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In silico structural and interactional study of wheat proteins gliadin and glutenin with comparison to wheat toxin alpha-purothionin

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Abstract

Wheat is most important component of food throughout the world. Wheat is full of different proteins but most abundant and most studied proteins are gliadin and glutenin. Interaction of gliadin and glutenin is important in biological properties of the wheat flour. Wheat also produce a toxin known as purothionin, which is used by the plant to show defense against different types of pathogen attack. Current *in silico* study was done to investigate the interaction between gliadin and glutenin proteins against wheat toxin alpha-purothionin solely. Methodology that was used in the current study consists of RaptorX tool for protein modeling, PROCHECK for predicted 3D model verification. CASTp 3.0 online tool for active site prediction. Online tool Cluspro and offline tool ligplus+v2.1 for protein-protein docking and visualization of docked complex respectively. Results showed high interaction between gliadin and glutenin, which supported the results of previous studies. While interaction between gliadin and purothionin proteins was high as compared to interaction between glutenin and alpha-purothionin proteins. Different helices were noted in the 3D structure of gliadin and glutenin proteins, where most of the time Gln amino acid was involved in making the helices. Form the results it has been concluded that greater interaction between gliadin and alpha-purothionin was due to similar sequence of gliadin with viral protein so the interaction of toxin purothionin against pathogens enhance the interaction of gliadin with alpha-purothionin. Current *in silico* study will enhance the understanding about the interaction of important wheat proteins.

Keywords: Wheat; Gliadin; Glutenin; Alpha-purothionin

1. Introduction

Wheat is a small plant and was originated in Tigris, Asia, and Euphrates river valley (Landriscina et al., 2017). Bread wheat also known as *Triticum aestivum*, produces one-seeded fruits, called as grains (Langridge, 2017). The pleasant and unique flavor and gluten-forming features of wheat products like bread, chapatti, pasta, etc. make them very attractive among other cereals (Lemmens et al., 2019). Wheat has been grown in areas with moderate temperature and is primarily used as food since ancient times (Li et al., 2014). Therefore, it is not an exaggeration to say that in the past, human life in many parts of the world depended on wheat and wheat-based foods (IWGSC, 2018). Both quality and quantity of wheat flour proteins being important in bread making quality.

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Main wheat storage proteins (endosperms) consists of gluten proteins. The gluten proteins consists of two main proteins i.e. gliadin and glutenin, which are studied intensively due to their bread making quality. Gluten was identified by Italian chemist, Beccari long ago, on the basis of wheat's unique dough-forming properties. Gluten is among the one of the protein, which was isolated in pure form from wheat endosperms (Lamacchia et al., 2018). Bread wheat, group *Triticeae* has five genera namely, *Triticum, Elymus*, *Aegilops*, *Secale,* and *Hordeum*, in it. There are 7 chromosomes in *Triticum* (Bush and Hefle, 1996).

Plants contain different bioactive compounds that are important for their protection against pathogens i.e. fungi and bacteria. Among these bioactive compounds are purothionins. Purotuionins are small and mainly basic globular proteins mostly found in the wheat epidermis (Triticum aestivum) (Duponte et al., 2007).

Major feature of purothionins is their harmfulness against many microorganism i.e. yeast, bacteria, and fungi (Constantin et al., 2008), different animals (Palosuo et al., 2001), cultured cells (mammalian) (Stec et al., 2004) and larvae of different insects (Sampson et al., 2006). Different in vitro studies have been documented to check the biological activities of purothionins, but the important biological purpose of purothionins seems to be involved in the defense of plants against their microscopic predators (Pahr et al., 2012).

Most of the documented biological properties result from the interaction of purothionins with the target cell membrane (Pahr et al., 2012). This is supported by the statement that some thionins interact with phospholipid bilayers (Hughes et al., 2000).

Current *in silico* study was done to investigate the interaction between the two important wheat proteins i.e. gliadin and glutenin and also to check the interaction of gliadin and glutenin with alpha-purothionin protein solely.

2. Methodology

In current in silico study protein 3D structure of gliadin, glutenin and purothionin was done using RaptorX online tools. RaptorX is a template-based protein structure modeling server. It requires protein sequence in FASTA format and provide 3D structure in PDB format (Källberg et al., 2012). Predicted 3D models were verified using PROCHEK tool (Laskowski et al., 1993), which provide results in the form of Ramachandran plot. 3D structures were visualized using Chimera1.13.1 (Goddard et al., 2007).

