated insulin secretion (GSIS) by participating in hydrogen peroxide-mediated signalling pathways. In doing so, they become indispensable contributors to the functionality and viability of β-cells [22, 23]. Ahmed et al., findings imply that the mitigation of renal damage in spontaneously hypertensive rats following the induction of hemeoxygenase-1 is linked to a reduction in renal oxidative stress and inflammation. The activation of hemeoxygenase-1 has a mitigating effect on glomerular injury and apoptosis induced by diabetes, and these benefits are linked to a reduction in inflammation and oxidative stress mediated by NF-κB [24].

Furthermore, our analysis unveiled specific binding sites and key amino acid residues involved in these interactions, providing valuable insight for future experimental validation. These finding could guide the development of novel colchicine-based compounds with enhanced antidiabetic properties. This interaction might explain colchicine's potential role in modulating oxidative stress, a key factor in diabetes pathogenesis.

Conclusion

Our In-silico study suggests that colchicine may exert its antidiabetic effects, at least in part, by interacting with key antioxidant signalling molecules. Further experimental studies are warranted to confirm these finding and explore the therapeutic potential of colchicine in the management of diabetes and associated oxidative stress-related complication. The extracellular and intracellular triggers for the production of reactive species play an important role in the defence mechanism of the cell against the pathogens. The strategy of inhibiting this stimulus needed more study in both invitro and in vivo for the better understanding of the antioxidant activity.

Conflict of Interest

The author hereby declares that there is no conflict of interest.

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