



Ethanolic leaf extract of *Langenaria breviflora* (bitter gourd) inhibits gastric onslaught in indomethacin-induced ulcerated rats.

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ABSTRACT

Objective: Gastrointestinal toxicity remains a barrier to applications of non-steroidal anti-inflammatory drugs in medical practice. Plant extract with excellent therapeutic potential may proffer solution to this menace. This study investigated the gastroprotective effect of *Lagenaria breviflora* leaves extract against indomethacin-mediated gastric ulcer in rats.

Methodology and result: Ulceration in the rat was induced by a single oral dose of indomethacin (60 mg/kg body weight). Ulcerated rats were orally administered with *Lagenaria breviflora* extract at 200 mg/kg body weight once daily for 21 days prior to and after ulcer induction. Gastric secretions and antioxidant parameters were then evaluated. The study indicates that indomethacin caused a significant increase in ulcer index, gastric acidity, malondialdehyde level and pepsin activity. Administration of *Lagenaria breviflora* in rats reversed these metabolic alterations. The extract also attenuated the reduced activity of catalase, pH and mucin content in the ulcerated rats.

Conclusions and application of findings: These findings are indicative of gastroprotective and antioxidative attributes of the extract, which is revealed in the percentage protection offered against ulceration. The supportive evidences in this study suggest that the effect of leaves extract of *Lagenaria breviflora* proved to be capable of modulating indomethacin-mediated gastric ulceration and could be harnessed as preventive therapy in the treatment of gastric ulcer related disorder.

Key words: Gastroprotective; H₂ receptor; Medicinal; NSAIDS; Proton pump inhibitor; Ulceration.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) like acetaminophen, ibuprofen, aspirin and indomethacin are effective agents for a variety of illnesses ranging from rheumatic, musculoskeletal to cardiovascular (Dae et al., 2014). They have shown proven benefits

as pain and anti-inflammatory drugs. Despite these therapeutic benefits, occurrence of gastrointestinal toxicity has been a factor militating against their applications in clinical practice (Hawkey, 1990). The use of NSAIDs accounts for approximately 25% of

gastric ulcer cases and has been identified as a major medical problem, ranking fourth in causing morbidity and mortality in the world (Halter *et al.*, 2001). Pronounced side effects, resistance and high cost of synthetic drugs are common limitations against their usage by considerable percentage of the world population (Hawkins and Hanks, 2000). In fact, an estimated 80% of the populations in developing nations rely on traditional systems of medicine (WHO, 2000). Little wonder, researches in recent times have accumulated evidences advocating parallel shift to therapeutic intervention of

medicinal plants to prevent and treat array of diseases including gastric ulcer. Such plants have excellent attributes ranging from non-toxicity, efficacious, easy accessibility to affordability (Edeoga *et al.*, 2005). The present study tested the healing effect of ethanolic leaf extract of *Lagenaria breviflora* on indomethacin-mediated gastric ulceration in rats.

Lagenaria breviflora, popularly called 'Tagiri' in the south-western part of Nigeria, is an important plant belonging to the family Cucurbitaceae (Yasuyuki, *et al.*, 2005).



Figure 1: *Lagenaria breviflora*

It is a perennial climber and occurs across Senegal, West Cameroon and the entire tropical African region (Oridupa *et al.*, 2011). The potency of its fruits against a wide range of gastrointestinal disorders and measles in experimental animal models has been documented in West Africa (Sonaiya, 1999). Ajayi *et al.* (2002) has also reported decoction from its stem to be effective as vermifuge and provide relief against stomach discomfort and headache. Its

broad spectrum antibacterial activity has also been reported (Tomori *et al.*, 2007). Phytochemical analysis of its whole fruit revealed the presence of saponins, phenolic acids (Elujoba *et al.*, 1991) and cucurbitacins (Miro, 1995; Wakimoto, 2008). Quite a number of cucurbitacins have been investigated for their cytotoxic (Seeram *et al.*, 2007), anti-inflammatory (Escandell *et al.*, 2007) and gastroprotective attributes (Miro, 1995).