CASTP 3.0 tool was used to predict the active site pockets of all the gliadin, glutenin and purothionin proteins (Binkowski et al., 2003). Top three active site pockets in the gliadin, glutenin and purothionin proteins were noted. For protein- protein interaction, another online tool Cluspro (Kozakov et al.,2017) and offline java based software LigPlot+ v2.1 (Laskowski et al.,2011) were used.

3. Results

3.1 Structural studies

Wheat gliadin protein was 290 amino acids long and its sequence was obtained from uniprot database to design its 3D model. Wheat Alpha-gliadin protein 3D model, consists of coils and helices while no beta-sheets were noted in its 3D structure.7 seven helices of different lengths were present in the 3D sequence of Wheat Alpha-gliadin protein i.e. Leu25-Gln29, Gln111-Ser153, Gln164-Gln180, Glu183-Gln211, Pro240-Gln243 and Gln250-Cys269. Most of the time amino acid Gln was involved in making helices in the wheat gliadin protein. Three Active site pockets were also predicated. Largest pocket consists of 20 residues, 2nd largest pocket consists of 17 residues and 3rd largest pocket consists of 12 residues (Table 1). 3D model, active site pockets and verification of 3D structure of gliadin protein through Ramachandran plot are shown in figure 1.

Wheat Glutenin protein was 660 amino acids long and its sequence was obtained from uniprot database to design its 3D model. Wheat glutenin protein 3D model, also consists of coils and helices, while no beta-sheets were noted in its 3D structure.17 seven helices of different lengths were present in the 3D sequence of glutenin protein i.e. Ala2-Glu24, Gln28-Ser38, Leu40-Ala52, Leu55-Trp57, Thr59-Asp71, Ala74-Gln87, Ser121-Val124, Pro224-Gln226,Glu237-Gly239, Gly272-Gln274, Ala354-Gln356, Pro414-Gln416, Gln473-Gln475, Pro490-Gln492, Ala625-Ala629, Ser631-Ala637 and Pro640-Gln643,while rest of the amino acids were involved in making coils. Most of the time amino acid Gln was involved in making helices in the glutenin protein. Three Active site pockets were also predicated. Largest pocket consists of 29 residues, 2nd largest pocket consists of 20 residues and 3rd largest pocket consists of 36 residues (Table

1). 3D model, active site pockets and verification of 3D structure of wheat glutenin protein through Ramachandran plot are shown in figure 2. Both glutenin and gliadin proteins were rich in Gln amino acid.

