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Volume 54, 2016 - [Issue 1](#)



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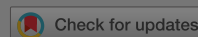
Original Article

# Indomethacin-induced gastric ulceration in rats: Ameliorative roles of *Spondias mombin* and *Ficus exasperata*

Saheed Sabiu ✉, Taofeeq Garuba, Taofik Olatunde Sunmonu, AbdulHakeem O. Sulyman & Nurain O. Ismail

Pages 180-186 | Received 02 Oct 2014, Accepted 10 Mar 2015, Published online: 27 Mar 2015

🗨️ Cite this article 🔗 <https://doi.org/10.3109/13880209.2015.1029050>



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**Results:** The significantly increased ( $p < 0.05$ ) ulcer index, gastric volume, malondialdehyde level, and pepsin activity by indomethacin were effectively reduced by 65.40, 36.47, 45.71, and 53.79%, respectively, following treatment with *F. exasperata* at 200 mg/kg b.w. *S. mombin* at this regimen also attenuated these parameters by 71.70, 46.62, 50.16, and 55.73%. Moreover, the extracts significantly increase the reduced activity of superoxide dismutase as well as pH and mucin content in the ulcerated rats.

Antacid    antioxidative    gastroprotective    NSAIDS    proton pump inhibitor    vagotomy

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simpler to severe side effects, prompting a search for non-toxic, easily accessible, and affordable antiulcer medication (Akah et al., [1998](#); Hawkins & Hanks, [2000](#)). Investigation on the phytotherapy of medicinal plants that are highly valued and widely used in the traditional systems of medicine might provide efficient formulation for better management. *Spondias mombin* (SM) and *Ficus exasperata* (FE) belongs to this class of therapeutic plants.

*Spondias mombin* Linn (Anacardiaceae), commonly known as “Iyeye” in south-western Nigeria, is a fructiferous tree. The plant grows in rain forests and coastal areas, attaining a height of 15–22 m (Ayoka et al., [2008](#)). It is commonly used in folk medicine to cure many diseases due to its potent bioactive principles including tannins, saponins, flavonoids, phenolics, and anthraquinone glycosides (Abo et al., [1999](#)). Antioxidant vitamins;  $\alpha$ -tocopherol, and ascorbic acid have been detected in its leaves extract (Maduka et al., [2014](#)). Tea from its flowers and leaves is taken as an analgesic and anti-inflammatory cure against stomach ache and discomfort (Villegas et al., [1997](#)). Ayoka et al. ([2008](#)) also reported that decoction from its leaves is therapeutic against urethritis, cystitis, as well as eye and throat inflammations. The gum from SM has also been exploited as an expectorant and vermifuge. The leaf extract of the plant has been strongly advocated for use in speedy wound-healing processes, hemorrhoids, and inflamed mucous membrane due to its tannin content (Njoku & Akumefula, [2007](#)). Its pharmacological potencies such as antioxidative, antimicrobial, antimalarial, and antibacterial have been reported (Ogundimu et al., [2004](#); Corthout et al., [2004](#)).

*Ficus exasperata* (Ficus ssp.) is a tree, commonly known as “sand papaya”, respectively. It is widely distributed in the coastal areas of Nigeria. The presence of the plant in the traditional medicine system has been researched. For instance, the infusion of the leaves of *F. exasperata* is used to treat diabetes mellitus, and the leaves are also used to treat stomach ache, and the cooked leaves are used to treat malaria. The leaf extract of the plant is also used as an antidote for snake bites (Ogundimu et al., [2004](#)).



With the remarkable attributes of SM and FE particularly in alleviating stomach ache-related disorders and wound-healing enhancement, the present study compared their therapeutic efficacy on indomethacin-induced gastric ulceration in rats.

## Materials and methods

### Chemicals and drugs

Indomethacin and esomeprazole were, respectively, procured from Kapit Pharmaceutical Limited, Abuja, Nigeria and Ranbaxy Laboratories, Mumbai, India. Trichloroacetic acid (TCA), dimethylaminobenzaldehyde, epinephrine, acetyl acetone, bovine serum albumin (BSA), gallic acid, aluminium chloride, quercetin, and thiobarbituric acid (TBA) were products of Sigma Chemical Co. (St. Louis, MO). Distilled water was obtained from Biochemistry Laboratory, Kwara State University, Malete, Nigeria. Assay kits used were from Randox Laboratories limited, United Kingdom. Other chemicals used were of analytical grade from reputable companies in the world.

