ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
June2025 • Vol. 38

# Formulation and evaluation of anti-inflammatory and antioxidant activities of *Glycyrrhiza glabra* (licorice) and Triphala based gum paint

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**Aim** : This study aimed to develop a herbal gum paint with potential anti-inflammatory and antioxidant benefits for treating oral conditions such as aphthous ulcers and periodontal diseases. By utilizing natural herbal extracts, this gum paint could provide a safer, possibly more effective alternative to conventional treatments.
**Materials and Methods :**The gum paint was formulated using concentrated extracts of Triphala and Glycyrrhiza glabra. Triphala, a blend of three fruits, and Glycyrrhiza glabra, known for its medicinal properties, were selected for their anti-inflammatory and antioxidant effects. Anti-inflammatory activity was assessed with the Bovine Serum Albumin Denaturation Assay, while antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay.
**Results:** The gum paint demonstrated promising anti-inflammatory and antioxidant properties. At a concentration of 50 µL, it showed the highest anti-inflammatory activity, comparable to the control group. The antioxidant activity was also optimal at this concentration. These findings suggest that the gum paint effectively reduces inflammation and oxidative stress, which are important in oral health conditions.
**Conclusion :** The herbal gum paint shows significant potential as an alternative to conventional treatments for oral health issues. By leveraging the combined effects of Triphala and Glycyrrhiza glabra, the formulation offers notable anti-inflammatory and antioxidant benefits. Further research and clinical trials are needed to confirm its efficacy and safety for broader clinical application.

**Keywords**: Antioxidant, Anti-inflammatory, Gum Paint, Herbal, Oral Ulcer, Periodontitis

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## Introduction

Recurrent aphthous ulcers (RAUs), which are also referred to as recurrent aphthous stomatitis, represent the most prevalent form of oral ulcerative disorder encountered in clinical practice.[1] These ulcers typically manifest as lesions that appear shallow and round to oval in shape, often accompanied by an erythematous border and a pseudomembrane. Their size can vary significantly, ranging from minute lesions measuring less than 1 mm to larger ulcers exceeding 1 cm in diameter.[2] Despite the characteristic clinical presentation of recurrent aphthous ulcers, the exact underlying cause remains elusive, perplexing both researchers and clinicians. However, it is widely acknowledged that immune and inflammatory processes play pivotal roles in the development and progression of RAUs. These mechanisms involve complex interactions within the oral mucosa, leading to the formation of recurrent ulcerative lesions. In addition to immune and inflammatory factors, various non-immunogenic elements have been identified as potential contributors to the etiology of RAUs. Among these factors, deficiencies in hematinic iron, certain bacterial components, and environmental or behavioral triggers have been implicated in the onset or exacerbation of recurrent aphthous ulcers.[1] The primary objectives in treating Recurrent Aphthous Ulcers (RAUs) focus on effectively managing pain and facilitating the healing process. For mild cases of aphthous ulcers, treatment typically involves the application of topical analgesics and protective bioadhesives. In contrast, both major and minor RAUs often require the use of topical corticosteroids to alleviate symptoms and promote healing.[1,3,4]

However, the widespread use of corticosteroids has diminished due to the potential for adverse effects, which can include oral candidiasis, mucosal atrophy, heightened susceptibility to infections, gastrointestinal disturbances, and the risk of systemic absorption. As a result, healthcare providers have become increasingly cautious about prescribing corticosteroids for the treatment of RAUs. The utilization of alternative therapies and preventive measures has gained traction in managing recurrent aphthous ulcers.[5]

The term "periodontitis" comprises a diverse range of inflammatory conditions impacting the periodontal ligament, bone, gingiva, and supportive structures surrounding the teeth. These periodontal diseases not only contribute to tooth loss but also provoke systemic inflammation. The onset and progression of periodontal disease occurs as a result of an imbalance in the commensal oral microbiota. This dysbiosis sets the stage for inflammation and disease by triggering interactions with the host's immune system. The pathophysiological state of periodontitis persists through cycles of activity and quiescence until the affected tooth is extracted or the microbial biofilm is effectively eradicated through medical intervention. During this process, the dysregulated oral microbiota perpetuates the inflammatory response, exacerbating the condition and posing challenges to its resolution.[6,7]

