

The effects of dimethyl sulfoxide on the acute phase of experimental acid and alkali corrosive esophageal burns

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Abstract. – BACKGROUND, AIM: The aim of this study was to investigate the effects of dimethyl sulfoxide (DMSO) on the acute phase of experimental corrosive esophageal burns.

MATERIALS AND METHODS: Fifty male rats were allocated into five groups (control, acid burn, alkali burn, acid burn + DMSO and alkali burn + DMSO) of ten rats each. Acid and alkali burns were created by burning the distal esophagus with 1 N hydrochloric acid and 50% sodium hydroxide solution, respectively. DMSO was applied intraperitoneally at 15 minutes after burn creation and then every 12 hours for four days. All animals were sacrificed at the end of the 7th day. Histopathological changes in esophageal tissue were scored by a single investigator who was blind to the burn group.

RESULTS: Application of DMSO resulted in a significant decrease in the severity of acute tissue damage as measured by macroscopic and microscopic assessments in both the acidic and alkaline esophagitis groups. The increased immunohistochemical Ki-67 proliferation index was significantly suppressed in the DMSO-treated alkaline esophagitis group, $p < 0.05$. Furthermore, the immunoreactivity of nuclear factor kappa B (NF- κ B) was significantly reduced in both the acid and alkali DMSO-treated groups, $p < 0.05$.

CONCLUSIONS: DMSO reduced the acute phase symptoms and decreased the severity of tissue damage in both acidic and alkaline corrosive esophagitis.

Key Words:

Corrosive esophageal burn; Dimethyl sulfoxide; NF- κ B immunoreactivity; Ki-67 proliferation index.

Introduction

Corrosive esophageal burns constitute a serious health problem during childhood. Corrosive substances are often found in cleaning agents such as alkali- or acid-containing solutions in the

form of granules or solid materials. In adults, intake of these materials is usually observed in the context of suicidal attempts, whereas children often ingest them accidentally. Eighty percent of corrosive esophageal burns occur in children under five years old. Steroids and antibiotics are currently used to reduce the inflammatory reaction and prevent secondary bacterial infections, respectively. In serious esophageal burns, nasogastric tube intubation, esophageal dilatation with bougies and balloon catheters, implantation of esophageal stents, myotomy, resection-anastomosis and esophageal replacement surgery have been added to the standard medical therapy¹⁻⁴.

Stricture formation is an important problem in corrosive esophageal injuries. Many drugs have been used in both clinical and experimental studies to prevent the development of strictures. However, antibiotics and steroids are the only pharmacological agents that are used in current clinical practice, and these agents have only limited success⁵⁻⁷. Therefore, new and effective agents that can be used in the treatment of patients with corrosive esophagitis are needed.

Dimethyl sulfoxide (DMSO) is an anti-inflammatory drug with a free oxygen radical binding capacity^{8,9}. DMSO has been used in the symptomatic treatment of interstitial cystitis in clinical practice¹⁰. The inhibitory effects of DMSO on nuclear factor kappa B (NF- κ B), which regulates the expression of cellular genes in inflammation, have been demonstrated in experimental models of sepsis¹¹⁻¹⁵. The suppressive effects of DMSO on activation of NF- κ B immunoreactivity (NF- κ B IR) are exerted by decreasing the production of tumor necrosis factor-alpha (TNF- α) and inhibiting the transcription of critically important adhesion molecules. The Ki-67 protein is a valuable marker reflecting cellular proliferation. Local inflammatory cell prolifera-

tion and an increase in the Ki-67 proliferation index (Ki-67 PI) are reported to be present in acute inflammation^{13,16-18}.

Experimental animal studies have documented the protective effects of DMSO as an antioxidant agent against septic shock and endotoxemia. Among its many proposed effects include the dissolution of collagen to decrease the amounts of connective tissue, the reinforcement of resistance against infections via nonspecific mechanisms and vasodilatation^{9,11,19,20}. In addition, the healing effects of DMSO in ischemia-reperfusion damage have been the subject of many experimental studies in recent years^{8,21-23}. However, application of this product in the treatment of corrosive esophageal injuries has not yet been reported in the literature. The anti-inflammatory and antioxidant activity of DMSO, in addition to its suppressive effects on NF- κ B IR, constituted the rationale for our present investigation. In addition, we used Ki-67 PI to investigate the effects of DMSO on cell proliferation in acute phase corrosive esophageal burns.

