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Effect of processing on resistant starch content of Indian rice varieties (*Sona Masuri* and *Mogra*) and its impact on postprandial blood glucose levels

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ABSTRACT

Introduction: Resistant starch has elicited new possibilities around the globe due to its plausible health benefits and functional properties. This research aimed to evaluate the effect of processing on the resistant starch content in selected Indian rice varieties and its effect on postprandial blood glucose levels. **Methods:** Two Indian rice varieties (*Sona Masuri* and *Mogra)* were evaluated for resistant starch in raw form, after boiling, steaming, and refrigeration. Thereafter, the increment in resistance starch content by different processing methods was validated by oral glucose tolerance test (OGTT) on ten healthy participants. **Results**: Among the two rice varieties, raw *Sona Masuri* had a higher resistance starch content (10.86%). After processing, resistant starch was observed to be high in steamed *Mogra* variety (3.52%). In the boiling process, *Sona Masuri* developed a higher resistant starch content (2.44%) as compared to *Mogra* variety (1.05%). The assessment done after refrigeration revealed a slight increase in resistant starch content in both rice varieties. *Mogra* variety had higher resistant starch (3.68%) than the other rice variety (2.56%) after refrigeration. Validation of increase in resistant starch content and its effect on blood glucose responses done through OGTT revealed that *Mogra* rice (test food) did not cause a swift spike in blood glucose level compared to glucose (reference food). Differences in blood glucose responses by test and reference food at 0, 30, 60, 90, and 120 minutes were statistically significant. **Conclusion**: Steamed and refrigerated *Mogra* rice did not cause significant increase in blood glucose.

Keywords: blood glucose, processing, resistant starch, rice

INTRODUCTION

Cereals are among the most consumed and economical source of carbohydrates (55–75%) and contribute extensively to energy intake (Vaidya & Sheth, 2011). The amount of cereals consumed in daily diet plays a crucial role in the prevalence of metabolic disorders like diabetes, obesity, and cardiovascular diseases (Rhee, 2015; Van Dam, 2020). Among them, diabetes, a chronic metabolic disorder, has been designated as a 'silent killer' due to its poor rate of detection. Research data have recorded many complications associated with diabetes.

Several researches have been done on the modification of dietary factors. Imposing restrictions on intake of high glycaemic foods like sugar and

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sugary foods, sugary soft drinks, white bread, potatoes, and white rice are one of the solutions given to diabetic patients by healthcare professionals. The elimination of so many foods has resulted in limiting the food options for the diabetic population (Nanri *et al.*, 2010).

Scientists have been working on the modifications of high glycaemic foods, especially rice, through various techniques such as genetic modification, altering its chemical composition in terms of amylose and amylopectin content, processing techniques, and storage conditions. One of them is the incubation of starch with enzymes without dispersing agents, which has given a unique classification of starches; the most popular one is resistant starch (RS). RS is resilient to breakdown by amylase and pullulanase, and is found in cereal grains, seeds, and heated starch or starch-containing foods (Charalampopoulos *et al.*, 2002). The process of retrogradation results in the formation of RS type 3, also known as retrograded starch.

Many researches stated the benefits of RS and its positive impact on prevention of colon cancer, diabetes, and obesity because of its high fibre content and slow digestion property in the intestine. It also acts as a soluble fibre in human body (Mikulíková, Masár & Kraic, 2008). It shows reduction in postprandial glycaemic response, abdominal fat, insulin resistance, and cholesterol, and improvement in the number of gut bacteria (Keenan *et al.*, 2013; Shen *et al.*, 2011).

Rice is a staple food in South Asian countries and because of its high glycaemic index (GI), it has become a source of calories; thus, not suggested for diabetic patients. In order to explore the possibilities for the inclusion of rice in diabetic diet, this study was planned and conducted to determine the impact of processing on RS content in rice. The study also aimed to validate the increment in RS content after processing through its impact on blood glucose levels. Thus, the objectives of this study included the estimation of RS content in selected Indian rice varieties (raw, after boiling, steaming, and refrigeration) and the impact of processing on blood glucose levels.

METHODOLOGY

The methodology opted for the current study can be discussed in two phases (Figure 1): Phase I (estimation of RS in selected Indian rice varieties before and after cooking, and after cooling i.e., by refrigeration) and Phase II (effect of processing on blood glucose level).

