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Hydroalcoholic Extract of *Terminalia pallida* Brandis for its Gastroprotective Effects in Wistar rats

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Abstract

The gastroprotective effects of a *Terminalia pallida* Brandis hydroalcoholic extract (HETP) were studied in Wistar rats utilizing ethyl alcohol, histamine, and indomethacin-induced ulcer models. At doses of 200 and 400 mg/kg (p.o.), HETP substantially reduced the incidence of indomethacin- and histamine-induced ulcers, with an effectiveness comparable to that of the oral reference drug, famotidine (30 mg/kg body weight). In addition, the risk of ethyl alcohol-induced stomach ulcers was dramatically decreased by *Terminalia pallida* extract. In ethanol-induced stomach ulceration, HETP successfully reduced the elevated lipid peroxide level of thio-barbituric acid reactive components and restored the altered glutathione level. The current study found that HETP considerably mitigated mucosal damage by increasing antioxidant capacity in the stomach mucosa.

Keywords: *Terminalia pallida*, Gastroprotective, Lipid peroxidation, Antioxidant

Introduction

Terminalia pallida Brandis is an endemic, tiny tree that can only be found in the Tirupati Hills of Andhra Pradesh. Diabetes, diarrhea, ulcers, and venereal disorders are regularly treated using the plant's fruits and leaves. (Akah, P.A et al., 1999) There is a slight diuretic effect in the bark. (Boyd, S.C et al., 1979) The fruits and leaves are used to cure liver disorders in Indian traditional medicine

as well. Extracts from both the fruits and the leaves showed strong antimicrobial and antifungal properties (Chopra, R.N et al., 1956). The extract from the fruits and leaves was shown to dramatically lower blood sugar levels in rats with alloxan-induced diabetes (Desai, J.K et al., 1996). Based on its historical usage, the present research evaluated the gastroprotective effects of a hydroalcoholic extract of *Terminalia pallida* on numerous Wistar rat ulcer groups.

Materials and Methods

Terminalia pallida Brandis leaves were gathered in Rakabganj, Uttar Pradesh, in March 2021, and identified by Dr. Navin Kumar Ambasht, Head of the Botany Department and Associate Professor at C.C. College in the state. The voucher specimen is stored in the herbarium of the relevant department. The leaves were air-dried in the shade before being ground up by a machine. After being sieved through a #40 mesh, powdered material should be stored in an airtight container.

Preparation of the extract

The chemical in the ground leaves has been extracted using hydroalcoholic solvent using a Soxhlet apparatus. A semisolid substance (10.5% yield) was produced by completely separating the solvent at decreased pressure. The material's fundamental chemical components were identified by qualitative analysis, and their authenticity was confirmed using TLC using authentic markers. Antioxidants such tannins, flavonoids, and steroids were found (Festa, F et al., 2001). After chilling the extract, certain quantity was suspended in 0.025% carboxy methyl cellulose before administration.

Animals

Boosts to health and quality of life for both sexes Wistar rats weighing 180-200g were bought from a local market and kept in a plastic cage with a 12-hour light/12-hour dark cycle at a constant temperature of 23.2°C. In the institute's animal home, they were fed a regular meal and given time to acclimate for a week. The Institute of Pharmacy, PSIT institutional animal ethics and welfare committee has approved all animal studies to ensure they are conducted in accordance with ethical standards of animal care.

Indomethacin-induced gastric ulcers in rat (Glavin, G.B et al., 1992)

Wistar rats were fasted for 12 and 18 hours, respectively. The subjects (n = 6) were randomly assigned to one of four groups. Group 1 operated the vehicle's controls while under a 0.025 percent CMC suspension. The second group had a dose of famotidine (30 milligrams per kilogram, orally).