MATERIALS AND METHODS

Plant collection and authentication: Fresh whole plant of *Lagenaria breviflora* comprising the leaves, fruits and roots were collected from farms in and around Oke Oyi, Ilorin, Kwara State, Nigeria. The plant was authenticated

at the Herbarium of Kwara State University, Malete, Nigeria where a voucher specimen was thereafter prepared and deposited.

Experimental animals: Male Wistar strain albino rats with a mean weight of 120.00 ± 2.33 g were purchased from Central Animal House of University of Ilorin, Nigeria. They were kept in cages in a well-ventilated room maintained at a temperature of $26 \pm 2^\circ\text{C}$ with a 12-hours light-dark cycle for 10 days to acclimatize, and were allowed free access to food and water *ad libitum*. The protocol conforms to the guidelines of the National Institute of Health for laboratory animal care and use (NIH, 1985), and in accordance with the principles of good laboratory procedure (WHO, 1998).

Preparation of ethanolic extracts: *L. breviflora* leaves were air-dried at room temperature for 10 days to constant weight. The dried samples were then pulverized with an electric blender (model MS-223; Blender/Miller III, Taiwan, China), weighed and kept airtight prior to extraction. The powdered sample (500 g) was extracted in 4 litres of 70% ethanol for 24 hrs with continuous shaking by orbital shaker maintained at 300 rpm. This was then filtered with Whatman No. 1 filter paper and the resulting filtrate lyophilized to give 12.0 g of the residue, corresponding to a yield of 2.4%.

Induction gastric ulceration: Gastric ulceration was induced in the rats by administering a single oral dose of indomethacin (60 mg/kg body weight, oral intubation) dissolved in distilled water. Rats were deprived of food 24 hours and water 2 hours before ulcer induction (Okokon et al., 2010, Clayton et al., 2006).

Animal grouping and treatments: Thirty five albino rats were randomized into five groups of seven rats each and were treated as:

Groups Treatment modes

- 1 Distilled water administered (normal control).
- 2 Indomethacin administered (ulcerated control).
- 3 Pre-treatment with *L. breviflora* extract followed by indomethacin administration.
- 4 Indomethacin administered and allowed for self recovery.
- 5 Indomethacin administered followed by treatment with *L. breviflora* extract.

Group 1 rats served as normal control and received only normal saline. Groups 2-5 comprised indomethacin ulcerated animals. Rats in group 2 served as ulcerated control and received only indomethacin while those in group 3 were pre-treated with therapeutic dose of *L. breviflora* leaf extract (200 mg/kg b.w) for 21 days prior to ulceration. Four hours after indomethacin administration, rats in groups 1-3 were sacrificed. Rats in groups 4 were

left to allow for self-recovery while those in group 5 were treated with 200 mg/kg b.w dose of *L. breviflora* extract once daily for 21 days. Administration of the extract was made to commenced 4 h after indomethacin administration. On the twenty second day, rats in groups 4 and 5 were sacrificed. All administrations were done orally with metal oropharyngeal cannula.

Stomach excision and collection of gastric juice: At the end of the experimental periods, the animals were humanely sacrificed by diethyl ether anaesthetization. The abdomen was opened and the stomach excised. The stomach was thereafter opened along greater curvature and gastric content was drained into a centrifuge tube. Five (5) ml of distilled water was added and the resultant solution was centrifuged at 3,000 rpm for 10 minutes. The supernatant obtained was thereafter used for biochemical analyses.

Determination of gastric ulceration parameters: Gastric acid output (volume) was determined in the supernatant by titration with 0.0025N NaOH. Free and total acidity were subsequently determined adopting the method of Grossman (1963). The pH of gastric juice was determined using a pH meter, while the procedures of Sanyal et al (1971) and Corne et al (1974) were used to determine specific pepsin activity and mucin concentration respectively.

Quantification of ulceration: Degrees of ulceration in the animals were quantified using the procedure outlined by Szabo et al (1985). Briefly, Cleaned stomachs were pinned on a corkboard and ulcers were scored using dissecting microscope with square-grid eyepiece based on grading on a 0-3 scale (depicting severity of hyperamia and hemorrhagic erosions) as follows:

- 0.0—almost normal mucosa
- 0.5—hyperemia
- 1.0—one or two lesions
- 1.5—severe lesions
- 2.0—very severe lesions
- 3.0—mucosa full of lesions

*Hyperamia: vascular congestions, Lesions: hemorrhagic erosions.