Phase I

Sample procurement

For the present study, two Indian rice varieties *(Sona Masuri and Mogra*) were selected. Both low-income and middle-income Indian families relish these rice varieties more because of their reasonable market price (as found during market survey done on 10 local grocery shops and online stores). The market price of *Sona Masuri and Mogra* were 50Rs/kg and 55Rs/kg, respectively, as compared to other rice varieties ranging from 80Rs/kg to 200 Rs/kg. *Sona Masuri* is a non-aromatic, Indian non-basmati rice variety with broken grains, while Mogra is an aromatic Indian basmati rice variety with broken grains. Hence, their market price were lower compared to other rice varieties. Samples of selected varieties of rice were collected from a local grocery store (selling different varieties of rice) in Faridabad, Haryana, India. All grains procured were weighed and collected in clean sample collectors.

The selected samples underwent two moist heat methods of processing, namely boiling and steaming. Boiling was done for 20 minutes in an open pan with filtered tap water (1:3 w/v) and extra water was drained. Steaming of rice was done in a pressure cooker with filtered tap water (1:2 w/v). RS content was analysed by using the RS Megazyme Assay Kit using spectrophotometer (Thermofisher, USA) (McCleary & Monaghan, 2002).

Statistical analysis revealed that the *Mogra* rice had increased RS content after steaming and the *Sona Masuri* had increased RS content after boiling. Therefore, boiled *Sona Masu*ri and steamed *Mogra* rice were selected for further study and they were stored in the refrigerator at 4^{0}C for ten hours. After refrigeration, RS content was analysed and steamed *Mogra* rice, which was rich in RS content was used for Phase II.

Phase II

Oral glucose tolerance test (OGTT)

To validate the results of phase I, OGTT was performed. Participants were fed with steamed + refrigerated *Mogra* rice.

