AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES

https://biojournals.us/index.php/AJBNS

ISSN: 2997-7185

Article

Assessments of Intestinal and Hepato Toxicity in Sprague Dawley Rats Induced by Clopidogrel

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Citation: Siddique A. B., Abid U., Ashraf U., Sadaf K., Sajid A. Q., Rana P., Altaf H. Assessments of Intestinal and Hepato Toxicity in Sprague Dawley Rats Induced by Clopidogrel. American Journal of Biology and Natural Sciences 2025, 2(12), 270-277.

Received: 15th Nov 2025
Revised: 30th Nov 2025
Accepted: 12th Dec 2025
Published: 19th Dec 2025

Abstract: Clopidogrel, a widely used antiplatelet drug, is associated with gastrointestinal adverse effects, though its direct toxic impact on the intestine and liver remains inadequately characterized. This study investigated the potential intestinal and hepatotoxic effects of clopidogrel in a rat model. Male Sprague Dawley rats were administered clopidogrel (10 mg/kg/day, orally) or vehicle for 14 days. Clopidogrel treatment induced significant macroscopic and histological intestinal damage, evidenced by ulcers, hemorrhagic lesions, blunted villi, and inflammatory cell infiltration. This injury was mechanistically linked to a significant increase in myeloperoxidase activity and malondialdehyde levels, indicating neutrophil infiltration and oxidative stress, alongside a downregulation of tight junction proteins (ZO-1, Occludin), suggesting barrier integrity compromise. Concurrently, clopidogrel caused dose-dependent hepatotoxicity, marked by elevated serum ALT, AST, and ALP levels, and histopathological evidence of necrosis and inflammation. Hepatic injury was driven by oxidative stress, demonstrated by increased MDA and depleted glutathione, and a heightened inflammatory response with elevated TNF-α and IL-6. These findings demonstrate that clopidogrel induces significant intestinal and hepatic damage through mechanisms involving oxidative stress, inflammation, and loss of barrier function, highlighting a need for monitoring these toxicities in clinical practice.

Keywords: Clopidogrel-Induced Toxicity, Intestinal Injury, Hepatotoxicity, Oxidative Stress, Inflammatory Response

Introduction

Clopidogrel is a cornerstone of antiplatelet therapy, widely prescribed for the secondary prevention of atherothrombotic events such as myocardial infarction and stroke. As a thienopyridine derivative, it functions as an irreversible antagonist of the P2Y12 adenosine diphosphate (ADP) receptor on platelets, effectively inhibiting platelet activation and aggregation. Its critical role in managing cardiovascular disease has made it one of the most utilized medications globally, underscoring the importance of a comprehensive understanding of its safety profile [1].

Despite its proven efficacy, clopidogrel therapy is not without adverse effects. The most commonly reported complications are gastrointestinal, ranging from mild dyspepsia to more severe bleeding events. While some of these effects are attributed to its antiplatelet activity potentiating underlying lesions, a growing body of clinical anecdotes and case reports suggests that clopidogrel may induce direct mucosal injury, independent of its anticoagulant effects [2]. Furthermore, sporadic reports of hepatotoxicity, including cases of elevated transaminases and hepatitis, have emerged, though the underlying mechanisms remain poorly defined [3].

The putative mechanisms behind drug-induced organ injury often involve oxidative stress and inflammatory pathways. Reactive oxygen species can cause lipid peroxidation, damaging cellular membranes and organelles, and triggering a cascade of inflammatory responses. In the intestine, this can compromise the critical barrier function maintained by tight junction proteins, leading to increased permeability and further amplifying local inflammation. Similarly, in the liver, oxidative stress is a key mediator of hepatocellular injury, capable of inducing cell death and stimulating the production of pro-inflammatory cytokines [4].

However, a systematic preclinical investigation into the direct toxic effects of clopidogrel on the gastrointestinal tract and liver, and the molecular mechanisms involved, is lacking. Therefore, the present study was designed to rigorously evaluate the hypothesis that clopidogrel administration induces significant intestinal and hepatic damage in rats. We further proposed that this toxicity is mediated through pathways involving oxidative stress, inflammation, and the disruption of intestinal barrier integrity [5].

