Anti-ulcerogenic effect of methanol fraction of Ocimum gratissimum leaves extract and honey on indomethacin-induced gastric ulcer in rats

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\textbf{ABSTRACT}

\textit{Ocimum gratissimum} (Og), a medicinal plant, and honey (H), a natural sweetener usually produced by bees, have historic importance in managing several ailments. This study aims at unraveling the anti-ulcerogenic effect of methanol leaves fraction (MEF) of Og and H on indomethacin-induced gastric ulcer in male rats. Crude methanol extract was partitioned between hexane, chloroform, ethylacetate and methanol successively using vacuum liquid chromatography. The anti-ulcerogenic effect of MEF of Og (100 and 400 mg/kg), honey (2.5 g/kg) and their combination was determined using indomethacin-induced gastric ulcer model. Gastric acidity, antioxidant assays and histopathology were conducted via titrimetric, spectrophotometric, H&E stain with microscopy methods respectively. Significant difference ($P < 0.05$) existed in ulcer score and gastric pH in all groups except MEF pre-treated relative to control. Significant increase and decrease existed in MDA level in untreated and pre-treated groups successively compared to control. CAT activity displayed no significant difference in 400 mg/kg MEF and 400 mg/kg/2.5 g/kgH proportionate to control. GST activity showed significant elevation in pre-treated groups compared to control. Meanwhile, GPx activity increased in 400 mg/kg MEF and 400 mg/kgMEF/2.5 g/kgH relative to control. There was no significant difference in TSH status in the pre-treated groups relative to control. Mild inflammatory cellular infiltration in the sub-mucosal layers in pre-treated groups occurred. The data suggests MEF effectively prevented ulcer better than fortification with honey and honey used singly.

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Introduction

Peptic ulcer is an injury found in the gastrointestinal tract as a result of the corrosion of the mucosal lining in response to the influence of excessive acidity, *Helicobacter pylori*, reduced bicarbonate reserve, prostaglandin synthesis, and even compromise in the microflora of the intestine [1]. This disease occurs from the compromise in the defense mechanisms of this tract [1]. It can also ensue from the cleavage activity of pepsin on the stomach, thus exerting its function on gastric and duodenum domains elongating via muscularis mucosae. This position of effect determines the names it is called, either duodenal or gastric ulceration [2].

The disease could be associated with mucosal damage caused by hyper-secretion of acid from the parietal cell of the intestinal tracts as well as environment-induced, lifestyle, diet and stress – either physiological or physical. Other causal factors of peptic ulcer are *H. pylori*, cigarettes, alcohol consumption, the use of non-steroidal anti-inflammatory drugs (NSAIDs) and Zollinger–Ellison syndrome [3]. However, only few people exposed to the major causative agents develop ulcer, partly because of predisposition or proneness as a result of genetic or environmental factors. Genetic polymorphisms in various inflammatory cytokine genes are related to peptic ulcer. For instance, IL-1β polymorphisms could influence the mucosal synthesis of the cytokine, which could play role in pathogenesis of *H. pylori*-induced gastroduodenal ulceration [4]. An understanding of these events might be of utmost relevance in designing new antiulcer drugs.

With the inherent adverse effects and considerably high cost of synthetic drugs, exploiting natural products of plant source which are believed to be nontoxic, efficacious, and affordable will be most appropriate in the treatment of gastric ulcer and other toxicity-related disorders (Table 1) [5]. Phytotherapy is rapidly gaining grounds in sustaining human health and in the prevention of certain diseases like gastric ulcer as a result of drug toxicity that could stem from the use of orthodox medication [6,7].

*Ocimum gratissimum* (Og) (Scent leaf), a perennial plant, is vastly scattered in the tropics of Africa and Asia. It belongs to the family *Labiatae* and it is the most abundant of the genus *Ocimum*. In the Southern part of Nigeria, it is called ‘Efirin nla’ by Yoruba tribe, ‘Nchanwu’ in Igbo land, and ‘Daidoya’ in the Northern side of the country [8]. This plant and its other species are known for their folkloric use in the traditional medicine for the treatment or management of numerous ailments because of the presence of bioactive agents which have physiological effect on biological system [9,10]. It has been discovered traditionally to be effective in the treatment of pile, diarrhea, dysentery, fever, malaria, diabetes mellitus and even epilepsy [11]. The phytochemicals present in this plant are tannins, saponins, alkaloids, glycosides, phenols and flavonoids. These active principles exert immune-defensive effects on diseases in the body. It can also be used as condiment for preparation of delicacies for human consumption [11,12].