Groups 3 and 4 received HETP at dosages of 200 and 400 milligrams per kilogram of body weight prior to the administration of indomethacin (20mg/kg p.o.). An hour later, there were sacrifices of animals. The stomachs were cut open at the larger curvature, inspected carefully, and washed with normal saline. The severity of the ulcers was measured using the following scale:

0 - [Normal Mucosa]
0.5- [Blushing]
1- [Spot Ulcers]
1.5- [Haemorrhage Streaks]
2- [Ulcers >3mm but <5mm]
2.5- [Ulcers >5 mm]

Ulcer index would be the average ulcer scoring per animals. The % of sore prevention were calculated: -

$$\% \text{ Protective} = \frac{[\text{Control means sore index}] - [\text{Test mean sore index}]}{[\text{Control means sore index}]} \times 100$$

Determination of acidity

$$\text{Acidity} = \frac{[\text{Vol. of NaOH}] \times [\text{Normality of NaOH}]}{[0.1]} \times 100 mEq./L.$$

Histamine-induced ulcers in rats **(Goyal, R.K et al., 1999)**

Both a 12-hour and an 18-hour water fast were imposed on the rats. There were a total of n = 6 animals, divided into five groups of 6. Group 1 normal, animals in Group 3 were given the reference medicine famotidine (30mg/kg p.o.) whereas those in Group 2 were given a CMC suspension vehicle control at 0.025 percent. The HETP dosages given to the animals in groups 4 and 5 were 200 mg/kg p.o. and 400 mg/kg p.o., respectively. Animals were dosed with 10mg/kg of histamine one hour after receiving the reference and test medications. After an hour, the animals were slain, and their stomachs were opened along their bigger curvature and washed with normal saline. The stomach lining was next evaluated and scored in the same way.

Ethanol-induced gastric ulceration **(Goyal, R.K et al., 2002)**

There were five groups, and each received six rats. Group 1 is normal, group 3 was given an omeprazole [10mg/kg p.o.] prescription, whereas Group 2 was given a 0.025 percent Carboxy Methyl Cellulose solution as a placebo. Rats in the group 4 and 5 were given HETP by mouth at

doses of 200 and 400 milligrams per kilogram, respectively. After the final dose of HETP and reference medicine, the animals were given 1 ml/200g of ethanol orally.

The animals were euthanized by cervical dislocation, and their stomachs were removed and examined for ulcers before the bigger arcs were eaten. The stomach's fundic mucosa were homogenized (at a 5 percent level) in 0.9% ice-cold normal saline using a homogenizer. Mitochondrial fraction was used to determine glutathione and lipid peroxide levels after the homogenate was centrifuged at 800g for 10 minutes and then at 12000g for 15 minutes to separate the supernatant. Lipid peroxidation (TBARS) was quantified using the Ohkawa et al. technique. (Gunasekhar, D. et al. 1993) Glutathione levels were determined using Ellman's technique and 5',5'-dithio-bis (2-nitrobenzoic acid) (DTNB), as reported by (Gupta, M et al., 2002)

2.7. Statistical analysis

Statistics are calculated using ANOVA and Dunnett's test, and results are presented as Mean±SEM. The cutoff for statistical significance was $p < 0.05$.

Histopathological evaluation

Samples of stomach tissue were preserved in neutral buffered formalin for a full day. Terminalia pallida's ulcerogenic or anti-ulcerogenic action in the stomach was evaluated by histopathology. The tissues were processed after being fixed in 10% buffered formalin. After the tissue was processed, it was fixed in paraffin and sectioned using a rotary microtome to achieve a thickness of 5 m. These sections underwent routine hematoxylin and eosin staining. Pathological abnormalities such as congestion, bleeding, edema, and erosions were rated on any scale after being observed under a microscope on glass slides (Harborne, J.B. et al., 1984)

Results

Indomethacin and histamine induced ulcer

Research on HETP's impact on indomethacin- and histamine-induced ulcers is summarized in Tables 1 and 2, respectively. Compared to placebo, HETP substantially reduced indomethacin- and histamine-induced ulcers. For indomethacin-induced stomach ulcer prevention, the extract was 33.24% effective at 200mg/kg and 52.21% effective at 400mg/kg ($p<0.05$), whereas famotidine was 64.16% effective at the same dosages ($p< 0.05$). The prophylactic effect of HETP at 400 mg/kg against histamine-induced ulcer development was 56.45%, whereas that of famotidine at 30 mg/kg was 68.82%.

Table 1: Effect of a hydroalcoholic extract of *Terminalia pallida* (HETP) on indomethacin induced ulcers in rats.