Areas of mucosal damage were expressed as a percentage of the total surface area of the glandular stomach estimated in square millimetres Mean ulcer score for each animal was expressed as ulcer index (U.I) and the percentage protection against ulceration was determined using the expressions:

$$\text{U.I} = [\text{Ulcerated Area/Total stomach area}] \times 100.$$

Ajani et al. J. Appl. Biosci. Ethanolic leaf extract of *Lagenaria breviflora* (bitter gourd) inhibits gastric onslaught in indomethacin-induced ulcerated rats.

% Ulcer Protection = $[U.I \text{ in control} - U.I \text{ in test}] \times 100 / U.I \text{ in control}$.

Preparation of stomach homogenate and assay of antioxidant parameters: The stomach was homogenized in ice cold 0.1 M phosphate saline buffer (1:4 w/v, pH 7.4) and the homogenate centrifuged at 2500 rpm for 10 min. The resulting supernatant was thereafter used for assay of lipid peroxidation (Devasagayam and Tarachand, 1987) in terms of malondialdehyde (MDA)

and catalase (CAT) activity (Sinha, 1972) in the stomach homogenate.

Statistical analysis: Level of protection against ulceration was expressed in percentage. Other results were expressed as mean of seven determinations \pm standard error of mean. Analysis of variance (ANOVA) using SPSS software package for windows (Version 16) for differences between means was used to detect any significant differences ($p < 0.05$) between the treatment groups in this study.

RESULTS

The effect of ethanolic leaf extract of *L. breviflora* on ulcer index and percentage protection offered against indomethacin ulceration in rats is shown in Table 1. Indomethacin administration predisposes rats to increased ulcer index corresponding to reduced protection against ulceration. The extract treated groups

had significant improvement ($p < 0.05$) for these parameters. The effect was more pronounced in the extract pre-treated groups with a 76.38% protection against ulceration compared to 17.92% and 49.77% respectively observed in the self-recovery and extract post-treated groups.

Table 1: Effect of *Lagenaria breviflora* leaves extract on ulcer indices of indomethacin ulcerated rats (n=7, $X \pm SEM$)

Group	Treatments	Ulcer index	% Protective index
1	Distilled water (Normal control)	00.00	-
2	IND (Ulcerated control)	10.71 \pm 0.30 ^a	-
3	LB + IND (Pre-treated)	2.53 \pm 0.20 ^b	76.38
4	IND (Self- recovery)	8.79 \pm 0.20 ^a	17.92
5	IND + LB (Post- treated)	5.38 \pm 0.10 ^c	49.77

Values with different superscripts along the same column for the parameters are significantly different ($p < 0.05$)

IND: Indomethacin (60 mg/kg b.w.), LB: *Lagenaria breviflora*

Table 2 depicts the effect of the extract on gastric secretion parameters of indomethacin-ulcerated rats. Indomethacin administration caused a significant elevation ($p < 0.05$) in free and total acidity and marked reduction ($p < 0.05$) in gastric volume and pH. Except for

gastric volume and free acidity for the extract post-treated groups, alterations were normalized for all the parameters in both extract treated instances compared with control. This was not the case for the self-recovery group.

Table 2: Effect of leaves extract of *Lagenaria breviflora* on gastric secretion indices of indomethacin ulcerated rats (n = 7, $X \pm SEM$)