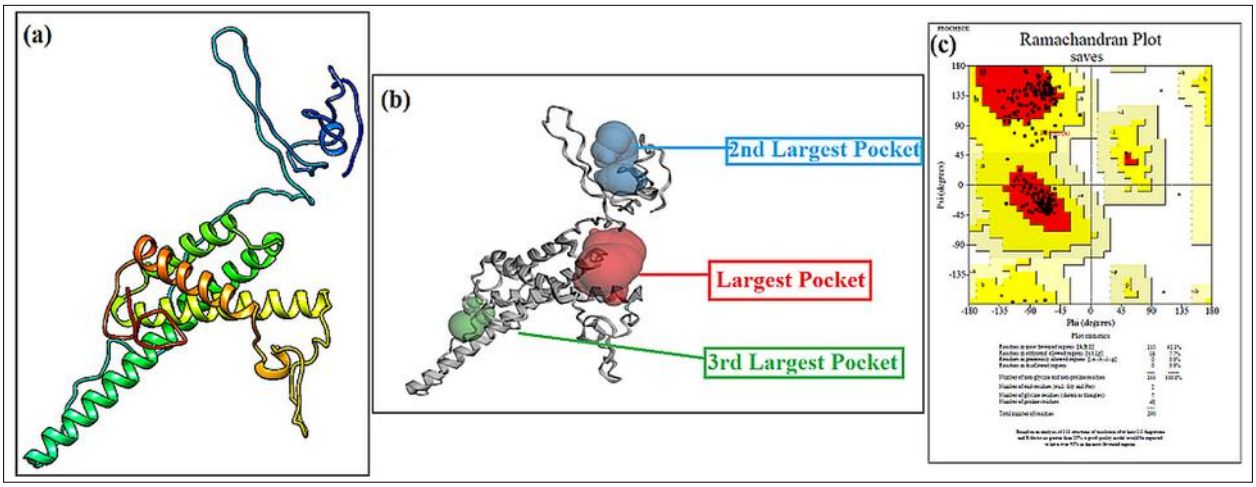

Figure 1 (a) 3D model of gliadin protein predicted through RaptorX tool. (b) 3D model of gliadin protein showing top three active site pockets (c) Predicted 3D model verified through Ramachandran plot

Table 1 Active site pockets of Gliadin, Glutenin and Alpha-Purothionin predicted through CASTp tool

Pocket Size	Active site pockets of Gliadin
1st	67Leu,68Pro,69Tyr,70Pro,71Gln,158Ser,159Ser,160Gln,163Gln,205Gln,206Gln,208Gln,209Gln,212Gln, 214Leu,215Ser,247Gln,249Gln,250Gln,253Gln.
2nd	11Pro,13Asn,15Ser,16Gln,17Gln,18Gln,19Pro,20Gln,21Glu,44Tyr,46Gln,47Pro,48Gln, 49Pro, 60Gln, 61Pro, 63Pro.
3rd	126Gln,129Gln,130Gln,133Leu,183Glu,184Gln,185Ser,187Cys,188Gln,274Pro, 276Tyr, 277Cys.
	Active site pockets of Glutenin
1st	565Ile,566Gly,568Val,570Gln,594Gln,597Gln,601Gly,618Asp,620Pro,621Tyr, 622His,623Val,627Gln,634Val,638Gln,642Thr,643Gln,644Leu,645Pro,648Cys, 649Arg,650Met,651Glu,654Asp,655Ala,656Leu,657Ala,658Ala,660Gln.
2nd	5Leu,8Phe,9Ala,12Val,15Thr,16Thr,19Gly,20Glu,25Leu,26Gln,27Cys,30Glu, 31Leu,34Ser,37Glu,38Ala,71Asp,72Val,73Ser,74Ala.
3rd	135Arg,136Gln,137Gly,139Tyr,155Pro,156Gly,157Lys,158Trp,160Glu,165Gln, 166Gln,168Tyr,169Pro,170Ser,172Ser,173Leu,174Gln,175Gln,176Pro, 178Gln,180Gln,181Gln,186Lys,189Tyr,191Pro,194Leu,196Gln,199Gln,201Gln, 208Gln,209Gly,212Pro,215His,217His,227Gly,229Gln.
	Active site pockets of Alpha-Purothionin
1st	6Ser, 8Leu, 30Arg.
2nd	1Lys, 2Ser, 13Tyr, 22Gln, 33Ile.
3rd	5Arg, 6Ser, 9Gly, 29Cys, 30Arg.