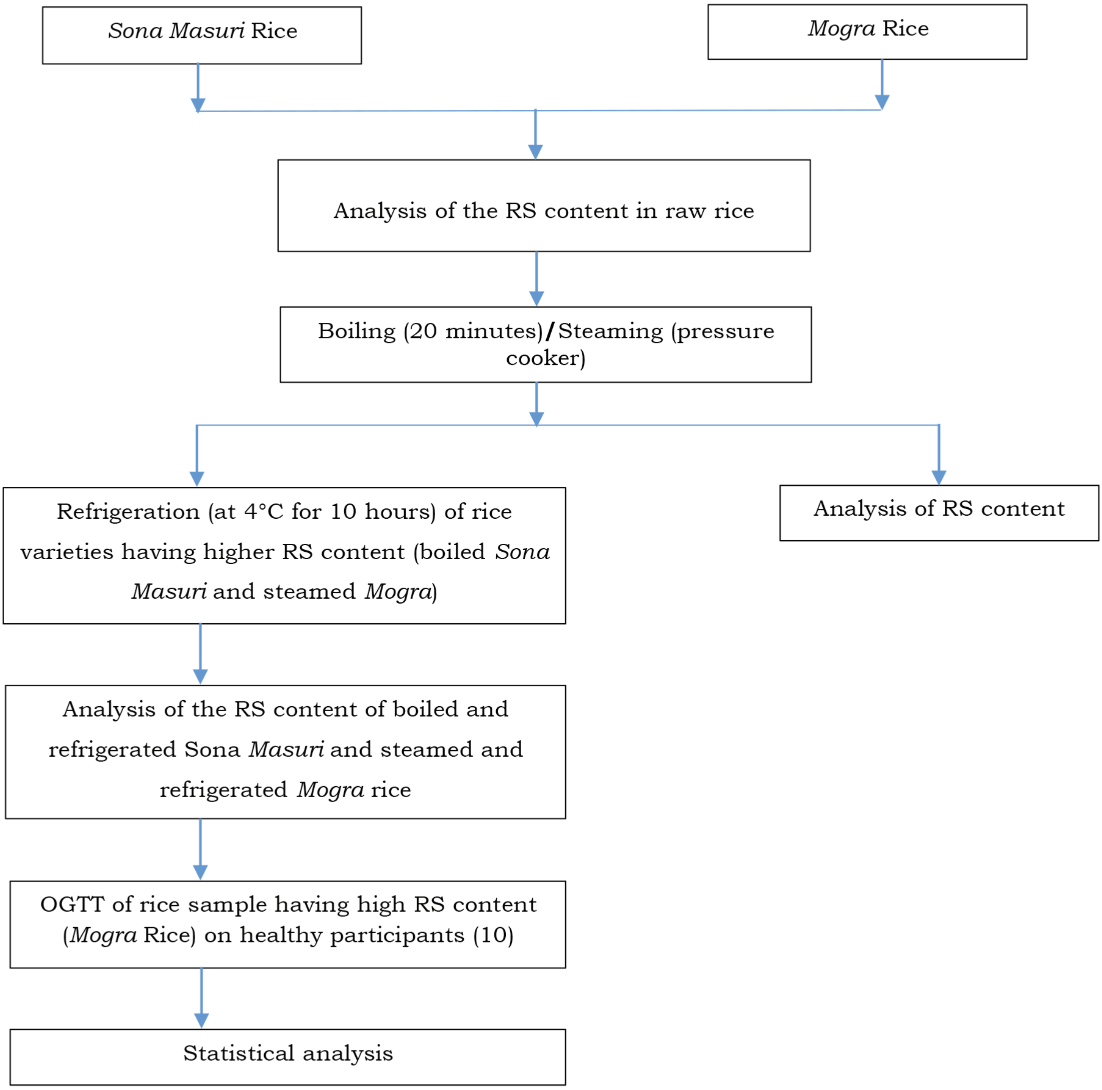

Figure 1. Flowchart of study methodology

For the study, ten healthy volunteers were selected from the Girls Hostel of Manav Rachna Campus. These participants were healthy and aged 20 to 30 years. Before proceeding with the intervention study, written and verbal consents were obtained from the participants.

Glucose solution (75 g of glucose dissolved in 250ml of water) was taken as reference food and rice variety was considered as test food. For blood glucose testing, ten healthy participants (after an overnight fasting of eight hours) were fed with glucose solution. The blood glucose levels of participants were tested after every half an hour i.e., at 0 minute (baseline), 30 minutes, 60 minutes, 90 minutes, and 120 minutes (Nelson & Blauvelt, 2015).

The next day, the same participants were tested first for fasting blood glucose and thereafter, the RS-rich steamed *Mogra* rice was fed to them. The amount of rice fed to the participants was calculated so that it provided 75 g of available carbohydrates. Subsequently, blood glucose levels of the participants were noted at half an hour intervals.

The experimental protocol was approved by the Ethical Committee, Faculty of Allied Health Sciences, Manav Rachna International Institute of Research and Studies (MRIIRS/FAHS/DEC/2021-22/N&D/M035).

Glycaemic Index (GI) of test food

The GI of test food was calculated in the study. The formula used to calculate GI: dividing the incremental blood glucose area of test food by the incremental area of reference food, multiplied by hundred.

Statistical analysis

The IBM SPSS Statistics for Windows version 28.00 (IBM Inc., Armonk, New York, USA) was used for statistical analysis. Study results were expressed as mean±standard deviations (*SD*) for RS content of *Sona Masuri* and *Mogra* rice. To determine the effect of processing on the RS content of raw, boiled, and steamed *Sona Masuri* and *Mogra* rice, one-way analysis of variance (ANOVA) was used; whereas to determine the effect of storage conditions on the RS content of steamed and refrigerated *Mogra* rice, *t*-test was performed.

Comparison of the spike in blood glucose levels by reference and test food against each time interval was done by independent *t*-test. To compare blood glucose responses with time at 0 minute, 30 minutes, 60 minutes, 90 minutes, and 120 minutes in one group (reference or test), one-way ANOVA was used and significance was reported at 5 percent level.

GI was calculated by dividing the incremental blood glucose area of test food by the incremental area of reference food, multiplied by 100. Incremental area under the curve (IAUC) was calculated by Graphpad Prism 9.

RESULTS

Phase I: Effect of processing on the RS content of Indian rice

RS content of raw and cooked rice varieties

Results of the RS content of raw and cooked rice varieties after boiling and steaming are presented in Table 1. Data in the table depicts that RS fraction was significantly ($p \leq 0.05$) influenced by variety.

