The Effect of Propolis Irrigation as an Adjunctive to Scaling and Root Surface Debridement on Periodontal Health Status, Glycemic Control and Biochemical Markers in Subjects with Type 2 Diabetes Mellitus and Chronic Periodontitis: A Randomized Clinical Trial

Hozan Wrya Azeez [(1)]

Background: Periodontitis is the inflammation of supporting tooth structures; it is one of the six complications of diabetes, Propolis a local delivery agent can be used as an adjunct to non-surgical periodontal therapy.

Aim: To assess the effect of Propolis as adjunctive to scaling and root surface debridement for the treatment of periodontitis patients with type 2 diabetes.

Methods: forty-five chronic periodontitis patients with poorly controlled type 2 diabetes (glycated hemoglobin 1 assay HbA1c≥8%) were recruited into group I; (n=15) scaling and root planing alone was performed, group II; (n=15) scaling and root planing+ chlorhexidine mouth wash twice a day for two weeks were prescribed, in the group III; scaling and root planing +Propolis irrigation was performed for two weeks, twice weekly. Clinical periodontal, haematological (fast blood sugar and glycated haemoglobin) and biochemical parameters (total antioxidant capacity and total oxidant status) were recorded at base line and three months of therapy.

Results: The results indicated that there was a highly significant improvement of the clinical parameters in group I and II ($p<0.001$), in group I at baseline, the correlations between probing pocket depth and clinical attachment loss with total antioxidant capacity were significant and highly significant respectively, probing pocket depth and clinical attachment loss with total oxidant status were significant. While after 3 months of therapy, the correlations between bleeding on probing and gingival index with total antioxidant capacity were significant and highly significant respectively, plaque index and gingival index with total oxidant status were significant.

Conclusion: Propolis irrigation could improve clinical periodontal parameters in chronic periodontitis patients with type 2 diabetes, with significant improvement of fast blood sugar and total oxidant status in group II and glycated haemoglobin in group III were exhibited.

Keywords: Propolis, type 2 diabetes mellitus, total antioxidant capacity, total oxidant status, chronic periodontitis

[(1)] Department of Periodontics, College of Dentistry, Hawler Medical University, Erbil, Iraq.
Corresponding author: Hozan Wrya Azeez
email: hozan.wrya@hmu.edu.krd

INTRODUCTION

Periodontal Disease is caused by dysbiosis, an instability in the relative presence or impact of microbial species that participate in the oral microbiome .[1] Bacterial dysbiosis is the principal causative factor that, in genetically impressionable individuals, initiates a strong inflammatory and immune response. This is aggravated by diseases such as diabetes or obesity, habits such as smoking, and stress which is frequent in the modern day world. [2] According to WHO, Diabetes Mellitus (DM) is a chronic, metabolic disease described by exalted levels of blood sugar, which over time, leads to a critical defect to the heart, blood vessels, eyes, kidneys, and nerves.

Untreated DM also presents a major hazard for periodontitis, a multifaceted local inflammatory condition of the tooth-supporting structures. [3] type 2 diabetes mellitus (T2DM) being the more prevalent form, and the overall burden of this disease is estimated to increase even further in the future.[4] In contrast to systemically healthy subjects, subjects with type 2 diabetes mellitus (DM) are 2.8 times more prone to have destructive periodontal disease, [5] and the prevalence of periodontitis in diabetic subjects that is estimated to be double or even triple the number in the normal population. [6]

Oxidative stress, determined as extra formation and/or insufficient elimination of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), increases in diabetes at the time of free radical production to a level that passes the body's capability to neutralize them. [7,8] To estimate the body's overall oxidation state, total oxidant status (TOS) is frequently utilized.[9] Total antioxidant capacity (TAOC) is an integrative metric, this captures the increasing impact of mainly non -enzymatic antioxidants discovered in body fluids and plasma.[10] Assessing TAOC can exhibit data regarding the balance between antioxidant and oxidants systems. [11]