### Plant collection and authentication

Fresh leaves of SM and FE were collected in April 2014 following identification of the two plants by Dr. A. A. Abdulrahman of the Department of Biological Sciences (Botany Unit) of the University of Lagos, Lagos State. The plants were identified and authenticated at the University of Lagos Herbarium (voucher number: K 0568) and the specimens were prepared for the study.

### Experiment

Albino rats of Wistar strain, weighing between 150 and 200 g, were used for the study. The rats were housed in a well-ventilated cage and fed with standard rat chow (2014), following the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Lagos. The rats were divided into four groups of equal number (n = 10) and kept in a well-ventilated cage for 7 days before the experiment.

### Preparation

Leaves of SM and FE were washed with distilled water to remove dust and dirt weight. The dried leaves were then ground into a fine powder using a mortar and pestle.



Powdered samples (500 g each) of both plants were separately extracted in 5 L of distilled water for 48 h with continuous shaking by orbital shaker maintained at 300 rpm. The solutions obtained were then filtered (with Whatman No. 1 filter paper) and the resulting filtrates were lyophilized to give 15.5 g (SM) and 12.4 g (FE) residues, corresponding to yields of 3.1 and 2.48%, respectively. The lyophilized samples were separately reconstituted in distilled water to give doses of 100 and 200 mg/kg body weight (b.w.) of each extract used in the study.

## Determination of total phenolics

Following the reported method of Wolfe et al. ([2003](#)), the total phenol contents in the plant extracts were determined. Briefly, an aliquot of each extract (1 mL) was mixed with 5 mL Folin–Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for color development. An absorbance was read at 765 nm using a spectrophotometer (Beckman, DU 7400, Beckman Coulter Inc, Brea, CA). Extracts were evaluated at a final concentration of 1 mg/mL. The total phenolic content was expressed as mg/g gallic acid equivalent using the equation obtained from a calibration curve of gallic acid.

## Determination of total flavonoids

Total flavonoid content was determined using the method of Zhishan et al. (2005). In brief, 0.5 mL of each extract was mixed with 0.5 mL of 10% NaNO<sub>2</sub> solution, 0.5 mL of 10% NaOH solution, and 0.5 mL of 10% AlCl<sub>3</sub> solution. After 1 h at room temperature, the color of the mixture was measured at 415 nm using a spectrophotometer. The color was compared with a standard curve of gallic acid. The total flavonoid content was expressed as mg/g gallic acid equivalent using the equation obtained from the calibration curve of gallic acid.

Ulcer formation was induced in rats by the method of Sayanti et al. (2005). In brief, gastric ulcers were induced in rats by the administration of indomethacin (10 mg/kg body weight) 24 h prior to the administration of the extract. The rats were sacrificed 4 h after the administration of the extract. The stomachs were removed and the ulcers were observed. The ulcer index was calculated as the sum of the ulcer scores in the stomach.

Forty-nine albino rats were randomized into seven groups of seven rats each. Group 1 (normal control) animals received only distilled water. Group 2 (ulcerated control) rats were given only indomethacin and were sacrificed 4 h after indomethacin administration. Animals in group 3 were given indomethacin and esomeprazole (20 mg/kg b.w.). Groups 4, 5, 6, and 7 comprised ulcerated rats treated with FE (100 mg/kg b.w.), FE (200 mg/kg b.w.), SM (100 mg/kg b.w.), and SM (200 mg/kg b.w.). Treatments with the reference drug and extracts commenced 4 h after indomethacin administration and lasted for 21 d. These were orally administered once daily using oral intubator with ad libitum provision of food and water throughout the experimental period.

## Isolation of stomach and collection of gastric juice

On the 22nd day, the animals were humanely sacrificed by cervical dislocation. The abdomen was opened and the stomach was excised. The stomach was thereafter opened along greater curvature and the gastric content was drained into a centrifuge tube. Distilled water (5 mL) was added and the resultant solution was centrifuged at 3000 rpm for 10 min. The supernatant obtained was thereafter used for biochemical analyses.