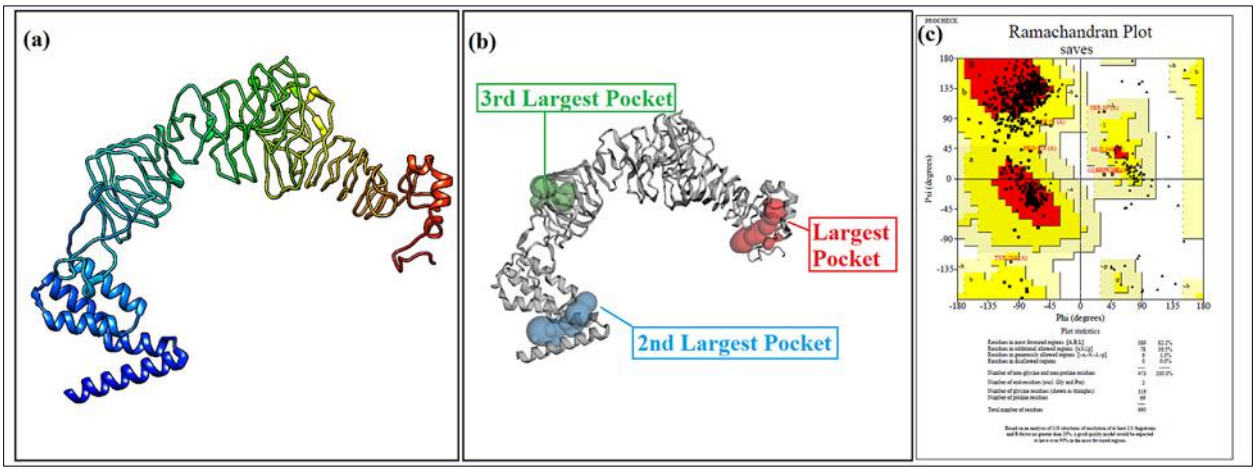

Figure 2 (a) 3D model of Glutenin protein predicted through RaptorX tool. (b) 3D model of Glutenin protein showing top three active site pockets (c) Predicted 3D model verified through Ramachandran plot

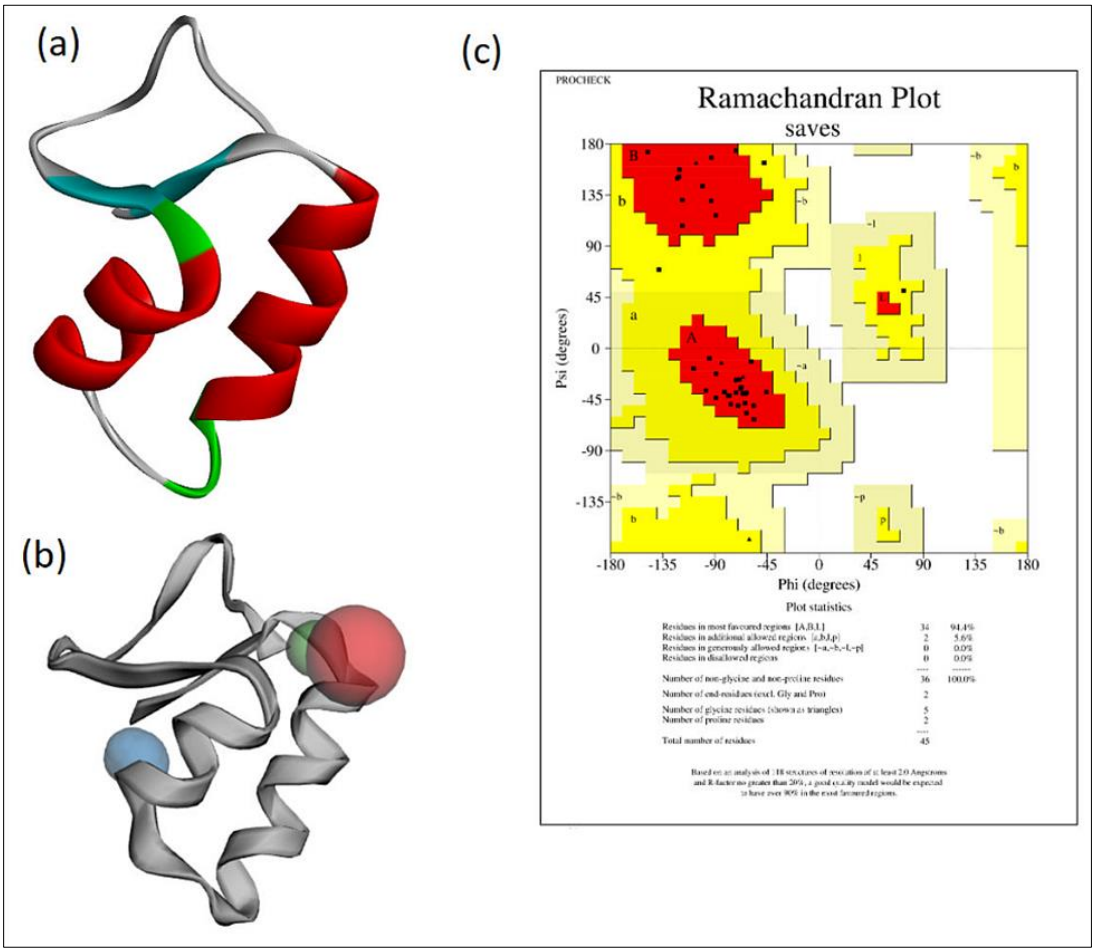

Figure 3 (a) 3D model of Alpha-purothionin protein (PDB id 2plh) (b) 3D model of Alpha-purothionin protein showing top three active site pockets (c) Predicted 3D model verified through Ramachandran plot

Alpha-purothionin protein was 45 amino acids long (PDB id 2PLH). Three Active site pockets were also predicated. Largest pocket consists of 3 residues, 2nd largest pocket consists of 5 residues and 3rd largest pocket also consists of 5 residues (Table 1). 3D model, active site pockets and verification of 3D structure of Alpha-purothionin through Ramachandran plot are shown in figure 3.