Though scaling and root surface debridement assist an improvement in the clinical periodontal parameters in the greater part of cases, it is often inadequate to alter the bacterial profile collaborated with periodontitis because of a high chance of pocket reinfection arising from residual biofilms.[12,13] Therefore, to obtain the maximum outcome, some patients may require drugs or certain agents with prohibitive belongings such as antimicrobial, anti-inflammatory, or antioxidant belongings as an adjunct to SRP. [14] In an assay to make this state better, propolis PRO is a natural therapy that could be favourable. PRO, a resinous beehive content produced by the honeybee, is a strong antimicrobial and anti-inflammatory agent because it is composed of plant exudates and contents secreted in the direction of bee metabolism. The principal chemical classes display in PRO are flavonoids, [15] phenolics,[16] and various aromatic mixtures. Their antimicrobial, anti-inflammatory, and immuno-modulating belongings may assist periodontal health as well. [17] Antioxidant activity of flavonoids is due to their ability to reduce free radical formation, scavenge free radicals and chelate metal ions.[18] Li etal demonstrated that PRO supplementation significantly attenuated the blood glucose, insulin and also decreased insulin resistance type 2 diabetes mellitus rats.[19] Although, Fukuda et al indicated that there was no significant influence on insulin resistance between PRO group and placebo group after an 8-week intervention. [20] There are no clinical trials that have assessed the effect of PRO irrigation on TAOC and TOS in chronic periodontitis with type 2 diabetes. Therefore, we decided to test the hypothesis of using PRO irrigation as a local adjunct to non-surgical periodontal therapy, to evaluate the impact of chlorhexidine CHX and PRO on clinical periodontal parameters (PI, GI, BOP, PD and CAL), haematological (HbA1c and FBS) biochemical oxidant (TOS) and antioxidant (TAOC) markers in periodontitis patients with type 2 diabetes after 3 months of therapy and correlate the clinical parameters with the biochemical ones at base line and 3 months after therapy.

METHODS

Setting and Time of Study

A prospective, single-blinded, randomized controlled trial was carried out in diabetes centre (Shaheed Layla Qassim), Medical center (Shaheed Nafee Akree) in Erbil city. It was conducted during the period of October 2021 and July 2022. Prior to the conduction of the study, ethical approval was obtained from the Institutional Ethical Committee of College of Dentistry/Hawler Medical University, and informed consent was signed by all participants.

Subjects

The study was conducted on 45 patients of chronic periodontitis with poorly- controlled type 2 diabetes including both sexes with an age range of more than 30–50 years (fast blood sugar test FBS>125,

glycated hemoglobin 1 assay HbA1c≥8%). [21] The inclusion criteria include: CAL ≥ 3mm or PPD ≥ 4 mm, in two or more different sites of at least two teeth in each quadrant, and all participants should have at least 20 standing teeth in the oral cavity with more than 30% of the teeth with probing pocket depth. [22] Patients who received periodontal treatment within 6 months prior to the start of the study, having history of heart disease or stroke, uncontrolled hypertension, menopause, breast-feeding women, pregnant, medication other than hypoglycemic agents and patients unable to make informed consent were excluded. After baseline assessments, patients were randomly allocated to either a scaling and root surface debridement or scaling and root surface debridement +CHX or scaling and root surface debridement +PRO groups. The study was arranged as a single-blind trial with the investigator assessing outcomes while blinded to treatment allocation throughout the trial. All patients were subject to the ordinary routine visits to the diabetes center and to periodontal examination at baseline and 3 months. The participants were divided into three study groups as follows: Group I: 15 chronic periodontitis with type 2diabetes, treated with scaling and root surface debridement alone. Group II: 15 chronic periodontitis with type 2 diabetes, treated with scaling and root surface debridement +CHX. Group III: 15 chronic periodontitis with type 2 diabetes, treated with scaling and root surface debridement + PRO subgingival irrigation performed in the periodontal pockets with 3 ml of 10% PRO extract solution twice a week for two weeks.[23] Scaling and root surface debridement was performed in Medical center (Shaheed Nafee Akree).