## Determination of gastric ulceration parameters

Gastric acid output (volume) was determined in the supernatant (2 mL) by titration with 0.0025 N NaOH. The volume of NaOH required for titration was determined. The gastric ulceration parameters were determined as follows: (1) Gastric ulceration index (G.U.I.) and (2) Gastric ulceration score (G.U.S.). The G.U.I. was determined as follows: (1) Gastric ulceration index (G.U.I.) and (2) Gastric ulceration score (G.U.S.). The G.U.S. was determined as follows: (1) Gastric ulceration index (G.U.I.) and (2) Gastric ulceration score (G.U.S.).

## Quantification of gastric ulceration

Degrees of gastric ulceration were scored as follows: (1) Gastric ulceration index (G.U.I.) and (2) Gastric ulceration score (G.U.S.). The G.U.I. was determined as follows: (1) Gastric ulceration index (G.U.I.) and (2) Gastric ulceration score (G.U.S.). The G.U.S. was determined as follows: (1) Gastric ulceration index (G.U.I.) and (2) Gastric ulceration score (G.U.S.).



Table 1. Ulcer scores and descriptive remark.

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## Preparation of stomach homogenate and assay of antioxidant indices

The stomach was homogenized in ice cold 0.1 M phosphate saline buffer (1:4 w/v, pH 7.4) and the homogenate was centrifuged at 2500 rpm for 10 min. The resulting supernatant was thereafter used for assay of antioxidants status.

The activity of superoxide dismutase (SOD) and the level of lipid peroxidation measured in terms of malondialdehyde (MDA) were, respectively, assayed in the stomach homogenate by the methods of Marklund and Marklund ([1974](#)) and Devasagayam and Tarachand ([1987](#)).

## Statistical analysis

Inhibition against ulceration was expressed in percentage. Other results were expressed as a mean of seven determinations  $\pm$  standard error of mean. One-way analysis of variance (ANOVA) complemented with Student's t-test using SPSS software package for windows (Version 16, SPSS Inc., Chicago, IL) for differences between means was used to detect any significant difference ( $p < 0.05$ ) between the treatment groups in this study.

## Results

Quantitative analysis of ulcer scores revealed the presence of ulcer in the control group.

Table 1. Ulcer scores and descriptive remark.

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The effect of the treatment on the inhibition of ulceration was evaluated by the ulcer scores.

mg/kg b.w. of indomethacin caused a significant ( $p < 0.05$ ) increase in the degree of ulceration (ulcer index) in the rats. A significant improvement in the level of inhibition against ulceration was, however, observed following treatment with the extracts. The extracts at 200 mg/kg b.w. offered better protection against ulceration than the 100 mg/kg b.w. regimens and compared well with the standard drug (Esomeprazole) used.

Table 3. Effect of aqueous leaf extracts of *S. mombin* and *F. exasperata* on ulcer indices of indomethacin ulcerated rats ( $n = 7$ ,  $\bar{X} \pm \text{SEM}$ ).

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Table 4 shows the effect of aqueous leaf extracts of SM and FE on gastric secretions of indomethacin ulcerated rats. Indomethacin administration caused significant ( $p < 0.05$ ) decrease in the pH value with a corresponding significant ( $p < 0.05$ ) increase in gastric volume of gastric content. Treatment with the extracts produced significant increase in the pH value coupled with significant decrease in gastric volume when compared with ulcerated control rats.

Table 4. Effects of aqueous leaf extracts of *S. mombin* and *F. exasperata* on gastric volume and pH of indomethacin ulcerated rats (

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Observable from [Figures 1 and 2](#) are the effects of aqueous leaf extracts of SM and FE on the lipid peroxidation and SOD activity of gastric mucosal of indomethacin-ulcerated rats. The MDA level was significantly increased ( $p < 0.05$ ) in the ulcerated animals ( [Figure 1](#)). A significant reduction ( $p < 0.05$ ) was also observed in the activity of SOD ( [Figure 2](#)) in the indomethacin-induced animals. Commendably, both extracts particularly at 200 mg/kg b.w. regimen attenuated these parameters and the observable effects compared favorably well with both normal control and standard drug employed in the study.