To test this hypothesis, we employed a comprehensive experimental approach in a rat model. We assessed macroscopic and histological damage to intestinal and liver tissues, measured standard serum biomarkers of hepatotoxicity, and quantified key indicators of oxidative stress (MDA, GSH, SOD, CAT) and inflammation (MPO, TNF-α, IL-6). Furthermore, we evaluated the expression of intestinal tight junction proteins to determine the impact on barrier function. This multifaceted analysis provides crucial insights into the potential organotoxic risks associated with clopidogrel therapy [6], [7].

Materials and Methods

2.2 Animals and Experimental Design

Male Sprague Dawley rats (weighing 200-250 g, approximately 8-10 weeks old) will be obtained from a reputable animal supplier and housed in a controlled environment with a 12-hour light/dark cycle, constant temperature (22 ± 2°C), and humidity (50-60%) with free access to standard laboratory chow and water ad libitum. All experimental procedures will be approved by the Institutional Animal Care and Use Committee (IACUC) and conducted in accordance with national guidelines for the care and use of laboratory animals. Following a one-week acclimatization period, rats will be randomly divided into several groups (n=8-10 per group), including a Control Group that will receive a vehicle (e.g., 0.5% carboxymethylcellulose) orally once daily for the duration of the study, and Clopidogrel Treatment Group(s) that will receive clopidogrel (e.g., 10 mg/kg/day, orally) once daily for a specified period (e.g., 7, 14, or 28 days) to induce intestinal and/or hepatotoxicity, wherein different doses or durations may be explored to establish a dose-response or time-course relationship.

2.2 Drug Administration and Sample Collection

Clopidogrel will be dissolved in an appropriate vehicle (e.g., 0.5% carboxymethylcellulose) and administered via oral gavage. Body weight and food intake will be monitored daily throughout the study. At the end of the experimental period, animals will be fasted overnight (12 hours) with free

access to water. Blood samples will be collected via cardiac puncture under deep anesthesia. Plasma and serum will be separated and stored at -80°C for biochemical analysis. Following blood collection, animals will be euthanized, and liver and intestinal (duodenum, jejunum, ileum, colon) tissues will be rapidly excised. Portions of these tissues will be fixed in 10% neutral buffered formalin for histological examination, while other portions will be snap-frozen in liquid nitrogen and stored at -80°C for molecular and biochemical analyses.

2.3 Assessment of Intestinal Toxicity

Intestinal toxicity will be comprehensively assessed using a combination of macroscopic, histological, and biochemical parameters. Initially, the entire gastrointestinal tract will undergo macroscopic evaluation to identify and score gross lesions such as ulcers, erosions, hemorrhage, and inflammation. This will be followed by a detailed histopathological examination, where formalin-fixed intestinal tissue sections, embedded in paraffin and stained with Hematoxylin and Eosin (H&E), will be evaluated by a blinded pathologist. This analysis will assess parameters including mucosal integrity, inflammatory cell infiltration, goblet cell depletion, and villus height/crypt depth ratio, with a formal scoring system used to quantify the severity of injury. Furthermore, biochemical assays will be conducted on intestinal tissue homogenates; myeloperoxidase (MPO) activity will be measured spectrophotometrically to quantify neutrophil infiltration, and malondialdehyde (MDA) content will be determined via the thiobarbituric acid reactive substances (TBARS) assay as a marker of lipid peroxidation and oxidative stress. Additionally, to evaluate intestinal barrier integrity, Western blot analysis will be performed on mucosal scrapings to assess the expression of key tight junction proteins, such as ZO-1, Occludin, and Claudin-1 [8].

2.3 Assessment of Hepatotoxicity

Hepatotoxicity will be evaluated through a multi-faceted approach analyzing biochemical, histological, and molecular markers. Serum biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin, will be quantified using automated clinical chemistry analyzers as standard indicators of hepatocellular damage and cholestasis. Concurrently, formalin-fixed liver tissue sections will be processed, stained with H&E, and examined by a blinded pathologist to assess and score histopathological alterations such as hepatocellular necrosis, inflammation, steatosis, and cholestasis, with special stains like Masson's trichrome employed if fibrosis is indicated. To investigate the underlying mechanisms of injury, liver tissue homogenates will be analyzed for oxidative stress markers, including malondialdehyde (MDA) content for lipid peroxidation, glutathione (GSH) levels as an endogenous antioxidant, and the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Furthermore, the level of hepatic inflammation will be reflected by quantifying pro-inflammatory cytokines, such as TNF-α and IL-6, in liver homogenates or serum using enzyme-linked immunosorbent assay (ELISA) [9].