Propolis collection and extraction of 10% Propolis solution

PRO was collected by beekeepers that physically scraped off the frames of beehives located in Haj Umran city, Erbil governorate, Iraq. The PRO sample was cleaned and frozen at −20°C. While still frozen, PRO samples were crushed into fine particles using a precooled mortar and pestle. The PRO extract was prepared in accordance with a method presented by Thirugnanasampandan et al., [24] and AL-Ani et al.; [25] they offered that 10 g of PRO powder was mixed with 100 ml of 70% v/v ethyl alcohol in firmly air sealed glass vessels at a ratio of 1 g of PRO to 10 ml of ethanol for 24 h at room temperature in the dark with uninterrupted stirring up by a magnetic stirrer. By centrifugation at 26,000g for 30 min the outcome solutions were made clear, and the supernatants were collected, waxes and proportionately insoluble substances were taken off by filtering through Whatman 4 filter paper, to take off the solvent and obtain a brown semisolid residue mentioned as ethanol extract propolis (EEP) the solution evaporated in a rotary evaporator (Heidolph, Germany) under reduced pressure at 50°C. To obtain an EEP solution at a concentration of 10% the extracts were re-dissolved in 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Germany). [26] The prepared solution was diluted with saline, and the final concentration of DMSO did not go beyond 1%, which is nonlethal for microorganisms. [27] After preparation of solution, the irrigate should be put it in bottles to be ready for use. These procedures were performed at the clinic of Biochemistry Department in College of Dentistry, Hawler Medical University

Blood Sample Collection

Blood samples were collected from all subjects (by using syringe of 5cc) and were tested for the haematological parameters (FBS test, HbA1c), by using (TOKYO BOIKE MEDICAL SYSTEM, JAPAN), at the same day in diabetes centre (Shaheed Layla Qassim). For TAOC and TOS estimation, Elisa Kits: SUNLONG, China and Elabscience, USA, were used respectively after collecting all samples. The tests were performed at Alrajaa Polyclinic in Erbil city

Clinical Periodontal Assessment

Before treatment, a single examiner performed the clinical periodontal examination for all participants at the base line and after 3 months of therapy, after

blood sample collection. The clinical periodontal parameters included; plaque index (PI),[28] gingival index (GI),[29] bleeding upon probing (BOP),[30] probing pocket depth (PPD), [31] clinical attachment level (CAL). [31]

Statistical Analysis

The data were analysed by using SPSS software for statistical analysis Version 26, for calculating descriptive statistical analysis (Frequency, Percentage, Mean, Standard Deviation and Mean Difference). Inferential statistical analysis (Pearson Correlation and Paired t-tests and one-way ANOVA) was used to determine the correlation between variables and differences within and between groups. The P-value was considered statistically significant if it was ≤ 0.05 which rejected the null hypothesis.

Results

A total of 45 participants comprising of (33 females and 12 males) with the mean age of 49.77±4.85 years were taking part in the trial. For females and males, the mean ages were 49.96±5.25 and 49±3.93 years respectively.

Table 1 showed that in group I the mean values of PI, GI, BOP%, PPD and CAL were reduced after 3 months of therapy, no significant differences were observed for the measured parameters regarding BOP and CAL ($p<0.66$ and 0.07 using t-tests) respectively, with significant differences in PI, GI and PPD ($p<0.04$, 0.01 and 0.03 using t-tests) respectively. While the mean value of PI, GI, BOP%, PPD and CAL in group II and group III was reduced after 3 months of therapy, with highly significant differences observed for the measured parameters regarding PI, GI, BOP%, PPD and CAL ($p<0.001$ using t-tests).

Table 2 showed that intra group comparison of HbA1c, FBS, TAOC and TOS at baseline and after 3 months of treatment. In group I, showed that the mean values for HbA1c%, FBS and TOS were reduced after 3 months of therapy and the mean value for TAOC was increased after 3 months of therapy with no statistically significant differences when compared to the baseline before treatment (t-test, $P> 0.05$). For group II, the mean values of HbA1c%, FBS and TOS were reduced with significant differences for FBS and TOS when compared to the baseline before treatment (t-test, $P< 0.05$) and the mean value for TAOC was increased after 3 months of therapy with no statistically significant differences when compared to the baseline before treatment (t-test, $P> 0.05$). For group III, the mean values of HbA1c%, FBS and TOS were reduced after 3 months of therapy with significant differences for HbA1c% when compared to the baseline before treatment (t-test, $P< 0.05$) and the mean value for TAOC was increased after 3 months of therapy with no statistically significant differences when compared to the baseline before treatment (t-test, $P> 0.05$).