The *in-vitro* antimicrobial screening of *O. gratissimum* revealed that it has potent bacteriocidal efficacy on *Staphylococcus aureus*, *E. coli*, *Streptococcus fecalis*, *Psudomonas aeruginosa* and *Lactobacilli* [13]. Other reports displayed that utilization of natural products is safer with less side effect compared to the synthetic substances [14].

Honey is a natural sweetener widely available across the world, extensively consumed and utilized for difference purposes most importantly medical [15]. Honey is a viscous solution containing approximately 200 distinct chemical compounds, such as fructose and

### Table 1. Ulcer scoring method.

| ULCER SCORE | CRITERIA |
|-------------|----------|
| 0           | Normal stomach/no ulcer. |
| 0.5         | Punctuate or pinpoint ulcers. |
| 1.0         | Two or more small hemorrhagic ulcers. |
| 2.0         | Ulcers greater than 3 mm in diameter. |
glucose (80–85%); water (15–17%); ash (0.2%); proteins and amino acids (0.1–0.4%) and trace amounts of enzymes, vitamins and other substances, such as phenolic compounds [16].

Nearly all honey worldwide contains similar types of phenolic acids, flavonoids and antioxidants. Each constituent has unique nutritional and medicinal properties, and the components act synergistically resulting in honey’s various applications [17]. The physical properties and chemical composition of honey fluctuate based on the plants from which the bees collect raw material, differences in the type of flora, climatic conditions, treatment during harvesting and geographical region [18–20]. Honey was meant to improve the organoleptic properties of plant concoction for easy ingestion. Some biological properties of honey such as antioxidant, anti-inflammatory, anti-bacterial, antiviral, anti-ulcer activities; and anti-hyperlipidemic, anti-diabetic and anticancer properties have been confirmed by various research studies [15]. Many studies have reported that the antioxidant capacity of honey is dependent on the presence of total phenolic compounds and flavonoids, which play an important role in ameliorating oxidative stress [21,22]. Although there is reduction in the mortality rate of peptic ulcer individuals, dropping from 327,000 demise in 1990 to 267,500 in 2015, due to the use of various anti-ulcer drugs such as antacids, proton pump inhibitors (omeprazole and lansoprazole), anticholinergics and histamine-2 (H2) receptor antagonist (nizatidine, cimetidine) [23], these drugs have been discovered to precipitate undesirable effects, relapses, and several drug reactions like food allergies, dizziness, headache, nausea, pneumonia and low libido among others [23,24].

On the other hand, medicinal plants such as turmeric, ginger and Og are useful in the prevention and treatment of numerous diseases such as cancer, diabetes mellitus, ulcer and other diseases with little or no adverse effect as a result of some bioactive components present in them [25].

The ulceropreventive potential of Og leaves extract in combination with honey in rat model has not been explored. Therefore, the study was designed to investigate whether methanol fraction of Og extract administered together with honey is capable of exerting improved anti-ulcerogenic influence on indomethacin-induced gastric ulcer in male Wistar rats.

**Materials and methodology**

**Chemicals and solvents used**

All chemicals used in this research were of analytical grade purchased from Sigma-Aldrich Ltd., USA.

**Collection of plant materials**

The leaves of Og were obtained at Iya-Laje Market, Ondo City, Ondo State, Nigeria. The plant was then authenticated in the Department of Pharmacognosy, University of Ibadan, Oyo State with authentication number 15,409. The research was approved by University of Medical Sciences, Ethical Committee, Ondo State, Nigeria with code number UNIMED-EC/19/005.

**Preparation of extract**

The leaves were washed, air dried for 4 weeks and pulverized using mortar and pestle. The weight of the powdered leaves was 520 g and this was soaked in 5.2 L of 100% methanol for 72 hours. The filtrate was obtained using muslin cloth and the process was repeatedly carried out three times [25]. The filtrate was then concentrated using rotary evaporator and 78.5 g (15%) extract yield was obtained. The crude methanol extract was then fractionated successively in increasing polarity between hexane, chloroform, ethyl acetate and methanol using vacuum liquid chromatography (VLC).
packed with silica gel (60 H) in order to obtain various fractions [26]. The methanol extract was adsorbed with silica gel at concentration 1:10 (W/W) before pouring it on silica gel (60 H) bed in sintered glass. Hexane was used to defat. Other fractions obtained were chloroform, ethyl acetate and methanol fractions with percentage yield of 26.3%, 5.4% and 24.5% respectively.