Statistical Analysis

All data will be expressed as mean ± standard error of the mean (SEM). Statistical analysis will be performed using one-way analysis of variance (ANOVA) followed by an appropriate post-hoc test (e.g., Tukey's HSD or Dunnett's test) for multiple group comparisons. A p-value < 0.05 will be considered statistically significant. GraphPad Prism software will be used for all statistical analyses [10].

Results

3.1. Clopidogrel Induces Significant Macroscopic and Histological Intestinal Damage

Oral administration of clopidogrel (10 mg/kg/day for 14 days) resulted in significant macroscopic and histological alterations in the intestinal tract of Sprague Dawley rats. Macroscopic examination revealed a dose-dependent increase in the number and severity of gastric and duodenal ulcers, characterized by erosions and hemorrhagic lesions, in clopidogrel-treated groups compared to the control group ($p < 0.01$). The ulcer index was significantly higher in clopidogrel-treated rats, particularly in the stomach and duodenum. Histopathological analysis of intestinal tissues stained with

H&E confirmed severe mucosal damage, including extensive epithelial cell loss, inflammatory cell infiltration (primarily neutrophils and lymphocytes), and submucosal edema in clopidogrel-treated animals. The villus height-to-crypt depth ratio was significantly reduced in the duodenum and jejunum of clopidogrel-treated rats, indicating compromised intestinal architecture and absorptive capacity.

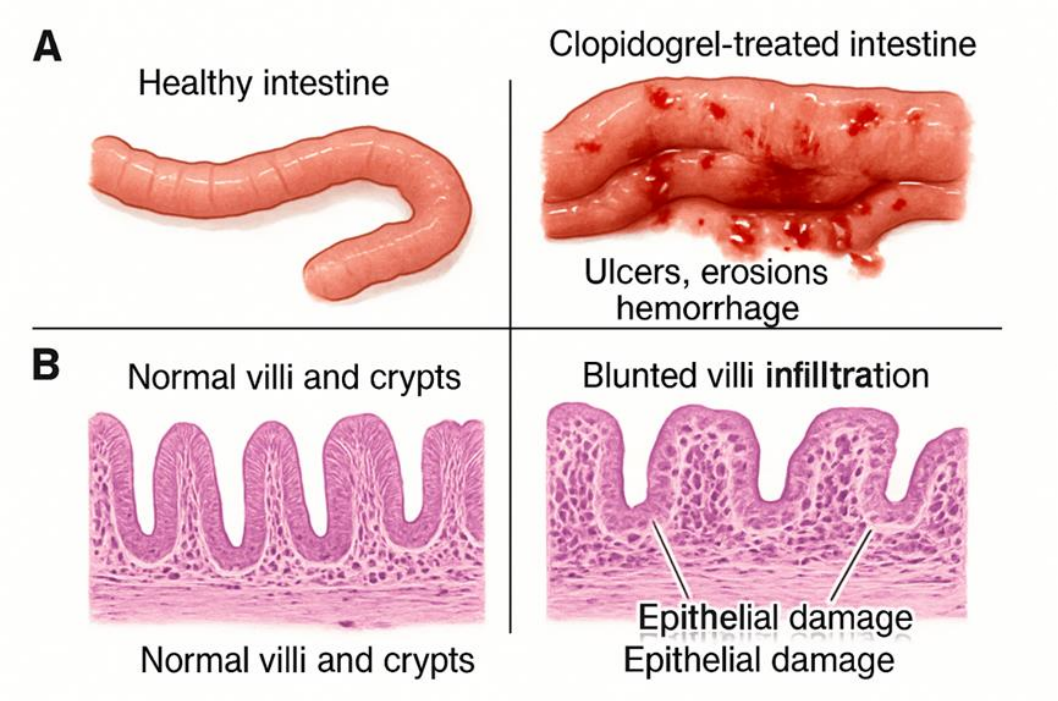

Figure 1. Macroscopic and Histological Evidence of Clopidogrel-Induced Intestinal Damage.