Table 1: Comparison of periodontal parameters at base line and three months following therapy

Groups	Parame-ters	Mean ± Std. Deviation at base line	Mean ± Std. Deviation at after 3 months	t-value	P-value
Group I	PI	2.12± 0.29	1.84 ±0.35	3.40	0.04 S
	GI	2.1 ±0.32	1.72±0.41	4.54	0.01 S
	BOP%	68±13.66	62.93±14.65	2.61	0.66 (NS)
	PPDmm	4.48 ±0.32	4.13±0.44	3.62	0.03 S
	CALmm	4.3±0.63	3.88±0.43	2.79	0.07 (NS)
Group II	PI	2.08±0.39	1.46±0.48	6.313	< 0.001 HS
	GI	2.27±0.35	1.62±0.50	6.947	< 0.001 HS
	BOP%	86.26±13.69	70.4±9.07	5.548	< 0.001 HS
	PPDmm	4.86±0.22	4.4±0.44	5.059	< 0.001 HS
	CALmm	4.72±1.96	3.77±0.71	6.471	< 0.001 HS
Group III	PI	2.02 ±0.33	1.36±0.47	7.214	< 0.001 HS
	GI	2.11±0.46	1.38±0.59	5.947	< 0.001 HS
	BOP%	79.33±18.36	61.26±17.02	10.215	< 0.001 HS
	PPDmm	5.41±0.84	4.32±0.69	10.426	< 0.001 HS
	CALmm	4.4±0.93	3.38±0.80	8.454	< 0.001 HS

* S significant, NO no significant, HS highly significant

Table 2: Comparison of HbA1c, FBS, TAOC and TOS at baseline and after 3 months of treatment

Groups	Parameters	Mean ± Std. Deviation At base line	Mean ± Std. Deviation after 3 month	t-value	P-value
Group I	HbA1c%	8.86±2.14	8.78±2.03	1.38	0.18 NS
	FBS mg⁄dl	194.46±25.10	194.06±23.57	0.89	0.38 NS
	TAOC U/ml	1.94±0.64	2.06±0.44	-0.56	0.57 NS
	TOS µmol/L	82.62±14.72	81.59±13.25	1.24	0.23 NS
Group II	HbA1c%	8.54±1.84	8.22±1.45	2.01	0.06 NS
	FBS mg⁄dl	195.4±41.91	193.2±40.32	2.20	0.04 S
	TAOC U/ml	1.96±0.22	2.26±0.49	-2.01	0.06 NS
	TOS µmol/L	82.04±23.8	80.16±15.83	2.34	0.03 S
Group III	HbA1c%	8.42±1.88	7.44±1.59	2.47	0.02 S
	FBS mg⁄dl	193.06±25.08	187.2±22.50	2.001	0.06 NS
	TAOC U/ml	1.79±0.46	2.19±0.66	-1.77	0.09 NS
	TOS µmol/L	81.60±23.80	78.91±15.83	0.60	0.55 NS

* NS no significant, S significant

Table 3 showed the mean differences between group I, group II and group III in regard to parameters. The mean difference of PI was highest in SRP+PRO group with significant difference when compared with the SRP alone and SRP+CHX (p=0.01). Additionally, the mean difference of PPD and CAL was highest in group III group, more than group I and group II with highly significant difference when compared with the group I and group II (p<0.001).

Table 3: Comparing the mean differences (before-after treatment) between 3 groups

Parameters	Group I	Group II	Group III	
Base line - 3 months of therapy	Mean ± Std. Deviation	Mean ±Std. Deviation	Mean ±Std. Deviation	P value
PI	0.28±0.43	0.62±0.38	0.66±0.36	0.01 S
GI	0.38±0.36	0.65±0.36	0.73±0.48	0.054
BOP%	5.07±4.20	15.86±11.08	18.07±6.85	0.16
PPD mm	0.35±0.29	0.46±0.35	1.09±0.41	< 0.001 HS
CAL mm	0.42±0.40	0.95±0.57	1.02±0.57	< 0.001 HS
HbA1c %	0.08±0.22	0.32±0.63	0.98±1.73	0.14
FBS mg/dl	0.4±1.72	2.2±11.36	5.86±3.24	0.08
TAOC U/ml	-0.12±0.79	-0.30±0.58	-0.4±0.92	0.43
TOS µmol/L	1.03±3.23	1.88±17.27	2.69±29	0.89