Materials and Methods

Experimental Animals

The experimental protocol was approved by the local Animal Ethic Committee of the Istanbul University. A total of 58 male Sprague-Dawley rats, weighing 150-200 g each, were used in this study. The animals were acclimatized for at least 10 days in the animal laboratory of our University Research Center, receiving a standard rat diet *ad libitum* and free access to water. Initially, five groups of 10 rats each were randomly formed. One rat in the acid burn group, 4 in the alkali burn group, and 3 in the alkali burn+ DMSO group died within the first 48 hours of the experiment due to anesthetic complications and aspiration of the corrosive material. Thus, these 8 rats were replaced by the same number of animals prepared in the same way.

Corrosive Esophageal Burns and Study Design

After 12 h of fasting, laparotomy was performed through a midline incision under anesthesia with 100 mg/kg of subcutaneous ketamine hydrochloride (Ketalar, Pfizer, Istanbul, Turkey) plus 15 mg/kg of subcutaneous xylazine hydrochloride (Rompun 2%, Bayer, Istanbul, Turkey). Standard corrosive esophageal burns

were created according to the model described by Gehanno *et al.* and later modified by Liu *et al.*^{24,25}. A 2 cm segment of the abdominal esophagus was mobilized. A 24 G cannula was inserted through the stomach up to the 2 cm abdominal esophagus and the abdominal esophagus tied with 3/0 Vicryl suture from the hiatal and cardiac ends. The distal esophageal segments of the groups were manipulated as described below:

Control group: 1 ml saline solution (0.9%) was given.

Acid burn group: 1 ml 1 N hydrochloric acid (HCl) solution from Sigma was instilled.

Alkali burn group: 1 ml 50% concentrated solution of sodium hydroxide (NaOH) from Riedel-de Haén was instilled.

Acid burn + DMSO group: The acid burn was created as in the acid burn group. Fifteen minutes after the acid burn, DMSO solution (10%, Sigma, Cryosure-10, 50 ml ampoule, 100 ml flacon form, Wak-chemie Med) was administered intraperitoneally at a dose of 10 ml /kg followed by successive doses with 12 hour intervals for 4 days.

Alkali burn + DMSO group: The alkali burn was created as in the alkali burn group. Fifteen minutes after the alkali burn, DMSO solution was administered as described previously.

In all groups, NaOH, HCl or saline solutions were infused at a pressure of 30 cm of water through the cannula for 90 seconds. In all acid and alkali burn groups, the distal esophageal segments were immediately irrigated with distilled water. Ligature sutures and catheters were removed. Abdomens were closed using uninterrupted 3/0 silk sutures. Three hours after the procedure all rats were fed *ad libitum* with special rat chow and tap water. At the end of the 7th day, all rats were sacrificed under anesthesia and their abdominal esophagi removed for histopathological examination. Esophageal tissue samples were fixed in 10% buffered formalin, embedded in paraffin and cut into 3-5 μ m thick slices for further histopathological evaluation and immunohistochemical staining.

Histopathological Evaluation

The slices were stained with hematoxylin-eosin and examined under the light microscope. Histological damage to esophageal tissue was scored by a single pathologist who was blind to the groups. Each group was evaluated for the

presence of the following parameters: ulceration, necrosis, inflammation, fibrosis, neovascularization, and muscular destruction. Each characteristic was scored as normal (0), minimal (1), moderate (2) or severe (3). The total tissue damage scores were calculated by summing the scores for each characteristic.

Immunohistochemical Evaluation

The esophageal tissue slices were prepared for the assessment of Ki-67 (Rabbit polyclonal ready to use 6 ml A00095 – IFU – IUD Syk Tek Laboratories Logan UTAH) and NF- κ B (P 50 Ab-2 RB – 1648 – R7 ready to use 7 ml NeoMarkers Ferment CA) antibodies. Tissue slices were incubated with monoclonal mouse and polyclonal rabbit antibodies. All steps were performed at room temperature. Antigens were retrieved for anti-Ki-67 and anti-NF- κ B antibodies. As positive controls, breast carcinoma and testicular tissues were used for the anti-Ki-67 and anti NF- κ B antibodies, respectively.