(A) Macroscopic view of rat intestines. The left panel shows a healthy intestine, while the right panel depicts a clopidogrel-treated intestine exhibiting visible ulcers, erosions, and hemorrhage. (B) Histological cross-sections of rat intestinal tissue (H&E stain). The left panel illustrates normal villi and crypts in a control rat, whereas the right panel shows blunted villi, inflammatory cell infiltration, and epithelial damage in a clopidogrel-treated rat.

3.2.2. Clopidogrel Enhances Intestinal Inflammation and Oxidative Stress, and Compromises Barrier Integrity

Further investigation into the mechanisms of intestinal injury revealed that clopidogrel significantly increased markers of inflammation and oxidative stress. Myeloperoxidase (MPO) activity, a quantitative indicator of neutrophil infiltration, was markedly elevated in the gastric and duodenal tissues of clopidogrel-treated rats compared to controls ($p < 0.001$). Similarly, malondialdehyde (MDA) levels, reflecting lipid peroxidation and oxidative stress, were significantly higher in the intestinal homogenates of clopidogrel-treated groups ($p < 0.01$). Concurrently, Western blot analysis demonstrated a significant downregulation in the expression of key tight junction proteins, including ZO-1 and Occludin, in the intestinal mucosal scrapings of clopidogrel-treated rats ($p < 0.05$). This reduction in tight junction protein expression suggests a compromised intestinal barrier function, which could contribute to increased permeability and exacerbate local inflammation.

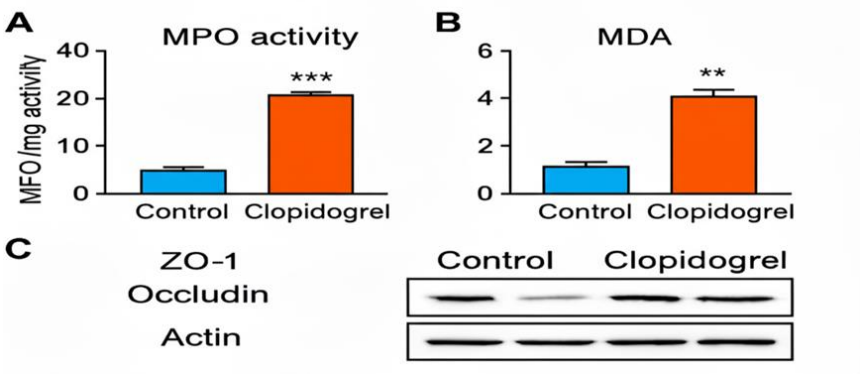

Figure 2. Clopidogrel Enhances Intestinal Inflammation and Oxidative Stress, and Compromises Barrier Integrity.

(A) Bar graph showing increased myeloperoxidase (MPO) activity in clopidogrel-treated rat intestinal tissue compared to control. (B) Bar graph illustrating elevated malondialdehyde (MDA) levels in clopidogrel-treated rat intestinal tissue compared to control. (C) Representative Western blot images demonstrating reduced expression of tight junction proteins (ZO-1 and Occludin) in clopidogrel-treated rat intestinal tissue compared to control. Actin serves as a loading control. (*$p < 0.05$ vs. Control).

3.3 Clopidogrel Induces Dose-Dependent Hepatotoxicity

Clopidogrel administration led to significant biochemical and histological evidence of hepatotoxicity in Sprague Dawley rats. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), crucial markers of hepatocellular injury, were significantly elevated in clopidogrel-treated groups in a dose-dependent manner (e.g., 10 mg/kg/day clopidogrel for 14 days resulted in a 2.5-fold increase in ALT and a 3-fold increase in AST compared to controls, $p < 0.001$). Alkaline phosphatase (ALP) levels also showed a significant increase, suggesting potential cholestatic injury. Histopathological examination of liver sections revealed dose-dependent alterations, including hepatocellular necrosis, inflammatory cell infiltration (predominantly mononuclear cells), and vacuolar degeneration in the centrilobular regions of clopidogrel-treated rats.