Treatment	Dose (mg/kg)	Ulcer index	Percentage inhibition
Normal	-	0	100
Indomethacin + vehicle	20	16.3 ± 1.3	-
Indomethacin + famotidine	30	5.8 ± 0.4*	64.16
Indomethacin + HETP	200	11.3 ± 0.8*	33.24
Indomethacin + HETP	400	8.4 ± 0.6*	52.21

Results are expressed as mean±S.E.M, Statistical significance was calculated by ANOVA followed by Dunnett's test. $*$ $p < 0.05$ was considered significant compared to the indomethacin treatment group.

Table 2: Effect of a hydroalcoholic extract of *Terminalia pallida* (HETP) on histamine induced ulcers in rats.

Treatment	Dose (mg/kg)	Ulcer index	Percentage inhibition
Normal	-	-	100
Histamine + vehicle	10	23.6 ± 1.8	-
Histamine + famotidine	30	6.7 ± 0.4*	68.82
Histamine + HETP	200	16.2 ± 0.8*	32.74
Histamine + HETP	400	11.5 ± 0.6*	56.45

Results are expressed as mean±S.E.M, Statistical significance was calculated by ANOVA followed by Dunnett's test. $*$ $p < 0.05$ was considered significant compared to the histamine treatment group.

Ethanol induced gastric ulcers

Table 3 shows the results of current studies evaluating the anti-ulcer effect of HETP on ethanol-induced gastric ulcer in rats. Alcohol intake resulted in harsh ulcers and markedly raised lipid-peroxide extent. Significantly lower glutathione extents were seen. In ethanol-induced ulcer model, the HETP dramatically decreased both the frequency and severity of ulceration. In comparison to the reference medicine omeprazole, the HETP provided 54.58 percent protection with dosage of 400mg/kg b.w. orally. The amount of material that reacts with thiobarbituric acid in animals given HETP with dosage of 400mg/kg b.w. considerably decreased 5.620.24 to 2.360.19 percent ($p < 0.05$).

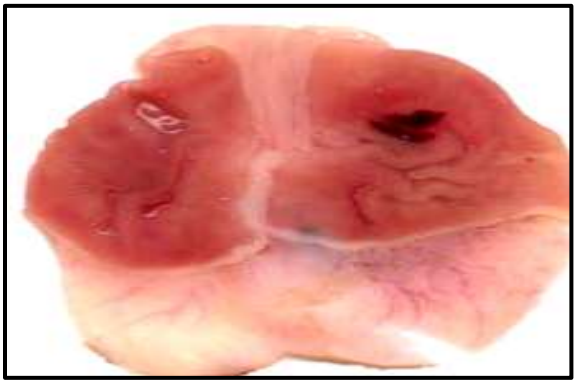
Table 3: Effect of ethanol induced ulcer formation in rats on a hydroalcoholic extract of *Terminalia pallida* Brandis.

Treatments	Dosage (mg/kg)	Ulcers index	Percentage inhibitions	TBARS (nmol MDA/mg protein)	Glutathione (nmol/mg protein)
Normal	-	0	100	1.45 ± 0.11	11.2 ± 0.9
Ethanol + vehicle	-	26.8	-	5.62 ± 0.24	5.3 ± 0.2
Ethanol + omeprazole	30	12.2	60.82*	1.82 ± 0.14*	6.3 ± 0.3*
Ethanol + HETP	200	18.3	33.07*	3.52 ± 0.20*	9.6 ± 0.8*
Ethanol + HETP	400	13.3	54.58*	2.36 ± 0.19*	7.8 ± 0.3*

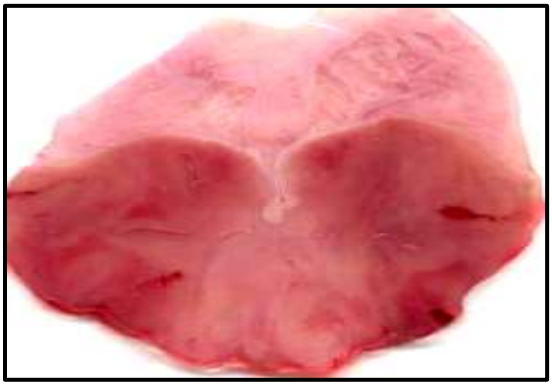
Results are expressed as mean±S.E.M, Statistical significance was calculated by ANOVA followed by Dunnett's test. $*$ $p < 0.05$ was considered significant compared with ethanol treatment group.