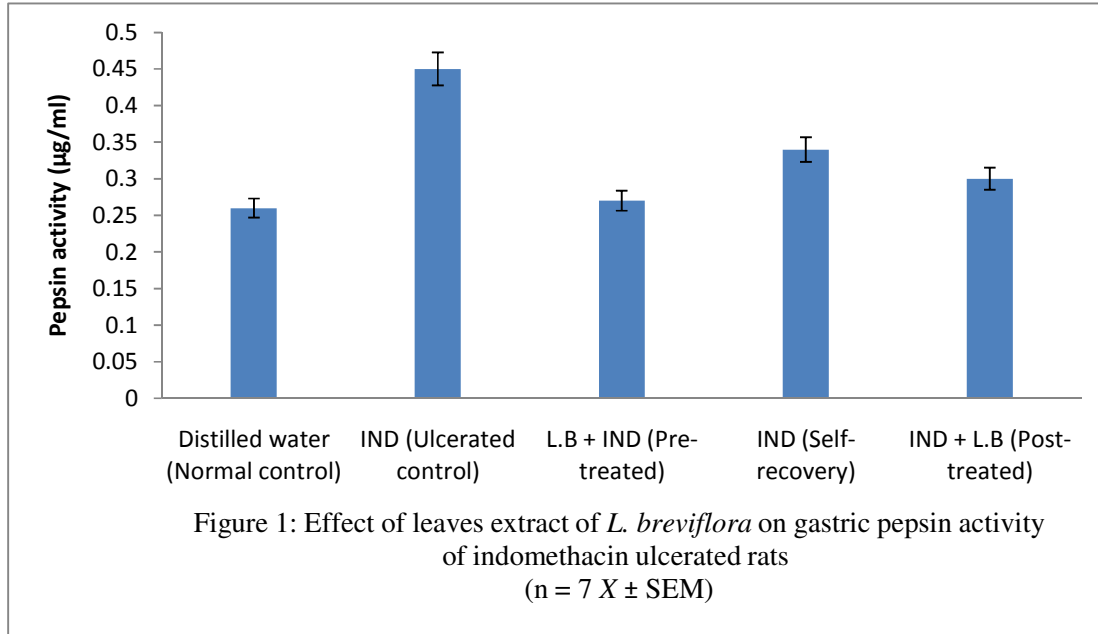
Treatments	pH	Gastric volume (ml)	Free acidity (mEq/L)	Total acidity (mEq/L)
Distilled water (Normal control)	4.16 \pm 0.19 ^a	2.00 \pm 0.09 ^a	4.80 \pm 0.30 ^a	15.70 \pm 0.40 ^a
IND (Ulcerated control)	2.59 \pm 0.11 ^b	5.83 \pm 0.15 ^b	9.00 \pm 0.90 ^b	20.20 \pm 0.90 ^b
LB + IND (Pre-treated)	3.27 \pm 0.10 ^a	2.54 \pm 0.06 ^a	5.50 \pm 0.80 ^a	16.30 \pm 0.20 ^a
IND (Self- recovery)	2.65 \pm 0.11 ^b	5.20 \pm 0.00 ^b	7.30 \pm 0.70 ^b	20.00 \pm 1.40 ^b
IND + LB (Post- treated)	2.99 \pm 0.05 ^c	2.77 \pm 0.26 ^a	5.00 \pm 0.70 ^a	18.00 \pm 0.40 ^c

Values with different superscripts along the same column for the parameters are significantly different ($P < 0.05$). IND: indomethacin (60 mg/kg b.w.), L.B: *Lagenaria breviflora* (200 mg/kg b.w.).