Figure 1. Effect of aqueous leaf extracts of *S. mombin* and *F. exasperata* on gastric Malondialdehyde (MDA) level of indomethacin ulcerated rats ( $n = 7$ ,  $\bar{X} \pm \text{SEM}$ ).  
<sup>a</sup>Significantly different from the normal control group ( $p < 0.05$ ). <sup>b</sup>Significantly different from the indomethacin-ulcerated control group ( $p < 0.05$ ). IND, indomethacin (30 mg/kg b.w.); ESP, esomeprazole (20 mg/kg b.w.); F.E1, *Ficus exasperata* (100 mg/kg b.w.); F.E2, *Ficus exasperata* (200 mg/kg b.w.); S.M1, *Spondias mombin* (100 mg/kg b.w.); S.M2, *Spondias mombin* (200 mg/kg b.w.).

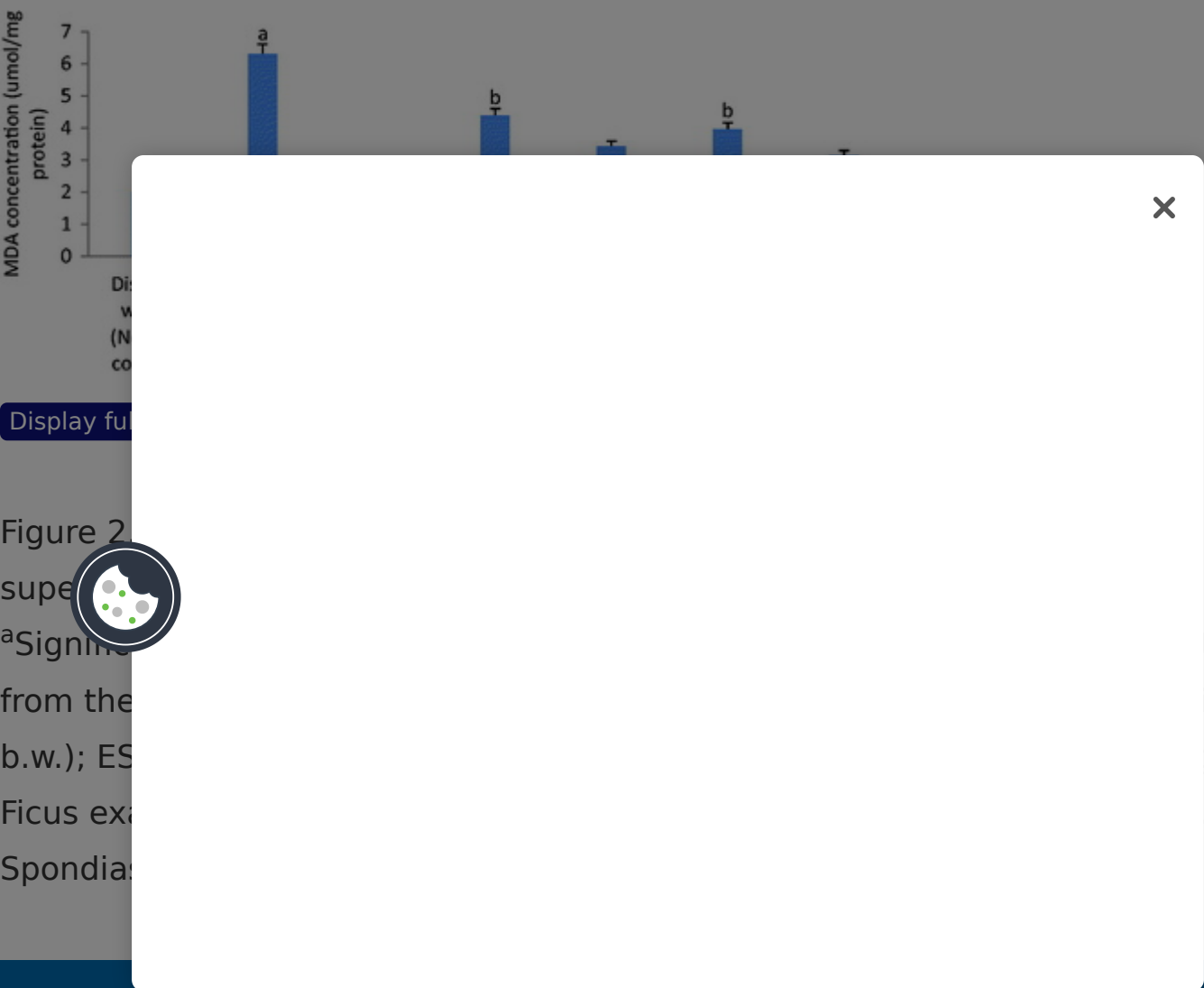
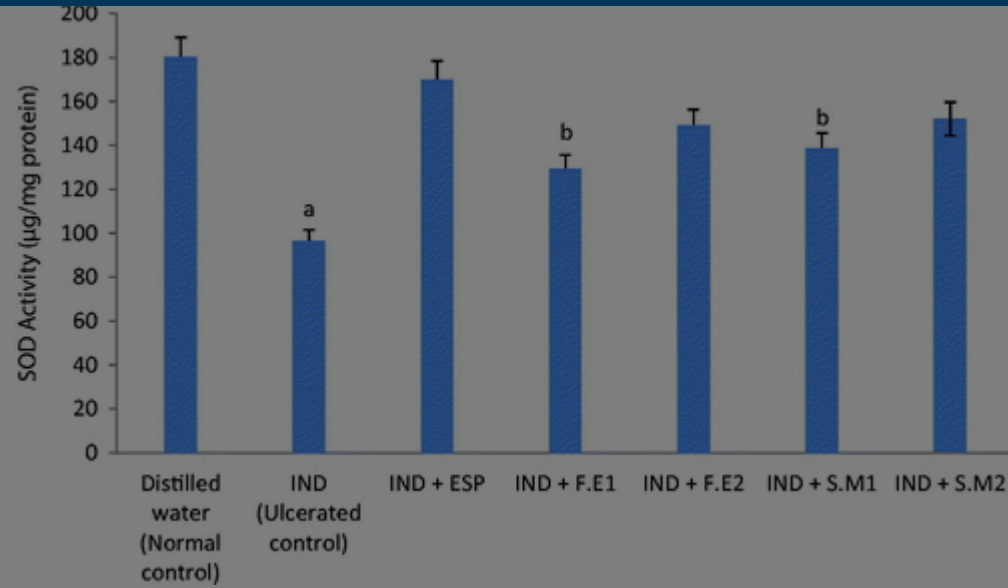