Macroscopical and Histopathological Evaluation

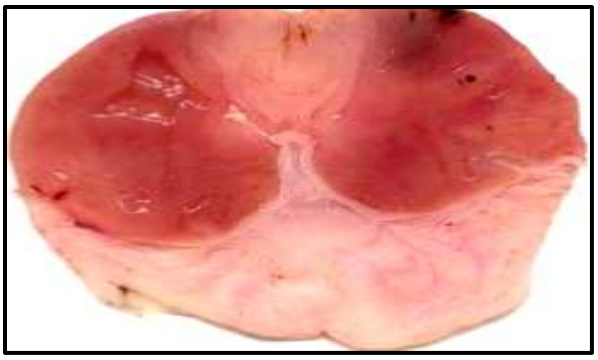
Major alterations in indomethacin-histamine-induced (1a, 1b, 1c) and ethanol-induced (2a, 2b, 2c) models. Compared to the indomethacin-histamine models, the HETP (400mg/kg) and Omeprazole (30mg/kg) treatment groups showed signs of resuscitation and prevented the emergence of hemorrhage and edema in gastric tissue (figures 3a, 3b, and 3c, respectively).



1a) Control (P.L.) shows severe damage to the mucosal layer

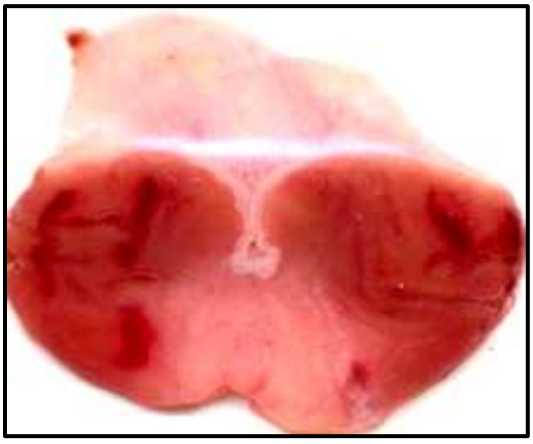


1 b) Omeprazole (30mg/kg) shows a protected mucosal layer

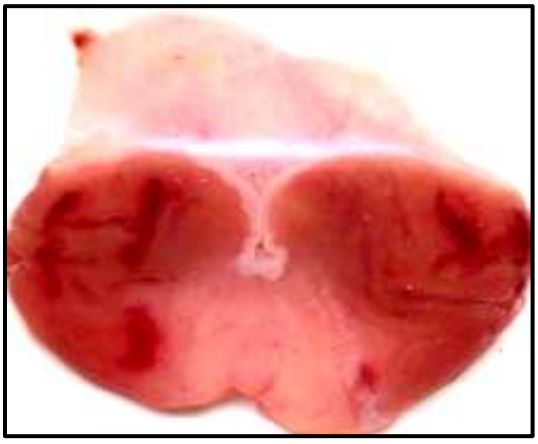


Hydroalcoholic extract (400mg/kg) 1c) HETP (400mg/kg) Shows protected mucosal layer

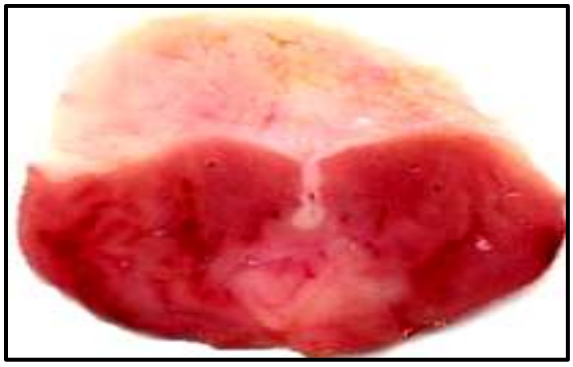
Macroscopical Views of Ethanol-induced Ulcers