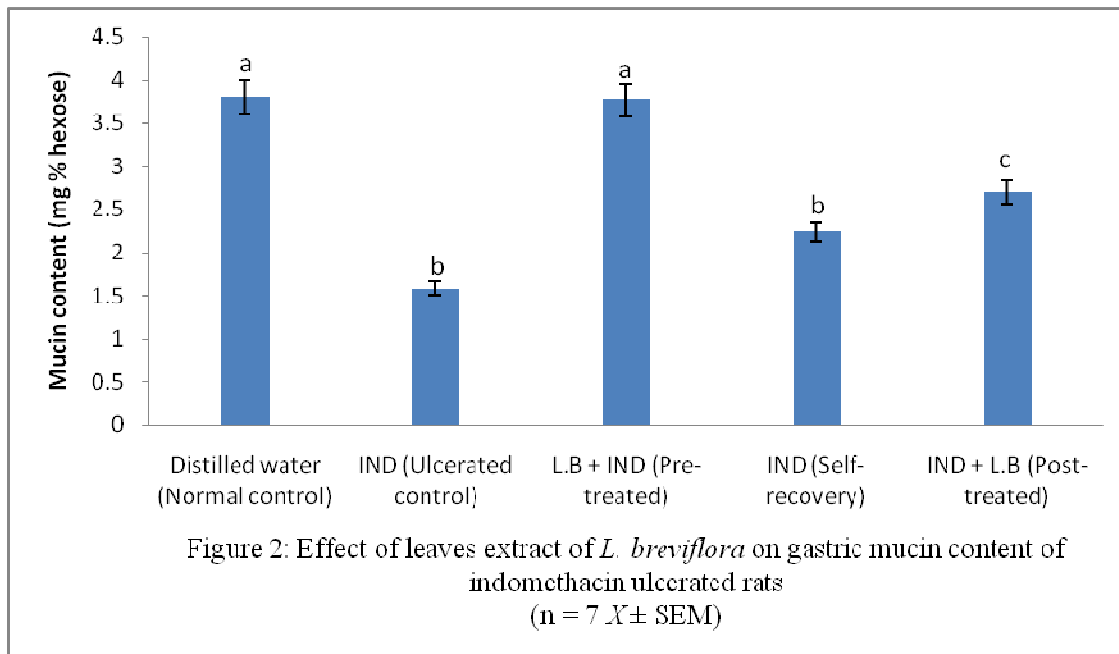
Ajani et al. J. Appl. Biosci. Ethanolic leaf extract of *Langenaria breviflora* (bitter gourd) inhibits gastric onslaught in indomethacin-induced ulcerated rats.

Observable from Figures 1-3 respectively is the effect of the extract on pepsin activity, mucin contents and MDA level and CAT activity of indomethacin-ulcerated rats. The extract treated groups had significant decrease ($p < 0.05$) in pepsin activity and MDA level coupled with significantly

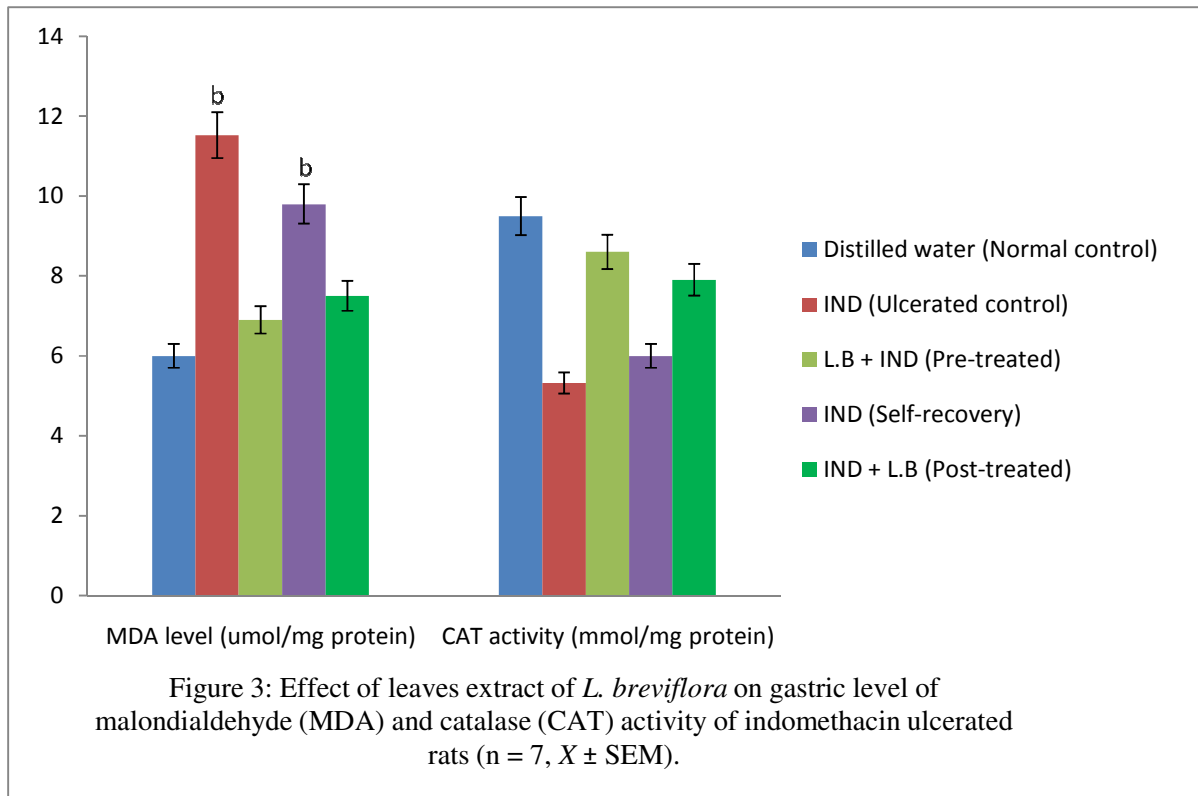
elevated ($p < 0.05$) mucin content and CAT activity when compared with both self-recovery and ulcerated control groups. The effects elicited in the extract treated groups were comparable to normal.