Ki-67-proliferation ratio: The prepared slides were examined for nuclear staining under x4 and x10 magnification. Based on the number of cells in the basal layers of esophageal squamous epithelium, where most of the stain is retained, proliferation was determined in terms of percent ratios. Proliferation ratios were scored as $< 50\%^1$, $50-75\%^2$ and $> 75\%^3$.

NF- κ B IR ratio: Cytoplasmic and nuclear uptake of the stain was considered primarily. Staining properties were evaluated of inflammatory cells only. Staining densities were expressed as percentages and scored as $< 25\%^1$, $25-50\%$ (2), $50-75\%^3$ and $> 75\%^4$.

Statistical Analysis

Intergroup comparisons for the total tissue damage, Ki-67 proliferation ratio and NF- κ B IR ratios were evaluated with the Mann-Whitney U test, and p values of < 0.05 were considered significant.

Results

Macroscopic Findings

Esophageal walls of the rats in the acid and alkali burn groups appeared edematous with white mucosal plaques, which were more frequent in the alkali burn group. However, in both the acid and alkali DMSO-treated burn groups, edema was only noted on the esophageal wall. Furthermore, edema was minimal in the acid burn+ DM-

SO group. The macroscopic appearance of the esophageal walls was unremarkable in the control group.

Histopathological Findings

Inflammation, fibrosis and total damage scores were significantly higher in the acid burn group than in the control group ($p < 0.05$). The DMSO-treated acid burn group displayed less inflammation, mucosal ulceration, necrosis and muscular destruction and lower total damage scores compared with the acid burn group ($p < 0.05$). The alkali burn group had more inflammation, fibrosis, mucosal ulceration, necrosis, fibrosis, neovascularization and muscular destruction and higher total damage scores with respect to the control group ($p < 0.05$). The total damage scores, inflammation and necrosis in the DMSO-treated alkali burn group were significantly better than the corresponding values in the alkali burn group ($p < 0.05$).

Graph 1: Comparative graphics demonstrating total esophageal damage scores in all groups

Immunohistochemical Findings

Ki-67 proliferation: The Ki-67 PI did not increase significantly in the acid burn group but did increase in the alkali burn group. Application of DMSO significantly changed the Ki-67 PI in the alkali burn group ($p < 0.05$) but not in the acid burn group.

Figure 1a and 1b. Comparison of Ki-67 PI.

Figure 2a and 2b. Comparatively more depressed Ki-67 PI in alkali burn + DMSO group due to the application of DMSO relative to alkali burn group.

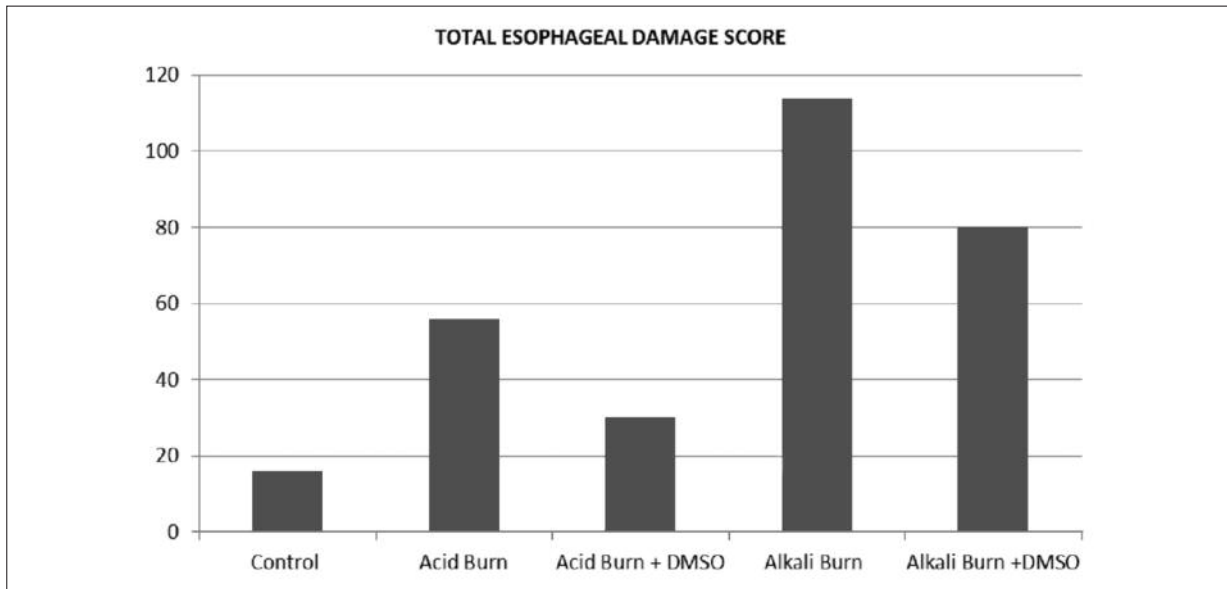
NF- κ B IR: The NF- κ B IR was significantly increased in both the acid and alkali burn groups. DMSO resulted in a significant decrease in the NF- κ B IR in both the acid and alkali burn groups ($p < 0.05$).