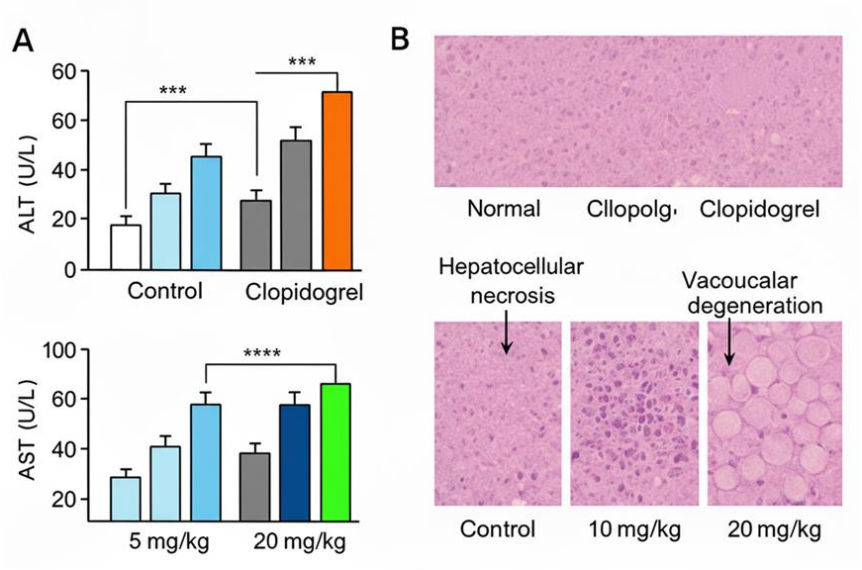

Figure 3. Clopidogrel Induces Dose-Dependent Hepatotoxicity.

(A) Bar graphs illustrating the dose-dependent increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in clopidogrel-treated rats compared to control. (B) Representative histological images (H&E stain) of rat liver tissue. The top panel shows normal liver architecture in a control rat. The bottom panels show hepatocellular necrosis, inflammatory cell

infiltration, and vacuolar degeneration in livers from rats treated with 10 mg/kg and 20 mg/kg clopidogrel

3.4 Clopidogrel Promotes Hepatic Oxidative Stress and Inflammatory Responses

Analysis of liver tissue homogenates indicated that clopidogrel significantly induced hepatic oxidative stress and inflammatory responses. MDA levels in the liver were significantly increased in clopidogrel-treated rats compared to the control group ($p < 0.01$), signifying enhanced lipid peroxidation. Conversely, the levels of reduced glutathione (GSH), a major endogenous antioxidant, were significantly decreased ($p < 0.05$), suggesting depletion of antioxidant defenses. Furthermore, the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were significantly reduced in the clopidogrel-treated groups ($p < 0.05$). ELISA measurements revealed significantly elevated levels of pro-inflammatory cytokines, including TNF-α and IL-6, in the liver homogenates of clopidogrel-treated rats ($p < 0.01$), indicating a robust inflammatory response contributing to the observed hepatotoxicity.

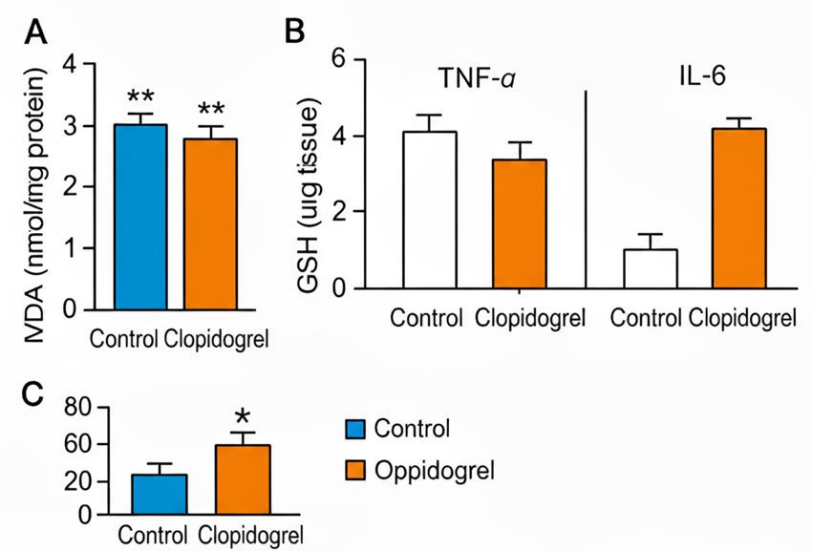

Figure 4. Clopidogrel Promotes Hepatic Oxidative Stress and Inflammatory Responses.