Figure 2. Effect of aqueous leaf extracts of *S. mombin* and *F. exasperata* on gastric superoxide dismutase (SOD) activity of indomethacin ulcerated rats ( $n = 7$ ,  $\bar{X} \pm \text{SEM}$ ).  
<sup>a</sup>Significantly different from the normal control group ( $p < 0.05$ ). <sup>b</sup>Significantly different from the indomethacin-ulcerated control group ( $p < 0.05$ ). IND, indomethacin (30 mg/kg b.w.); ESP, esomeprazole (20 mg/kg b.w.); F.E1, *Ficus exasperata* (100 mg/kg b.w.); F.E2, *Ficus exasperata* (200 mg/kg b.w.); S.M1, *Spondias mombin* (100 mg/kg b.w.); S.M2, *Spondias mombin* (200 mg/kg b.w.).



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## Discussion

Inhibitory action of indomethacin on prostaglandin synthesis coupled with free radicals formation has been opined as critical biochemical events in the pathogenesis of gastric ulceration (Ajani et al., [2014](#); Hong et al., [2014](#); Inas et al., [2011](#); Lichtenberger, [2005](#)).

An understanding of these events might be of utmost relevance in designing new antiulcer drugs. With the inherent adverse side effects and considerably high cost of synthetic non-toxic antiulcer drugs, the search for natural products has been intensified. Phytotherapy has been opined as a promising approach in the management of gastric ulceration (Ajani et al., [2014](#); Rajkumar et al., [2014](#)).