The fundamental methods of prevention include consistent self-performed oral hygiene and periodic professional removal of bacterial biofilms, ideally conducted every three to six months. However, a significant portion of the population may lack the motivation and manual dexterity necessary to uphold optimal dental care practices.[8]

Chlorhexidine gluconate is considered the gold standard antiplaque agent. However, its long-term use has been correlated with unpleasant side effects, such as changes in taste perception, tooth

discoloration, and the development of resistant microbial strains

There has been a constant requirement for the development of alternative methods for combating these inflammatory conditions.[9] One promising approach involves investigating plant-based remedies containing naturally occurring active components. The emphasis lies in verifying the efficacy of these herbal treatments, especially considering that there have been no or minimally reported negative effects associated with their use thus far.[8] Licorice (Glycyrrhiza glabra), one of the earliest recognized medicinal herbs, has a broad spectrum of therapeutic applications, ranging from wound healing to asthma, urinary tract infections, and gastric ulcers. Its primary components, glycyrrhizic acid and glabridin, play pivotal roles in its medicinal properties. Glycyrrhiza glabra and its active constituents inhibit enzymatic pathways such as 5-cyclooxygenase and lipoxygenase, consequently halting the synthesis of reactive oxygen species (ROS) and cell migration. This inhibition leads to the suppression of arachidonic acid metabolism, vascular permeability, and inflammatory responses.[10]

Triphala is a blend of three herbal fruits derived from Indian gooseberry Amalaki (Embilica officinalis), Bibhitaki (Terminalia beleria), and Haritaki (Terminalia chebula). Previous research suggests that Triphala demonstrates promising effects on inflammation. It possesses the capacity to inhibit a variety of microbes and enhance oral flora. The primary polyphenolic compounds found in this plant extract include phenolic acids, flavonoids, and tannins which attributes to its anti-inflammatory property.[10,11] It inhibits inflammatory mediators and pathways, thereby attenuating inflammation. Furthermore, its antioxidant properties play a crucial role in inhibiting inflammation by neutralizing harmful free radicals and lowering oxidative stress levels.[11]

This study aimed to formulate a gum paint using Triphala and Glycyrrhiza glabra and assess its anti-oxidant and anti-inflammatory activities in an invitro background.

## Material & Methods

The present study was conducted in the Department of Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Chennai (Saveetha University). The study was conducted in an vitro setting and received approval from the scientific review board (SRB number: SRB/SDC/FACULTY/24/OPATH/008).

### Preparation of Extract

To initiate the extraction process, 1 gram each of Licorice and Triphala powders were meticulously measured and combined. Subsequently, 100 milliliters of distilled water was carefully added to the powders, and the mixture was brought to a boil. After boiling, the resulting extracts were meticulously mixed and subjected to a double filtration process to effectively separate any residual particles from the solution.

Following the filtration process, the solution underwent further concentration. To achieve this, the filtered solution was once again boiled until its volume was significantly reduced, ultimately yielding a concentrated extract. This reduction in volume was monitored until the quantity reached 15 milliliters, indicating the successful extraction and concentration of the desired components.

To enhance the formulation's stability and texture, glycerol was incorporated into the concentrated extract. Subsequently, the mixture underwent centrifugation to ensure thorough mixing and homogenization of the ingredients. As an additional step to enhance the sensory experience and palatability of the gum paint, peppermint and lemon essential

oils were thoughtfully selected and added as flavoring agents. These oils not only impart a pleasant taste but also contribute to the overall appeal and user experience of the final product (Figure 1).


**Figure 1- a) Licorice powder; b) Triphala powder; c) Licorice and Triphala extracts; d) Filtration of the extracts; e) Concentrated extract post filtration; f) Formulated Gum Paint**

### Anti-inflammatory activity

**bovine serum albumin denaturation assay**

Formulation of various fixations at 10, 20, 30, 40, 50ul were added to 0.45ml Bovine serum albumin. The pH was brought to 6.3 with 1N hydrochloric acid and incubated in room temperature. It was then heated for 20 minutes at 55 degrees Celsius. The samples were then cooled. The absorbency was estimated by spectrometry at 660 nm. A commercial gum paint was used as the standard for comparison.