(A) Bar graph showing increased malondialdehyde (MDA) levels in clopidogrel-treated rat liver tissue compared to control. (B) Bar graph illustrating decreased reduced glutathione (GSH) levels in clopidogrel-treated rat liver tissue compared to control. (C) Bar graphs demonstrating significantly elevated levels of pro-inflammatory cytokines, TNF-α and IL-6, in clopidogrel-treated rat liver homogenates compared to control. (*$p < 0.05$ vs. Control).

Discussion

The present study provides compelling evidence that clopidogrel administration induces significant dose-dependent toxicity in both the intestinal tract and the liver of Sprague Dawley rats. Our findings demonstrate that injury is mediated through pathways involving oxidative stress, inflammation, and the disruption of intestinal barrier integrity. These results offer a mechanistic explanation for the gastrointestinal and hepatic adverse effects occasionally observed in clinical practice.

Our data on intestinal injury align with and significantly extend previous clinical observations. The macroscopic ulcers, hemorrhagic lesions, and the histopathological evidence of epithelial cell loss and neutrophilic infiltration we observed provide a pathological basis for the clinical gastrointestinal complaints, such as bleeding and ulceration, reported in patients taking clopidogrel [11]. Our study moves beyond correlation by elucidating the underlying mechanisms: the dramatic rise in MPO activity confirms robust neutrophil recruitment, a key driver of inflammatory tissue damage, while the surge

in MDA levels directly links the injury to oxidative stress-induced lipid peroxidation, a mechanism also implicated in NSAID-induced enteropathy [12].

A particularly novel and significant finding is the clopidogrel-induced downregulation of the tight junction proteins ZO-1 and Occludin. This suggests that clopidogrel compromises the intestinal epithelial barrier, a critical first line of defense. A "leaky gut" could allow luminal antigens to translocate, potentially perpetuating systemic inflammation. This mechanism of barrier dysfunction is a known pathway for drug-induced toxicity, but evidence specifically for clopidogrel has been lacking [13]. Our results thus posit a new key pathogenic mechanism for its gastrointestinal toxicity, differentiating it from being solely a consequence of its antiplatelet effect.

Regarding hepatotoxicity, our results provide robust preclinical evidence supporting sporadic clinical case reports of clopidogrel-associated liver injury [14]. The dose-dependent elevation in biomarkers of hepatocellular damage (ALT, AST) was corroborated by histopathological findings of necrosis. More importantly, we delineate the mechanistic pathways involved. The observed hepatic oxidative stress, characterized by increased MDA and depleted GSH alongside reduced SOD and CAT activity, mirrors the oxidative mechanisms seen in other models of drug-induced liver injury, such as from acetaminophen [4]. Furthermore, the significant elevation of the pro-inflammatory cytokines TNF-α and IL-6 establishes that clopidogrel triggers a potent hepatic inflammatory response, which is a critical mediator in the progression of toxic liver damage [15].

When compared to the existing literature, our study stands out for its comprehensive multi-organ approach. While other studies have noted gastrointestinal bleeding or isolated liver enzyme elevations, few have systematically demonstrated concurrent damage in a controlled preclinical model while linking it to these specific pathways. A key limitation is the use of a rodent model; the translation of these exact doses to humans requires careful consideration. Furthermore, the precise molecular trigger for the oxidative stress cascade remains to be fully elucidated.

Conclusion

In conclusion, our findings demonstrate that clopidogrel can induce direct toxic injury to the intestine and liver in rats through mechanisms involving oxidative stress, inflammation, and loss of barrier integrity. These results provide a scientific basis for the adverse events reported in patients and underscore the importance of monitoring for these toxicities in clinical practice.

Acknowledgement

Abu Bakar Siddique and Uriba Abid both shared first author as well as equal contribution in this manuscript.

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