Phytotherapy has been opined as a promising approach in the management of gastric ulceration (Ajani et al., [2014](#); Rajkumar et al., [2014](#)). The present study was designed to evaluate the antiulcer activity of the leaf extracts of *S. mombin* (IND + S.M1 and IND + S.M2) and *F. esculentum* (IND + F.E1 and IND + F.E2) against the ulcer induced by indomethacin (IND) in rats. The results of the present study showed that the leaf extracts of *S. mombin* and *F. esculentum* significantly reduced the ulcer index and increased the SOD activity in the gastric mucosa of rats. The results of the present study are in line with the findings of Ajani et al. (2014) who reported that the leaf extracts of *S. mombin* and *F. esculentum* significantly reduced the ulcer index and increased the SOD activity in the gastric mucosa of rats. The results of the present study are also in line with the findings of Rajkumar et al. (2014) who reported that the leaf extracts of *S. mombin* and *F. esculentum* significantly reduced the ulcer index and increased the SOD activity in the gastric mucosa of rats. The results of the present study are also in line with the findings of Inas et al. (2011) who reported that the leaf extracts of *S. mombin* and *F. esculentum* significantly reduced the ulcer index and increased the SOD activity in the gastric mucosa of rats. The results of the present study are also in line with the findings of Lichtenberger (2005) who reported that the leaf extracts of *S. mombin* and *F. esculentum* significantly reduced the ulcer index and increased the SOD activity in the gastric mucosa of rats.


Biochemical analysis of gastric secretions (for pH, gastric volume, bicarbonate, and pepsin) and mucosal integrity for stomach is usually employed to ascertain its status following exposure to pharmacological agents (Biplab et al., [2011](#)). The pH gives an idea of the level of acidity and volume of gastric secretions. Low pH value is a manifestation of decreased hydrogen ion concentration in gastric juice. This has been linked to pathogenesis of ulcer and gastric damage in experimental animals (Lüllmann et al., [2000](#)). Inas et al. ([2011](#)) have also attributed gastrointestinal injury to eroded mucin content. This erosion is facilitated by onslaughts of both internal (pepsin and oxidants produced in the gastric lumen) and external (drugs and chemicals) aggressive agents on mucosal epithelia.

In the present study, the significant increase in ulcer index and gastric volume following oral administration of indomethacin in the ulcerated rats may be attributed to either free radicals formation or inhibition of prostaglandin synthesis. Decreased prostaglandin level has been attributed to impaired gastroprotection and increased gastric secretion which are important events in the etiology of mucosal ulceration. This agrees with the reports of Bech et al. ([2000](#)), Biplab et al. ([2011](#)), and Muhammed et al. ([2012](#)) where indomethacin was reported to have caused alterations in gastric secretions of rats. Conversely, treatments with the two extracts significantly reduced these parameters. In fact, the effects noticed for pH compared favorably well with both normal control and standard drug used in this study and indeed suggestive of their possible

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Tissues are in a stable state if the rates of free radical formation and scavenging capacity are essentially constant and in equilibrium. However, an imbalance between them results in oxidative stress which further deregulates cellular functions leading to different pathological conditions (Sabiou et al., [2014](#)). In the present study, the increased concentration of MDA as well as reduced activity of SOD in the stomach of indomethacin-ulcerated rats is a manifestation of facilitated lipid peroxidation and overproduction of free radicals resulting in mucosal damage. Free radicals deplete antioxidant enzyme activities and initiate lipid peroxidation which is an important event in the toxicity mechanism of indomethacin (Halici et al., [2005](#)). Indomethacin has previously been reported to decrease antioxidant enzymes (SOD, CAT, and GST) activity in rat stomach thereby inducing gastric ulceration (Odabasoglu et al., [2006](#)). This is associated with overpowering of the cellular antioxidant defense systems by free radicals



by modulating cells in the mucosal lining of the stomach against excess acid secretion (Fornai et al., [2011](#); Tulassay et al., [2008](#)).

## Conclusion

Overall, the attenuation of gastric affronts of indomethacin by administration of aqueous leaf extracts of SM and FE at 200 mg/kg b.w. regimen is indicative of their excellent gastroprotective and antioxidative potentials in rats. Efforts are ongoing to investigate the exact antiulcerogenic principle(s) in these extracts and also harness their possible synergistic efficacy against gastric ulcer.

## Declaration of interest

The authors report that they have no conflicts of interest.

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