**Antioxidant activity** DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

Free radical scavenging was evaluated. 0.2mM DPPH was added to methanol with extract (10-50ug/ml). The sample was incubated for 30 minutes and the absorbance was analyzed at 517nm.

### Statistical analysis

Three repeats of the experiments were run, and Microsoft Excel was used to record the findings. Using IBM SPSS Statistics (Version 26.0), statistical analysis was performed. A one-way ANOVA was used to assess the changes in inhibitory means between the gum paint and control at different concentrations. Then, in order to identify significant differences, a Bonferroni post-hoc test was conducted.

## Results

### Antioxidant activity

The antioxidant activity of both the standard and the gum paint was evaluated by measuring the percentage of inhibition at various volumes (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL). For the standard, the inhibition percentages were 44.6%, 56.6%, 74.6%, 77.3%, and 83.4%, respectively, demonstrating a dose-dependent increase in antioxidant activity. Similarly, the gum paint exhibited increasing inhibition percentages with increasing volume: at 10 µL, the average inhibition was 47.3%; at 20 µL, it was 60.6%; at 30 µL, it was 72%; at 40 µL, it was 77.6%; and at 50 µL, it was 83.6%. There was no statistical significance (p value >0.05) between the inhibition percentage exhibited by the Gum paint and the standard. These results indicate that the gum paint's antioxidant activity closely matches that of the standard, particularly at higher volumes, suggesting that the gum paint possesses comparable antioxidant properties to the standard (Figure 2).

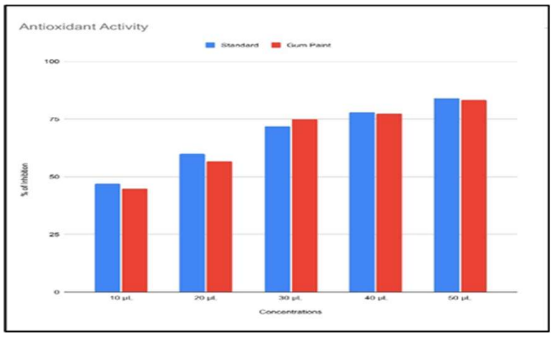


**Figure 2: Graph illustrating the Antioxidant activity of the Gum paint at different concentrations against the standard.**

**Table 1** displaying the results of antioxidant activity exhibited by the gum paint and the standard.

| Concentrations (µL) | Standard % of inhibition (Mean) | Standard Deviation (SD) | Standard Error Mean | Gum Paint % of inhibition | Standard Deviation (SD) | Standard Error Mean | P value |
|---|---|---|---|---|---|---|---|
| 10 | 44.6667 | 0.57735 | 0.33333 | 47.33 | 0.57735 | 0.33333 | >0.05 |
| 20 | 56.6 | 0.52915 | 0.30551 | 60.6667 | 0.57735 | 0.33333 | |
| 30 | 74.6333 | 0.55076 | 0.31798 | 72 | 1 | 0.57735 | |
| 40 | 77.3333 | 0.28868 | 0.16667 | 77.6667 | 0.57735 | 0.33333 | |
| 50 | 83.4333 | 0.51316 | 0.29627 | 83.6667 | 0.57735 | 0.33333 | |

**Table 2** displaying the results of antiinflammatory activity exhibited by the gum paint and the standard.

| Concentrations (µL) | Standard % of inhibition (Mean) | Standard Deviation (SD) | Standard error Mean | Gum Paint % of inhibition | Standard Deviation (SD) | Standard Error Mean | P value |
|---|---|---|---|---|---|---|---|
| 10 | 70.5 | 0.5 | 0.28868 | 76.52 | 0.5012 | 0.28937 | >0.05 |
| 20 | 74.4667 | 0.50332 | 0.29059 | 77.84 | 0.77253 | 0.44602 | |
| 30 | 83.2333 | 0.25166 | 0.1453 | 85.5433 | 0.5056 | 0.29191 | |
| 40 | 86.3 | 0.26458 | 0.15275 | 88.3933 | 0.35233 | 0.20342 | |
| 50 | 91.7567 | 0.66905 | 0.38628 | 93.05 | 0.0866 | 0.05 | |