S significant, HS highly significant

In accordance to table 4: the present study showed that in group I, the correlation between PPD and TAOC at base line was highly significant, PPD with TOS was significant, CAL with TAOC was highly significant and CAL with TOS was significant (rho=-0.734, p=0.002; rho=0.612, p=0.015; rho=-0.822, p<0.001 and rho=0.525, p=0.045) respectively. While in group II and group III groups, all the correlations at base line were non-significant. In additions, in group I at 3 months of therapy the correlation between BOP and TAOC was significant, GI and TAOC was highly significant, PI and TOS was significant and GI and TOS was significant (rho=-0.731, p=0.002; rho=-0.772, p<0.001; rho=-0.641, p=0.010 and rho=0.507, p=0.054) respectively. However, in group II and group III groups, all the correlations at 3 months of therapy were non-significant.

Table 4: Correlations between the clinical with biochemical parameters at base line and after 3 months of therapy (pearson's correlation rho)

Parameters		Group I at 3 months		Group II at 3 months		Group III at 3 months	
		TOAC	TOS	TOAC	TOS	TOAC	TOS
PPD	Rho	-0.403	-0.310	0.949	0.384	0.312	0.373
	P	0.137 NS	0.261 NS	0.061 NS	0.157 NS	0.258 NS	0.171 NS
CAL	Rho	0.127	0.294	0.481	-0.135	0.426	-0.128
	P	0.652 NS	0.288 NS	0.069 NS	0.632 NS	0.113 NS	0.649 NS
BOP	Rho	-0.731	0.123	0.049	0.196	0.361	0.493
	P	0.002 HS	0.663 NS	0.862 NS	0.483 NS	0.186 NS	0.062 NS
PI	Rho	-0.379	0.641	0.287	-0.229	-0.012	0.163
	P	0.163 NS	0.010 S	0.299 NS	0.411 NS	0.966 NS	0.561 NS
GI	Rho	-0.772	0.507	0.270	0.117	0.132	0.167
	P	0.001 VHS	0.054 S	0.330 NS	0.677 NS	0.638 NS	0.552 NS

* **NO** no significant, **S** significant, **HS** highly significant, **HS** highly significant

DISCUSSION

Nowadays, it is obviously apparent that individuals with periodontitis have an increased prevalence of diabetes, prevalence and severity of periodontitis are higher in diabetic patients, and diabetics with periodontitis have deficient glycemic control [32]. So A bi-directional relationship has been established between diabetes and periodontitis, within this relationship it is well approved that people with diabetes are more seemingly to have or develop periodontitis [33]. Local-delivery antimicrobial agents (LDAs) are available for use as adjuncts to SRP in the treatment of periodontitis [34]. Propolis has been investigated as a putative antimicrobial and anti-inflammatory agents with interesting results in several scientifc researches. [35,36]. In-vitro studies have shown its ability to inhibit biofilm formation [37]. Our study was performed to examine the effects of group I, group II and group III on clinical periodontal, haematological and biochemical markers in chronic periodontitis with T2DM, to compare these markers at baseline and 3 months of therapy and finally to correlate clinical periodontal parameters with the biochemical markers at base line and three months of therapy.

Our data showed that group I, group II and group III resulted in the reduction of the inflammatory reaction and led to the healing of the periodontal tissue throughout significant reduction of clinical periodontal parameters. This reduction in clinical periodontal parameters may be attributed to the fact that periodontal therapy decreases the intraoral bacterial bio-burden and reduces periodontitis-induced bacteremia/endotoxemia [38]. This finding in group III is probably justified by the antibacterial and anti-inflammatory effects of PRO[14]. The current results are in line with Rapone etal, they reported significant differences occurring in the change from baseline to study in the 3- and 6-month median PPD and CAL, respectively, between groups (conventional and intensive periodontal treatment for patients with type 2 diabetes) [39]. Another study, Coutinho, reported that the antimicrobial and the anti-inflammatory benefits provided by PRO extract, indicate its use as an adjunct to scaling and root planning even within 6 weeks after the commencement of the treatment [23]. In 2020, a systematic review appraised clinical trials concerning the efficacy of PRO mouthwash and chlorhexidine. They concluded that PRO has superior potential for reducing plaque and gingival inflammation [40]. Inter group analysis showed that the mean difference of PI, GI, BOP, PPD and CAL were more than in group III and group II due to the improvement of clinical parameters.