Figure 3a and 3b. Comparatively more depressed NF- κ B IR in acid burn + DMSO group due to the application of DMSO relative to acid burn group.

Figure 4a and 4b. Comparatively more depressed NF- κ B IR in alkali burn group due to the application of DMSO relative to alkali burn + DMSO group.

Discussion

This study revealed the beneficial effects of DMSO on acute inflammatory injury after experimental corrosive esophageal burns. DMSO sig-



Graph 1. Comparative graphics demonstrating total esophageal damage scores in all groups.

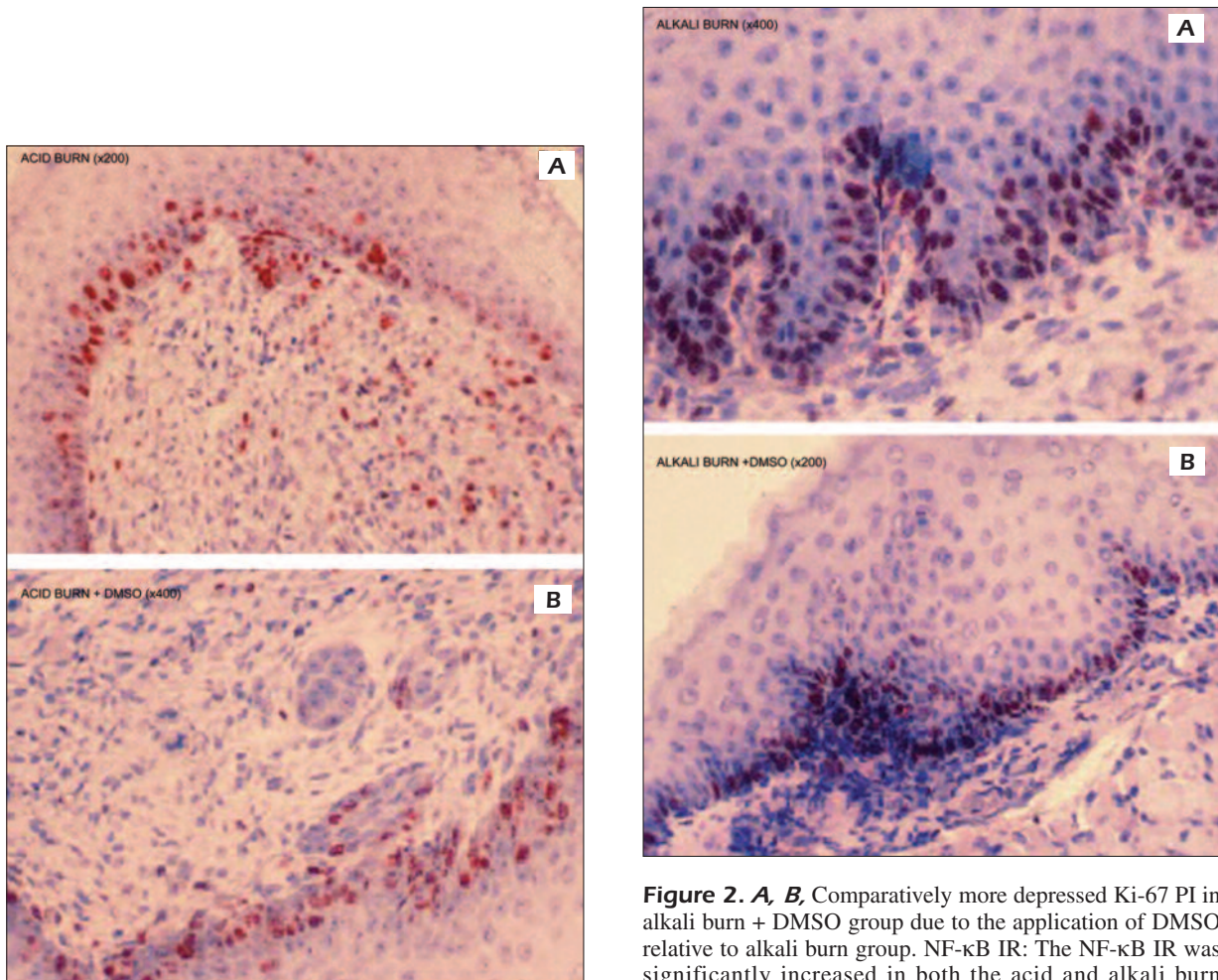


Figure 1. A, B, Comparison of Ki-67 PI.

Figure 2. A, B, Comparatively more depressed Ki-67 PI in alkali burn + DMSO group due to the application of DMSO relative to alkali burn group. NF- κ B IR: The NF- κ B IR was