RS content as analysed in raw rice was higher (10.86) in *Sona Masuri* rice than in the *Mogra* rice variety (3.76). Furthermore, when the raw rice varieties underwent boiling, *Sona Masuri* showed higher RS content (2.44) compared to the latter. After steaming, *Mogra* rice variety showed a higher RS content (3.52) in comparison to steamed *Sona Masuri*. Among all treatments, boiled *Sona Masuri* and steamed *Mogra* rice, due to their higher RS content, were further refrigerated.

Table 1. Effect of cooking and refrigeration on the resistant starch (RS) content of Indian rice varieties

Rice varieties	*Sona Masuri*	*Mogra*
Effect of cooking		
Raw	10.86±0.01	3.76±0.01
Boiled	2.44±0.01	1.05±0.01
Steamed	2.02±0.01	3.52±0.01
F-value	746092.00	67513.00
p-value One-way ANOVA	<0.001*	<0.001*
Effect of refrigeration		
Boiled+refrigerated	2.56±0.05	-
Steamed+refrigerated	-	3.68±0.05
t-test	<0.001*	<0.001*

RS values presented as percentages
*Significant at p<0.05

RS content in the rice varieties after refrigeration

The effect of storage conditions on the RS content of Indian rice, *Sona Masuri* and *Mogra*, is depicted in Table 1. The mean values depicted that the steamed and refrigerated *Mogra* rice had significantly higher RS content, i.e., 3.68±0.005 than the boiled and refrigerated *Sona Masuri* variety (2.56±0.005). Considering the result analysis, the *Mogra* rice variety after steaming was further used for Phase II of the study and was analysed for its effect on blood glucose level because of its high RS content.

Phase II

Effect of rice processing on postprandial blood glucose level

Blood glucose concentrations (mmol/L) for reference (glucose) and test (*Mogra* rice) foods at every 30 minutes are shown in Table 2. At 0 minute before intervention, differences in blood glucose spikes by reference and test foods were not significant. However, a sharp spike in the blood glucose level was observed after 30 minutes in the case of reference food. After that, blood glucose levels started to decline.

Table 2. Difference in blood glucose concentrations (mmol/L) after consumption of reference and test foods at 30 minutes intervals

Time	*Reference food (glucose solution)*	*Test food (steamed and refrigerated Mogra Rice)*	*p-value*
0 minute	5.1±0.7 ^{bcd}	5.1±0.7	0.884
30 minutes	8.8±1.6 ^{ade}	5.7±0.9	<0.001*
60 minutes	8.6±1.3 ^{ae}	5.5±0.9	<0.001*
90 minutes	6.9±1.6 ^{ab}	5.2±0.8	0.004
120 minutes	5.8±1.4 ^{bc}	5.1±0.7	0.145
p-value	<0.001**	0.264	

*Data expressed as mean±SEM
**Values in the same row with different superscripts (a,b,c,d,e) are significantly different (*p*<0.05) by one-way ANOVA and post-hoc test
^{a,b,c,d,e}Means with different superscripts in the same row differ significantly (*p*<0.05)

Table 3. IAUC and calculated glycaemic index for reference and test foods

Groups	*IAUC±SEM*	*p-value*	*Glycaemic index*
Reference food (glucose)	4979.0±588.5	0.001	-
Test food (steamed and refrigerated *Mogra* Rice)	963.4±483.4		19.34

After intervention, a significant difference (p<0.05) was reported in blood glucose responses every 30 minutes. The data revealed that the glucose level of participants administered with test food (*Mogra* rice) had better glucose control throughout the 2 hours as compared to blood glucose levels raised by reference food. The reason for such a difference is because of the property of resistant starch as soluble fibres that slow digestibility in the intestine.

A clear inference can be made from Figure 2, which showed the variation in glycaemic response of glucose solution and *Mogra* rice. The line chart depicted that the glucose solution caused a sharp rise in blood glucose levels, whereas the release of glucose by *Mogra* rice variety was slow and steady, thereby not causing any instant spike in blood glucose levels. The graph also showed that after 120 minutes, blood glucose levels of the participants fed with reference and test foods were almost similar. Further, it can also be inferred from the line graph that glucose solution (reference food) was able to spike blood glucose till 90 minutes of consumption, while blood glucose upsurged by the test food (*Mogra* rice) was limited to 60 minutes with a slow rise.