Bars with different superscripts for the parameters are significantly different ($p < 0.05$), IND: Indomethacin (60 mg/kg b.w). LB: *L. breviflora* (200 mg/kg b.w)



Bars with different superscripts for the parameters are significantly different ($p < 0.05$), IND: Indomethacin (60 mg/kg b.w). LB: *L. breviflora* (200 mg/kg b.w)



Bars with different superscripts for the parameters are significantly different ($p < 0.05$), IND: Indomethacin (60 mg/kg b.w). LB: *L. breviflora* (200 mg/kg b.w)

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. This irritates the mucosa lining of the stomach, decreasing hydrophobicity of mucus gel layer, which causes elevated gastric acid secretion, reduced mucosal blood flow and induced bicarbonate secretion (Feldman et al 1992; Bandyopadhyay et al., 2000; Lichtenberger, 2005; Wallace, 2008). Disequilibrium in secretions, depletion or damage and altered permeability of gastric mucosa and elevated pepsin activity has also been attributed to gastrointestinal toxicity of indomethacin (Salim, 1990). Despite the rapidly changing concept of gastric ulcer management from conventional anticholinergic drugs, histamine H_2 receptor antagonists and antacids to proton pump inhibitors, gastrointestinal toxicity remains an impediment to their application in the medical world (Hawkey, 1990). Researchers in recent times are now paying considerable attention to plants based products that possess phytonutrients with excellent

antioxidant properties and play a significant role in managing toxicity related disorders. Interestingly, our preliminary finding on the phytochemical constituents of leaf extract of *L. breviflora* revealed the presence of flavonoids, tannins, terpenoids, saponnins and phenolics (Ajani et al., 2014). These bioactive principles have been reported to sustain, promote good health and exhibit antioxidative and gastroprotective potentials (Akah et al., 2008; Ayoka et al., 2008; Okarter et al., 2009; Onasanwo et al., 2011). Thus, the present study examined the possible gastroprotective and antioxidative effects of the extract on indomethacin ulcerated rats.

Analysis of biochemical indices (gastric volume, pH, pepsin activity, bicarbonate and mucus level) for stomach is often used to find out its integrity following exposure to pharmacological agents (Raji et al., 2011). Low gastric pH value is a manifestation of increased hydrogen ion concentration (back diffusion of hydrogen ions) in gastric juice and has been linked to genesis of ulcer and gastric damage in experimental animals (Lüllmann et al., 2000).

In this study, the significantly increased ulcer index, gastric juice acidity and reduced pH 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 is associated with impaired gastroprotection and implicated in increased gastric secretion that is an important event in the etiology of mucosal ulceration. This agrees with the report of Bech et al (2000) where indomethacin was reported to have caused alterations in gastric secretions of rats. Conversely, treatments with the extract significantly attenuated these alterations and thus suggestive of its probable gastroprotective potential.