### Anti-inflammatory activity

The anti-inflammatory activity of both the standard and the gum paint was measured by assessing the percentage of inhibition at various volumes (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL). For the standard, the inhibition percentages were 70.5%, 74.4%, 83.2%, 86.3%, and 91.7% respectively, showing a clear dose-dependent increase in anti-inflammatory activity. Similarly, the gum paint also demonstrated increasing inhibition percentages with increasing volume: at 10 µL, the inhibition was 76.5%; at 20 µL, it was 77.8%; at 30 µL, it was 85.5%; at 40 µL, it was 88.4%; and at 50 µL, it was 93.05%. The differences in inhibitory percentages between the gum paint and the standard were not statistically significant ($p > 0.05$). These results demonstrated that the gum paint's anti-inflammatory activity was similar to that of the standard, especially at higher volumes, indicating that the gum paint is highly effective and comparable to the standard in its anti-inflammatory properties.

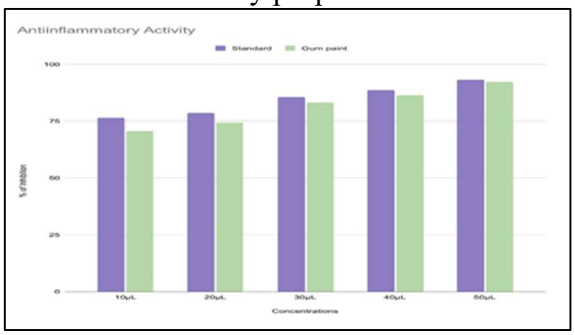


**Figure 3-** Graph illustrating the Anti-inflammatory activity of the Gum paint at different concentrations against the standard**.**

## Discussion

A growing number of researchers are examining the relationships between the antimicrobial, antifungal, anti-inflammatory, and cytotoxic properties of diverse herbs and food components to understand their potential in lowering the risk of chronic diseases.[12, 13] Previous research has shown that plant-based bioactive compounds are effective in treating various oral lesions such as recurrent aphthous stomatitis, chemotherapy and radiotherapy-induced mucositis and Lichen Planus.[14] Various studies have also evaluated the potential benefits of Licorice and Triphala individually, while the combined action of these two compounds have not been studied. This study aimed to incorporate Triphala and Glycyrrhiza glabra into a gum paint formulation and evaluate its symbiotic anti-inflammatory and antioxidant activities.[15]

In the current study, the formulated gum paint demonstrated high anti-inflammatory activity and moderate to comparable antioxidant activity. These results are similar with those reported in several prior studies. In a study by Assar et al, which aimed to assess the wound healing properties of Glycyrrhiza glabra exerted through its antioxidant and anti-inflammatory activities, it was found that the application of Glycyrrhiza glabra extracts

orally enhanced the defense mechanisms in rodents. It was additionally noted that the utilization of Glycyrrhiza glabra resulted in an improved antioxidant status and a significant reduction in oxidative stress markers compared to the untreated control group. This could be attributed to the presence of sterols and polyphenols, particularly Glycyrrhizic acid, which contributes to accelerated wound healing. It further improves wound contraction, epithelization rate, vascularity, and reduces cell necrosis and skin damage through its antioxidant properties.[16]

Veratti et al, in his study demonstrated that 18ß-glycyrrhetinic acid and glabridin inhibited DNA damage in human keratinocytes induced by UV radiation. The core reason behind this action likely stems from Glycyrrhiza glabra's potent antioxidant properties, primarily attributed to its rich phenolic content, encompassing flavonoids, sterols, polyphenols, and isoflavones.[17]

The high anti-inflammatory action of Glycyrrhiza glabra was substantiated by Shinada et al in another study in which administration of Glycyrrhiza glabra root resulted in elevated leukocyte infiltration and blood flow, effectively reducing inflammation through actions resembling those of steroids and cortisone. This was explained by the presence of Glycyrrhetinic acid, which inhibits the release of pro-inflammatory cytokines, such as TNFα and IL-6, while increasing the production of anti-inflammatory cytokines, such as IL-7 and IL-10.[17,18]