significantly increased in both the acid and alkali burn groups. DMSO resulted in a significant decrease in the NF- κ B IR in both the acid and alkali burn groups ($p < 0.05$).

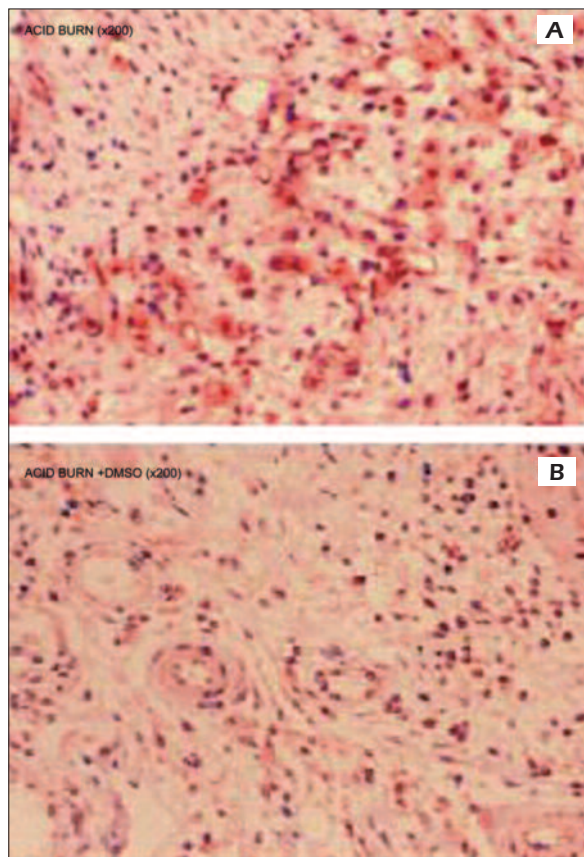


Figure 3. A, B, Comparatively more depressed NF- κ B IR in acid burn + DMSO group due to the application of DMSO relative to acid burn group.

nificantly decreased the severity of histologic lesions and suppressed the NF- κ B IR. To date, the effects of several pharmacologic agents have been investigated in experimental corrosive esophagitis models. Nearly all of these studies were conducted with alkaline substances; however, acidic substances are responsible for 15-25% of this burns²⁶⁻²⁸. We investigated the effects of DMSO on both acid and alkali esophageal burns.