Changes in blood glucose levels within time intervals for each group (reference and test) were also assessed. Post-hoc test revealed that there was a significant difference (p<0.05) in the rise of blood glucose level by reference food at every 30 minutes interval. However, no significant difference was observed in the test food after every 30 minutes of blood glucose analysis.

GI and incremental area under the curve (IAUC)

The study assessed the GI of the rice varieties having maximum resistant starch by calculating the incremental area under the blood glucose response curve (IAUC). The results revealed that

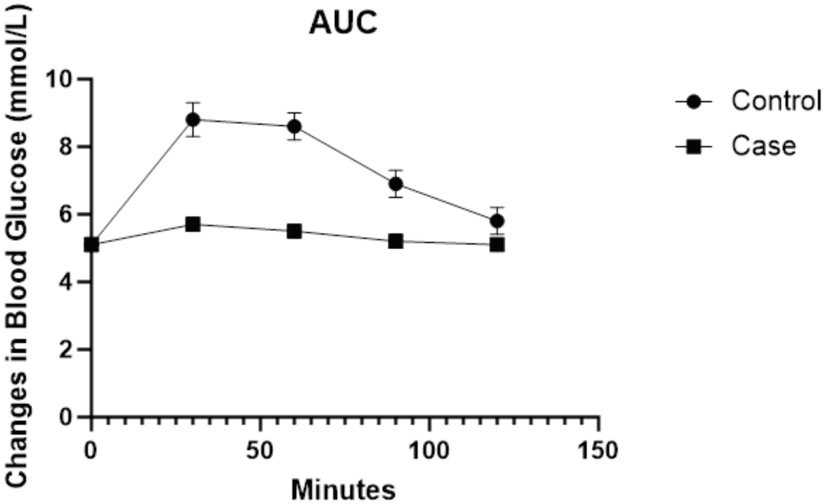

Figure 2. Glycaemic response curves for reference and test foods

GI of *Mogra* rice (steamed and refrigerated) was 19.34 and is represented in Table 3. Table 3 and Figure 2 determined the IAUC for *Mogra* rice (steamed and refrigerated). Significant difference ($p<0.05$) was found in the IAUC between standard and *Mogra* rice (steamed and refrigerated).

DISCUSSION

There was a significant variation in the RS content of different rice varieties, which can be inferred from the values of RS content obtained after analysis with one-way ANOVA. *Sona Masuri* rice was observed with a higher RS content in the raw form than *Mogra* rice. Variation in the RS content of rice varieties in the present study may be caused by changes in starch hierarchical structures, protein compositions and physical barriers, lipid compositions and amylose-lipid complex, cell wall architecture and compositions of the rice grains (Yi & Li, 2021).

The *Mogra* rice variety showed higher RS content after steaming than boiling. On the contrary, *Sona Masuri* rice had greater RS after boiling in contrast to steaming. The reason here could be the destruction of RS structure while steaming, in the case of *Sona Masuri,* that reduced the RS content. Many factors, including amylose chain lengths, granule size, type of crystalline polymorphs, physical insulation of starch by thick-walled cells, porosity, and physical distribution of starch in relation to the dietary fibre components, may have influence over it (Yadav, Sharma & Yadav, 2009). It is not imperative that a rice variety having a higher RS content in the raw form will have a higher RS content post-processing. Although raw *Sona Masuri* rice had a higher RS content, but steamed *Mogra* rice in comparison to steamed *Sona Masuri* rice had a greater RS content after processing. In a study conducted on sweet corn, it was revealed that boiling and steaming did not affect the total starch and water-soluble carbohydrates of sweet corn, but there was a reduction in resistance starch content (Zhang *et al.*, 2022).

A study conducted on resistant starch content after cooling of cooked white rice and its impact on glycaemic response concluded that cooling of rice after cooking can be opted to increase its resistant starch content. This study was conducted in China with three different treatments (treatment 1: freshly cooked rice; treatment 2: cooked rice cooled at room temperature for 10 hours; treatment 3: cooked rice cooled for 24 hours at 4°C then re-heated) (Sonia, Witjaksono & Ridwan, 2015).