The effectiveness of dipyridamole (DPG) as a treatment strategy for reducing intestinal inflammation was investigated by Vitali et al. The effect of DPG on high mobility group box 1 (HMGB1), an early pro-inflammatory cytokine released from damaged cells during inflammation, was examined in their study. In vitro tests showed that DPG dramatically reduced the expression levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 and suppressed the release of HMGB1. Moreover, in vivo tests showed that DPG attenuated intestinal inflammation and lessened the severity of colitis induced by dextran sulfate sodium (DSS) by suppressing the expression of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, as well as the HMGB1 receptors RAGE and TLR.[19]

The effects of Glycyrrhiza uralensis on mouse skin treated with 12-O-tetradecanoylphorbol-13-acetate and macrophages treated with lipopolysaccharide have been studied in lab settings by Cho et al. Glycyrrhiza uralensis showed a reduction in the secretion and mRNA levels of pro-inflammatory cytokines TNF-α, IL-6, cyclooxygenase-2 (COX-2), and IL-1β, as well as a decrease in the release of prostaglandin E2 (PGE2) and nitric oxide (NO) in the model of lipopolysaccharide treated macrophages. Furthermore, it suppressed the transcriptional activity and protein expression of PLA2 and inducible nitric oxide synthase (iNOS), as well as the degradation of inhibitor of NF-κB α (IκBα) and the translocation of p65 into the nucleus. It decreased the expression of COX-2 and iNOS and lessened dermal inflammation in a mouse model of inflammation.[20]

Rackova et al. discovered that Glycyrrhiza glabra exhibited antioxidant properties, capable of combating free radicals in the body. Specifically, Glycyrrhiza glabra demonstrated efficacy in neutralizing the stable1,1′-diphenyl-2-picrylhydrazyl radical. Moreover, it reduced the generation of reactive oxygen species, detrimental molecules implicated in cellular damage, induced by various stimuli. Additionally, a protective effect on damaged liposomes and cellular structures was observed. These beneficial effects were attributed to specific compounds present in Glycyrrhiza glabra,

such as chalcones, coumarins, and isoflavan derivatives, which function as antioxidants.[21]

In our study the anti-inflammatory activity exhibited by the gum paint was similar to that of the control. The highest anti-inflammatory activity was seen at a concentration of 50ul. The effect of Triphala on the immune cells have been contradictory in the literature. Phetkate and associates analyzed the immune-stimulating qualities of Triphala in their study.[22] The findings demonstrated that, in comparison to control samples, Triphala significantly increased the activity of natural killer cells and cytotoxic T cells. On the other hand, no appreciable changes in cytokine secretion were noted. Triphala's three constituent fruits- Phyllanthus emblica, Terminalia chebula, and Terminalia belerica, have immunostimulatory properties that may explain the increase in cytotoxic T lymphocytes and natural killer cells associated with the herb. Phyllanthus emblica has been shown in earlier research to improve immunity, specifically NK cell-induced cytotoxic activity. Its fruit has substances with immune-stimulating qualities, such as quercetin, gallic acid, and vitamin C. Immunity in rodents has been demonstrated to be improved by quercetin, and the immune system is modulated by vitamin C through increased NK cell activity and an improved cell-mediated immune response. In studies on animals, it has been discovered that Terminalia chebula, which is high in chebulinic acid, increases T and B lymphocytes and activates NK cells. Furthermore, studies on mice have demonstrated that Terminalia belerica stimulates T lymphocytes.[22,23, 24]

In a study by Sireeratawong et al., Triphala demonstrated a notable ability to inhibit the development of ear edema caused by ethyl phenylpropiolate. Additionally, the Triphala formulation significantly decreased carrageenan-induced hind paw edema across various dosage levels (300, 600, and 1,200 mg/kg).[24,25]