A combination of complex events including release of preformed mucus, wound retraction and re-epithelialization is involved in ulcer-healing process after toxicological injury (Szabo, 1985; Naito et al., 1995). Besides providing significant buffering capacity for the neutralization of luminal acid, the mucus also offers protection against both endogenous aggressors (pepsin and oxidants produced in the gastric lumen) and exogenous gastro-toxic agents, such as indomethacin, enhancing the rate of the local healing process (Alanko et al., 1999). In the present study, the increased pepsin activity coupled with decrease in mucin secretion in the indomethacin-ulcerated rats showed an altered hydrophobicity and reduced ability of the mucosal membrane to protect the mucosa lining against hemorrhagic erosion of ulceration, thus, resulting in tissue damage. This further proved the decreased ability of the gastric mucosa to withstand the offensive onslaught of indomethacin. Besides antioxidant action that protects the mucus layer and arrests ulcer progression, drugs that increase the synthesis and secretion of gastric mucus would accelerate gastric ulcer healing. Treatment with the extract however, speed up the ulcer healing process, which is associated with decreased pepsin activity and elevated mucin level in the gastric mucosa. This indicates enhanced mucus modulation by the extract and suggestive of its significant role in ulcer healing process. Healing of mucosa epithelia cells was prominently displayed by the extract pre-treated animals than those of post-treated group, depicting a better ulcer healing capacity and compared favourably well with normal control. A disequilibrium between free radicals and antioxidant status in animals result in oxidative stress which further deregulates cellular functions leading to various pathological conditions (Sabiu et al., 2014). Gastric mucosal damage has been attributed to free radicals generation by indomethacin administration (Wada et al., 1997; Hong et al., 2014). In the present

study, the increased concentration of MDA and reduced activity of CAT in the stomach of indomethacin-ulcerated rats is an obvious reflection of enhanced lipid peroxidation and excessive free radicals formation resulting in tissue injury. Reactive oxygen species reduces activity of antioxidant enzymes and initiates lipid peroxidation which is an important event in the toxicity mechanism of indomethacin (Dae et al., 2014). However, the significantly reduced concentration of MDA and elevated activity of the CAT following treatment with the extract is a pointer to its antiperoxidative attribute and thus antioxidative potential. Chen et al. (1998), Halici, et al. (2005), Odabasoglu et al (2006) and Sayanti et al. (2007) have also reported enhancement of gastric mucosa integrity and speedy ulcer healing ability by CAT and reduced glutathione (GSH) through increased prostaglandin synthesis and reduction in MDA level.

The therapeutic efficacy of leaf extract of *L. breviflora* displayed in this study may be attributed to its excellent mucus secretory potential which might have facilitated increased mucin content. This in turn has encouraged speedy wound healing of the ulcerated areas of the mucosal epithelia and shielded the gastrointestinal membrane, thus abrogating the deleterious influence of indomethacin in the ulcerated rats. An increase in mucus production has been opined to protect ulcer crater against irritating stomach secretions (HCl and pepsin) thereby enhancing the rate of local healing process (Naito et al., 1995). Studies have also shown that *L. breviflora* is rich in antioxidants and phytochemicals which promote good health (Elujoba et al., 1991; Onasanwo et al., 2011, Ajani et al., 2014). Hence, the protection offered by the extract against indomethacin-induced gastric ulceration may be linked to its beneficial medicinal attributes occasioned by phytochemical constituents. These include ability to scavenge free radicals and regulate mucosal membrane permeability thereby countering the effect of indomethacin on gastric acid secretion. This is in agreement with the submissions of Inas et al (2011), Muhammed et al (2012) and Gege-Adebayo et al (2013), where gastroprotective potentials of plant extracts against indomethacin ulcerated rats were associated with their various phytonutrients. Overall, research is currently ongoing to unravel the exact mechanism of anti-ulcerative action displayed by the extract in this study. It could perhaps, have antagonized histamine binding to H₂ receptor on the parietal cells or inhibited proton pump, thereby countering indomethacin effect on acid secretion and enhance healing process. The inherent gastroprotective and antioxidative attributes of *L.*

breviflora leaf extract against indomethacin ulceration is recommendable as preventive therapy in the

management of gastric ulcer cases.

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