Processing techniques, such as baking, boiling, and roasting, can enhance RS content along with shallow frying. Steaming and frying decrease RS content in cereals, specifically wheat, rice, maize, and pearl millet, as verified in another study (Vaidya & Sheth, 2011). Research data have shown that moist heat treatment can have an incremental effect in RS content. On the contrary, the application of dry heat can cause decrease in RS content. A considerable amount of increment in RS content was also seen when rice and grain products were stored overnight (Nigudkar, 2014).

In the current study, the statistical analysis of blood glucose levels by reference and test foods revealed that steaming and refrigeration had an overall positive impact on the rise in blood glucose levels due to the increment in RS content. Application of independent sample *t*-test revealed that the blood glucose levels of participants from both groups significantly increased after 30 minutes and 60 minutes as compared to 90 minutes and 120 minutes. However, a major change in the blood glucose values at 30 and 60 minutes was observed in the case of reference food (glucose solution). It was clear from the one-way ANOVA analysis that RS-rich *Mogra* rice after processing showed a slow and steady rise in blood glucose levels, while a sharp rise was observed in the case of reference food. There was no clear sudden spike in the blood glucose levels by test food (RS-rich *Mogra),* demonstrating a lower GI due to increment in RS content.

GI was calculated by using IAUC. The results revealed that GI of *Mogra* (steamed and refrigerated) was 19.34, low in comparison to the GI of 5 other Indian rice varieties without any processing treatment (*Sampata*: 56.38; *Dhanrasi*: 59.23; *DRR Dhan* 42: 71.73; *DRR Dhan* 43: 87.40; *Jarava*: 94.05) and thus, can be recommended to diabetic patients (Azam *et al.*, 2020). Variation caused in RS due to the proportion of amylose to amylopectin in grains may decrease total GI in foods after cooking.

The consumption of high GI foods is higher in the South Asian populations, thus making them more prone to type-2 diabetes mellitus and cardiovascular diseases. Factors, such as inherent starch characteristics (amylose: amylopectin ratio), processing techniques after harvesting (particularly parboiling), and cooking methods including storage and then reheating lead to changes in rice, in turn affecting postprandial glycaemic responses in the population. In general, the observed GI of rice ranges from 48 to 93, indicating it as a high-glycaemic food (Blaak *et al.*, 2012). Moreover, the fluctuation in blood glucose levels is further influenced by consumer characteristics like chewing habit and ethnicity. Hence, the cooking time of rice and choosing rice with high amylose content will be beneficial for diabetic people as it will help in bringing a lower postprandial glucose response to the body.

In this study, RS fractions were influenced by the variety of rice, its processing and storage conditions. The combined effect of steaming and refrigeration had a positive impact on the glycaemic response of rice due to increased RS content. Results of the blood glucose level estimation further validated that increased RS content in food varieties can delay glycaemic response in the body.

Although this study had covered vital points with reference to the RS content of rice using two of the most widely used methods of rice preparation, other household methods used to prepare rice like microwaving, soaking then autoclaving, etc. can be used to determine which method yields higher RS content. Other factors, such as botanical source, moisture content, amylose: amylopectin ratio, etc. that have a crucial impact on the RS content of rice should also be considered. The present study estimated postprandial glycaemic response after a half an hour interval. Research data are also available where significant blood glucose spikes were reported within 15 minutes interval. There is a possibility of different inferences in the present study through early inception of postprandial glucose responses in the case of both foods. In this research, for the validation of the variation in increased RS content of rice on blood glucose levels, only female participants were included for the facilitation of the study. However, future research can also include male participants to study any changes on blood glucose response that may be affected by sex differences.

CONCLUSION

The inferences of the results from this study clearly showed that processing methods and low temperature storage of selected rice varieties had an impact on resistant starch content. Furthermore, these methods significantly affected blood glucose levels as observed by OGTT. This study highlighted the possibilities for pre-diabetic and diabetic people to relish white rice by changing its cooking methods, followed by low temperature storage.

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Authors’ contributions

Mishra S, performed the lab experiments and clinical study; Awasthi M, supervised the study, data analyses, critically reviewed the manuscript and had overall responsibility; Sharma M, performed the statistical analysis and interpretation.

Conflict of interest

None

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