In a similar study by Naik et al., Triphala and its components (Emblica officinalis, Terminalia chebula, and Terminalia belerica) were assessed for their antioxidant activity in a study conducted by Naik et al. They discovered that gamma radiation-induced DNA damage and lipid peroxidation were successfully inhibited by these extracts. They also displayed the ability to scavenge free radicals. With Terminalia chebula demonstrating higher radical scavenging activity and Emblica officinalis being more effective in lipid peroxidation, each component displayed marginally different activities. Triphala derives its antioxidant and radioprotective attributes from its polyphenols, which mitigate oxidative stress by converting reactive oxygen free radicals into inert substances. Because of the combined influence of its three constituents, the triphala blend exhibits heightened potency.[26]

In another investigation by Ronzio et al., treatment with Triphala substantially decreased the production of free radicals, leading to decreased levels of lipid peroxidation. This outcome is linked to the presence of phytochemicals like flavonoids and phenolic compounds in Triphala extract. These compounds have been found to inhibit neutrophil respiratory burst, improve redox potential, eliminate singlet oxygen, and release lysosomal enzymes.[26,27] Jadhav et al's study demonstrated that Triphala extract exhibited notable effectiveness in scavenging free radicals, thereby mitigating cell damage and senescence induced by hydrogen peroxide.[28]

The elevated polyphenol content in both components likely contributes to the enhanced anti-inflammatory and antioxidant effects of the gum paint.[29] These effects are mediated through several mechanisms, such as inhibiting pro-inflammatory enzymes,

suppressing inflammatory mediators, modulating immune responses, scavenging free radicals, boosting endogenous antioxidant defenses, and reducing the production of pro-inflammatory substances. Collectively, these actions help diminish inflammation, prevent oxidative stress, and safeguard against DNA damage.[30-32] A flowchart below illustrates the mechanisms of polyphenols (Figure 4).

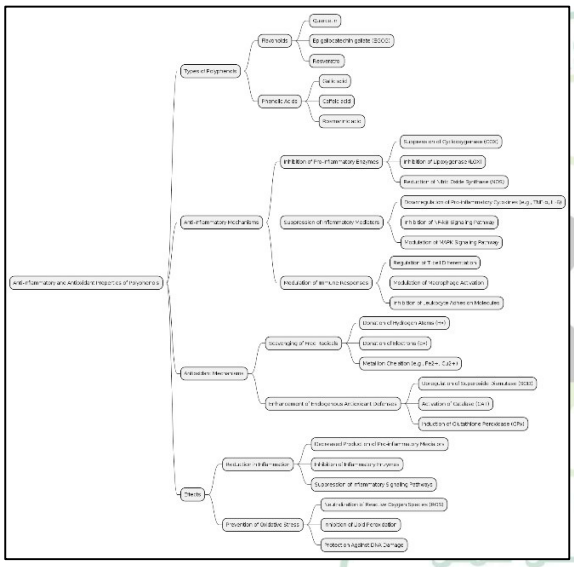

**Figure 4: Antioxidant and Anti-inflammatory actions of polyphenols**

The gum paint formulated with Glycyrrhiza glabra and Triphala demonstrated promising characteristics and biocompatibility, indicating its potential for treating various oral lesions safely and effectively. However, the study had limitations, such as being conducted in an in vitro setting, which affected the generalizability and long-term assessment of the gum paint's efficacy and safety. Additionally, comparisons were limited to a standard, possibly overlooking other influencing factors. Further regulatory standards and long-term adverse effects and interactions with other treatments should be extensively monitored in the future studies.

## Conclusion

In conclusion, the findings of this study demonstrate the positive outcomes of gum paint in terms of its anti-inflammatory and antioxidant activities. Numerous scientific investigations have provided empirical validation for the traditional uses of Triphala and Glycyrrhiza glabra, shedding light on their potential therapeutic benefits. However, further research is imperative to corroborate these findings through human clinical trials and to elucidate the underlying biological mechanisms associated with plant-based medicines. This ongoing exploration will not only enhance our understanding of the therapeutic potential of Triphala and Glycyrrhiza glabra but also pave the way for their integration into mainstream healthcare practices.

**Funding**: The project was intramurally funded by Saveetha Dental College and hospitals (SIMATS), Chennai

**Data availability**: Available on request

**Declarations:**

**Ethics approval and consent to participate**: Not applicable for in vitro studies

**Competing interests:** Authors declare that they have no conflict of interest

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