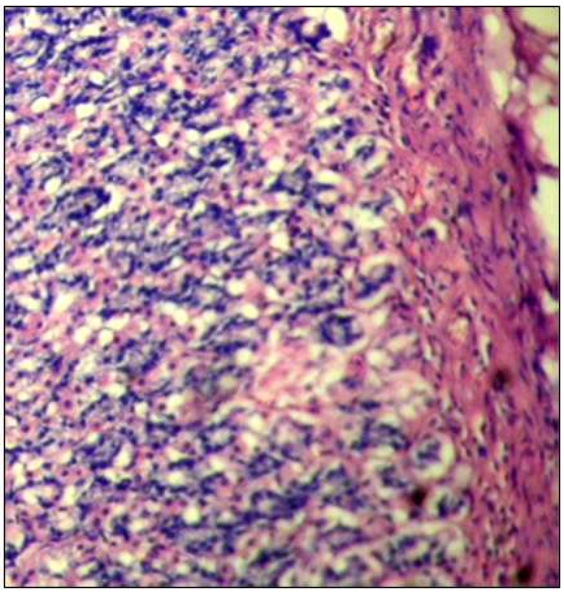
2 a) Ethanol Treated rats shows congestion, oedema, mucosal damage



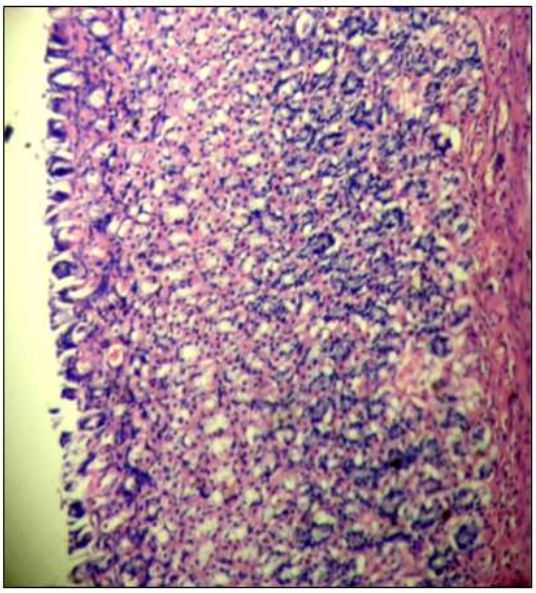
2 b) Omeprazole (30 mg/kg) shows protection of mucosal layer



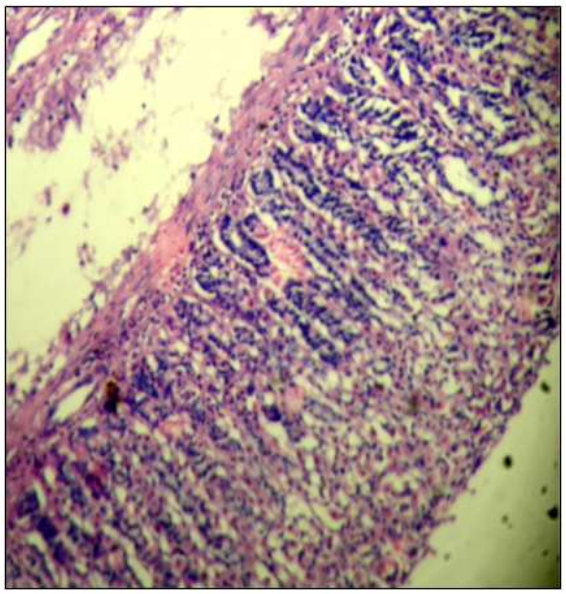
Hydroalcoholic extract (400mg/kg) **2c) HETP (400mg/kg) shows protection of mucosal layer**



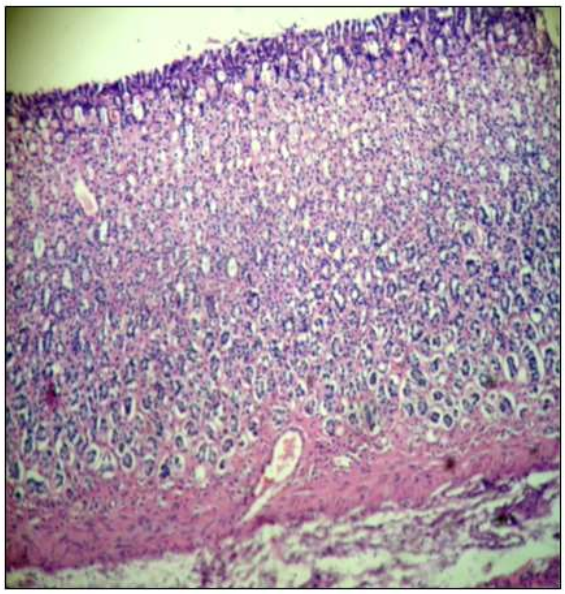
3a) Section of gastric mucosal layer Shows normal appearance Control



3b) Indomethacin-histamine groups show mucosal ulceration and inflammation



3 c) Omeprazole (30mg/kg) shows no significance changes in histopathology almost normal appearance.