3.2 Interaction Studies

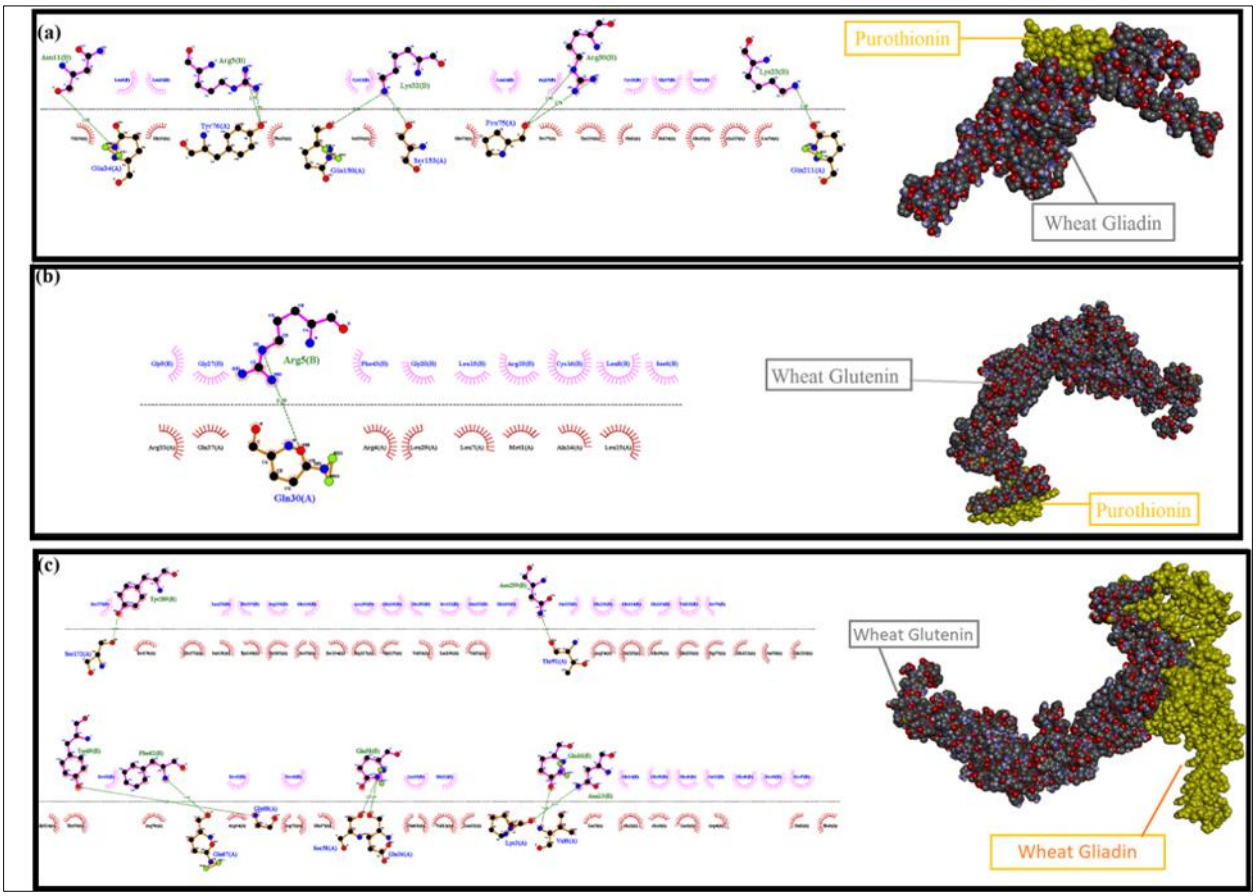

Figure 4 (a) Protein-protein interaction between alpha-purothionin and gliadin (b) Protein-protein interaction between alpha-purothionin and glutenin (c) Protein-protein interaction between glutenin and gliadin