The underlying pathophysiology of alkali and acid ingestion differs. Alkaline substances cause mucosal liquefactive necrosis, and the subsequent submucosal destruction allows for deeper penetration that may even penetrate through the muscularis propria layer. Acidic substances tend to cause a coagulation necrosis of the mucosa, and the resultant scar formation tends to limit penetration and subsequent injury to the esophageal wall. However, ingestion of acidic substances, especially in high concentrations, may also cause severe esophageal damage. In our study, DMSO decreased the tissue damage in

both acid and alkali burns. One of the main objectives in treating corrosive esophagitis is to prevent stricture formation. In these types of burns, diffuse esophageal inflammation is present in the first four days. Based on the degree of the burn, inflammation may penetrate all layers. Thrombosis within submucosal vessels causes local necrosis. The impact of reactive oxygen radicals formed during the acute phase is also important in esophageal damage. The extent of the acute inflammatory reaction plays the most critical role in stricture formation^{2,3,6,29,30}. Pharmacologic agents with antioxidant activity^{3,31-36}, anti-inflammatory effects^{6,24,37,38}, anti-platelet aggregation effects^{2,6,29} and inhibition of collagen accumulation^{7,25,30,39} have been used to decrease esophageal tissue damage in an attempt to prevent esophageal stricture in experimental animal models. We show that DMSO, which possesses all of these properties, is a promising agent in the treatment of experimental corrosive esophagitis. The NF- κ B protein family includes transcription fac-

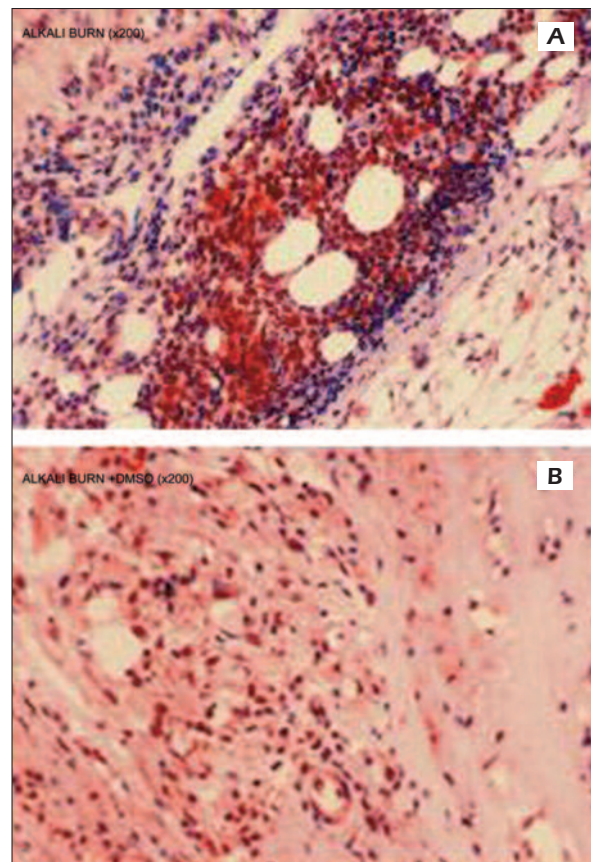


Figure 4. A, B, Comparatively more depressed NF- κ B IR in alkali burn group due to the application of DMSO relative to alkali burn + DMSO group.

tors that can rapidly activate expression of genes critical in the inflammatory acute phase response. NF- κ B was also the first eukaryotic transcription factor found to respond directly to oxidative stress¹⁴. A few studies have demonstrated that DMSO can inhibit NF- κ B in hemorrhagic shock, endotoxin-induced liver injury and peritonitis models^{11, 12, 20}. These studies showed that application of DMSO resulted in a decrease in inflammatory biochemical markers, but histopathologic assessments were not reported. To the best of our knowledge, we are first to demonstrate an increase in NF- κ B IR together with its subsequent suppression by DMSO in both acidic and alkaline esophagitis models. Furthermore, DMSO decreased histopathological inflammatory findings in both models. The Ki-67 protein is reported to indicate cellular proliferative activity with a higher sensitivity and reliability than the number of mitotic divisions¹⁶. We demonstrated an increase in the Ki-67 PI in alkaline esophagitis only, and DMSO suppressed the Ki-67 PI in this group. Our inability to show any corresponding increase in the acid burn group may be related to the lower level of tissue damage.

Conclusions

In conclusion, we reveal DMSO to be a promising agent in treating acid and alkali corrosive burns via the control of acute inflammatory injury as seen on macroscopic and microscopic assessments. However, long-term studies should be conducted to reveal the effects of DMSO on esophageal stricture formation. The NF- κ B IR increased in corrosive esophagitis and seemed to be an important mediator in the beneficial effects of DMSO. The Ki-67 protein should also be more thoroughly investigated in these inflammatory processes.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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