3 d) HETP (400mg/kg) shows no significance changes in histopathology almost normal appearance.

Discussion

Ulcers form when the destructive elements of the stomach mucosa are out of whack. Mucin secretions, mucosal glycoproteins, cell dissociation, cell proliferation, and prostaglandin all attenuate the proteolytic effects of pepsin and stomach acid (Hollander, D et al. 1985). Medications and botanical extracts are used to inhibit the production of prostaglandin, lower stomach acid production, stimulate mucosal defense system mucus production, and protect surface epithelial cells (Hoppenkamps, R et al., 1984). Several different types of chemicals can harm the digestive tract (Kameswara Rao et al., 2003). Diseases of the digestive tract have been related to lipid peroxidation and oxygen-derived free radicals (Kritikar, K.R et al., 1998). Lesions caused by certain ulcerogens are halted in their development by antioxidants (Loguercio, C et al., 1993). The current investigation was carried out to assess the HETP's anti-ulcer efficacy using a variety of animal models of ulcers.

Significant reductions in prostaglandin synthesis caused by indomethacin correspond with the earliest stages of damage to mucosal, parietal, and endothelial cell membranes. Mucosal lesions caused by indomethacin have been linked to increased production of stomach acid. The HETP significantly reduced the ulcers index and provided substantial protection against stomach ulcers brought on by indomethacin. In addition, HETP was beneficial in preventing histamine-induced ulcers. It is hypothesized that histamine causes stomach ulcers by increasing gastric acid production and vasospastic activity (Goyal, R.K et al., 1999).

Ethanol-induced gastric ulcers are commonly used as a model to gauge the effectiveness of gastroprotective agents. Alcohol reduces blood flow across the gastric mucosa and increases mucus formation in the gastrointestinal lumen. It also increases vascular permeability in the stomach, acid "reverse diffusion," histamine release, sodium and potassium outflow, calcium inflow, free radicals, and leukotrienes. Endogenous glutathione and prostaglandin levels are lowered as well (Mizui, T et al., 1987). Oxygen-derived free radicals have been linked to the development of both acute and chronic ulcers, and neutralizing them helps speed healing. Lipid peroxide levels rise due to ethanol's free radical formation, and glutathione levels fall due to decreased cysteine (Moron, M.A et al., 1979). Rats have a lot of reduced glutathione in their stomach mucosa, and humans do too. (Murakami, S et al. 1991). Mucosal integrity is maintained in part by glutathione, hence its depletion in the stomach mucosa may lead to macroscopic mucosal ulcers (Nadar, T.S et al., 1989).

When ethanol was administered orally, it caused significant damage to the stomach and increased lipid peroxide levels in comparison to the control animals. The stomach mucosa's glutathione levels were low. When compared to untreated rats, HETP was able to dramatically lower high lipid peroxide levels and restore depleted glutathione levels.

HETP's antioxidant-activities help to explain why it has such strong effect on ethanol-induced stomach injury. Several Terminalia species have been credited in the past with having anti-ulcer effects. [Ohkawa, H., et al. 1979 & Rajeshkumar, N.V et al., 2001). Following a phytochemical analysis, it was discovered that this therapeutic plant contained both gallic acid and ellagic acid. (Sarkar, T.T et al. 1976). HETP's TLC and HPTLC phytochemical analysis identified ellagic acid and gallic acid as major components (data not shown). Ellagic acid, a common polyphenol, has potent anti-oxidant effects (Van Kolfschoten et al. 1983). Show a strong inhibitory impact at HCL secretion and development of stress-induced gastric ulcers because it inhibits H+- and K+-ATPase activity. [Murakami, S et al., 1991]

CONCLUSION

The biochemical evaluation results demonstrated a significant antiperoxidative effect. By increasing the stomach mucosa's antioxidant capability, the current study found that HETP considerably decreased mucosal damage.

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None

AUTHOR CONTRIBUTIONS

All the authors contributed equally

COMPETING INTERESTS

The authors have declared that there are no competing interests.

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