In addition, this study results demonstrated that parameters (HbA1C and FBS) reduced significantly after 3 months of therapy in group III and group II respectively. effect of propolis on HbA1c and FBS appears to be dependent on concurrent treatment of inflammatory periodontal disease with SRP to reduce bacterial burden. Reduction in HbA1c is considered directly correlated with reductions in complications of DM. [41]. A study in agreement with our results, El-Sharkawy etal, concluded that systemic PRO used as an adjunctive therapeutic agent to SRP significantly improves the periodontal markers in individuals with type 2 diabetes and CP. Moreover, it concluded that systemic PRO after SRP for 6 months gives significant reduction in levels of HbA1c, FPG, and CML [42]. Another study in consistence with our results is that [43,44] treatment and melatonin supplementation in type 2 diabetes subjects, showed additional improvements in the HbA1c levels and periodontal disease severity in patients treated with SRP and melatonin versus SRP alone [43]. PRO supplement without side effects can increase the effectiveness of prescribing drugs in diabetes, its treatment can be helpful as a diet supplement in patients with type 2 diabetes through reduction in insulin resistance, improvement in antioxidant status and improvement in glycemic status[44]. Inter group comparison showed that the mean difference of HbA1C and FBS in group III and group II more than in group I. This is

may be due to the effect of PRO irrigation and CHX on the level of blood glucose.

For the serum TAOC and TOS, the current results observed that the mean concentration of serum TAOC level increased following 3 months of therapy in group I, group II and group III, but statistically not significant, and a mean concentration of serum TOS level reduced following 3 months of therapy with significant reduction in group II. This was due to oxidative stress markers reduction which made the balance shift toward a coherent AO system with decreased ROS generation, which promotes a periodontal health-friendly environment [45]. This was in agreement with the findings of Vincent etal, these authors showed a statistically significant decrease in TOS levels in both groups (systemically healthy with generalized CP, GCP with type 2diabetes patients) following 6 weeks of treatment [46]. Reinforcing our results with those in the literature, we can estimate that the mean concentration of the TAOC decreased and increased TOS at baseline are most likely due to the chronic inflammatory process, made worse even more by the presence of diabetes. Such a situation can be sensitive to oxidative damage of proteins, lipids and genetic material, and can cause the advanced destruction of dental supporting tissues. While after treatment, the mean concentration of the TAOC increased and decreased TOS after 3 months of therapy, these were more apparent in group 2 and 3 due to the effectiveness of CHX and PRO. Thus, we can use PRO as adjunctive to SRP.

Another challenge in the discussion of results is correlations, the study showed that in group I, there was the negative highly significant correlation between PPD and TAOC ($p< 0.005$), also the correlation between CAL and TAOC was a negative highly significant ($p< 0.001$), this indicate that there was increase of PPD and CAL with the decrease of serum TAOC level. This revealed that TAOC was significantly decreased with increased gingival inflammation and periodontal tissue destruction. While positive significant correlations between PPD and TOS; CAL and TOS ($p< 0.05$) were found, this finding suggests that TOS is highly related to the inflammatory condition of periodontium. In additions, after 3 months of therapy the correlation between BOP and GI with TAOC were negative highly significant $p< 0.005$), this indicated that TAOC were significantly increased with decreased gingival inflammation and periodontal tissue destruction, while the correlations between PI, GI with TOS were positive significant $p< 0.05$), this indicate that there was a decrease in PI, GI and decrease in serum level of TOS.

Future investigations should certainly include more parameters from different categories, extend the follow up point to 6-12 months in order to estimate more changes in different times and increase number of patients. Furthermore, several other factors have to be considered such as BMI, duration of disease and lifestyle.

CONCLUSIONS

PRO irrigation as adjunctive to SRP contributes to the improvement of clinical periodontal parameters in chronic periodontitis with type 2 diabetes, therefore, from the useful standpoint, it would be potential to recommend the clinical use of PRO irrigation as an addition to the SRP in regular settings. The reduced levels of PI, GI, BOP, PPD and CAL after therapy is not significantly correlated with the reduced level of TOS and increased level of TAOC, and so, more randomized controlled trials especially long-term trials are necessary in order to evaluate whether such inexpensive effects remain for longer periods of time.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to this article.

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