Table 2 Protein-protein interaction between Gliadin, Glutenin and Alpha-Purothionin

Interacting Proteins	Interacting residues of Gliadin	Interacting residues of Glutenin	Interacting residues of Purothionin	Number of bonds
Gliadin and Purothionin	Gln34,Tyr76, Gln150, Ser153, Pro75, Gln211	__	Asn11,Arg5, Lys32,Arg30, Lys23	8
Glutenin and Purothionin	__	Gln30	Arg5	1
Gliadin and Glutenin	Tyr289,Asn259,Tyr69,Phe62,Gln58, Gln16, Asn16	Ser172,Thr91, Gln67,Gly60,Ser38, Glu38,Lys3,Val6	___	8

Protein-protein interaction between gliadin and glutenin, gliadin and alpha-purothionin and glutenin and alpha-purothionin was done. Gliadin and glutenin were interacting through 8 hydrogen bonds. Residues that were involved in making interaction present in gliadin protein were Tyr289, Asn259, Tyr69, Phe62, Gln58, Gln16 and Asn16 and residues involved in making interactions that were present in glutenin protein were Ser172, Thr91, Gln67, Gly60, Ser38, Glu38, Lys3 and Val6. Gliadin and alpha-purothionin were interacting through 8 hydrogen bonds. Gliadin protein residues that were involved in making interaction were Gln34, Tyr76, Gln150, Ser153, Pro75, Gln211 and residues involved in making interactions in alpha-purothionin protein were Asn11, Arg5, Lys32, Arg30 and Lys23. Glutenin and

Purothionin were interacting through only 1 hydrogen bond. Residues that were involved in making interaction in glutenin protein was Gln30 and residue involved in making interactions in alpha-purothionin protein was Arg5 (figure 4). Description of all the interacting residues in three proteins are summarized in table 2.

4. Discussion

Our findings showed high interaction between gliadin and glutenin proteins. Which supported the studty of Lagranin and his co-workers (2008) that for initial RVA viscosity the interaction of gliadin and glutenin is important. Loss of hydrogens bonds (break on high temperature) or other conformational changes in the gluten proteins can happens if the RVA values decreases (Lagrain et al., 2008).

The viscosity rise in the RVA profile at temperatures exceeding 90 °C was caused by formation of large glutenin polymers with the incorporation of gliadin through SS bonds impacting the rotation of the RVA paddle. The sudden decrease in apparent viscosity during cooling was due to the protein polymers aggregating tightly and sticking to the paddle caused by the loss of kinetic energy from heating (Lagrain et al., 2008). The balance and interaction between gliadins and glutenins is responsible for important rheological properties such as viscosity and elasticity (Gomez et al., 2011).

In 3D models of gliadin and glutenin high ratio of glutamine (Gln) were present in forming helices, studies showed that amino acids Glu, Gln, Ala, Met and Leu are most often found in helices formation (Krivoshapko and Ivanov, 2015).

Our *in silico* interaction study showed high interaction between gliadin and alpha-purothionin protein, which supported the study of Kagnoff et al., 1984. Kangnoff et al., compared the amino acid sequence of adeno virus and alpha-gliadin proteins and found significant similarity between E1b viral protein and some part of alpha-gliadin protein (Kagnoff et al., 1984). So the possibility is that the sequence similarly between viral and alpha gliadin protein is responsible for high interaction between gliadin and alpha-purothionin proteins because the major function of alpha-purothionin protein is to act against different pathogens including bacteria, fungi and virus. So therefore gliadin and alpha-purothionin protein showed high interaction as compared to glutenin and alpha-purothionin protein.

5. Conclusion

Current In silico study was done to find out the interaction between different wheat proteins. From the results it has been concluded that the interaction between purothionin and gliadin protein was higher as compared to interaction between glutenin and purothionin protein, we conclude that as the gliadin protein resembles with viral protein so the purothionin, which is actually a toxin against different pathogens, shows highs interaction for gliadin protein.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no financial as well as competing interests.

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