**Experimental animals**

The animals used for this study were locally bred male Wistar rats from University of Medical Sciences, Ondo State Animal House. The animals were acclimatized for 1 week and maintained under the standard environmental conditions on 12 h-day/night cycle and given rat chow and water *ad libitum*. They were then pre-treated every morning through oral route of administration with methanol fraction of Og leaves extract and honey for 2 weeks.

**Experimental design**

Exactly 56 rats weighing between 100 g and 120 g were distributed randomly into seven groups containing eight rats each. The animals were grouped as follows:

- Group 1: Control
- Group 2: Ulcerative Control
- Group 3: Ulcerogenic + 100 mg/kg body weight methanol fraction of Og leaves extract.
- Group 4: Ulcerogenic + 400 mg/kg body weight methanol fraction of Og leaves extract.
- Group 5: Ulcerogenic + 100 mg/kg body weight methanol fraction of Og leaves extract and 2.5 g/kg body honey.
- Group 6: Ulcerogenic + 400 mg/kg body weight methanol fraction of Og leaves extract and 2.5 g/kg body honey.
- Group 7: Ulcerogenic + 2.5 g/kg body weight honey.

**Induction of ulcer**

Twenty four hours before induction of ulcer, food and water were withdrawn from the animals in order to empty the stomach of all food substances. The animals were induced with 30 mg/kg body weight indomethacin dissolved in 0.1 M phosphate buffer using an oral cannular with five minutes interval between each animal. They were left for 4 h from their respective induction so that the indomethacin could exert its ulcerative effect. Gastric ulceration was induced in the animals according to the procedure described by Sayanti et al. [27].

**Samples collection**

The animals were euthanized and sacrificed by cervical dislocation and opened up from the peritoneal region to excise the stomach. The excised stomach was injected with 1 ml of 0.9% normal saline and the contents were collected. The collected wash solutions were centrifuged at 3000 rpm for 5 minutes and the supernatant was preserved at 4°C for gastric acidity assay. The stomach was harvested, homogenized using 0.1 M phosphate buffer in 1:5 dilution factor and centrifuged at 10,000 rpm for 15 minutes to obtain the post-mitochondria fraction stored at 4°C.

**Determination of ulcer scores**

The stomach of the animals were opened through the lesser curvature and examined macroscopically using a magnifying lens for the degree of ulceration. The ulcers were scored using the Alphin and Ward [28] method. The scoring technique used for the induced-ulceration was based on the following criteria: Alphin and Ward [28].
Ulcer index

It was quantified as described by Mousa et al. [29].

\[
\text{Ulcer Index (UI)} = \frac{\text{Total area of mucosal ulcer}}{\text{Total mucosal area}} \times 100
\]

\[
\% \text{Inhibition of ulceration} = \frac{1}{\text{UI(Indomethacin untreated) - UI(treated) \times mu}} \times 100
\]

Measurement of gastric secretion

Gastric supernatant (1 ml) was titrated against 0.1 M NaOH using phenolphthalein as the indicator to get a faint pink coloration. The total gastric acid secretion was determined using the formula:

\[
M_A V_A = M_B V_B
\]

where \( M_A \) = Molar Concentration of Acid.
\( M_B \) = Molar Concentration of NaOH.
\( V_A \) = Volume of acid.
\( V_B \) = Titer Value.

Protein determination

It was determined in various samples by Biuret method [30]. Post-mitochondrial fraction was diluted five times. Precisely 1 ml of the sample was taken and added to 3 ml of Biuret reagent. The mixture was incubated at room temperature for 30 minutes after which the absorbance was read at 540 nm using distilled water as blank.

Antioxidant enzyme assay determination of superoxide dismutase (SOD) activity

The activity of SOD was determined by the method of Misra and Fridovich [31]. Change in absorbance was evaluated at 30 seconds interval for 2.5 minutes at 480 nm spectrophotometrically.

Calculation

\[
\% \text{inhibition} = \frac{100 - \left( \frac{\text{Increase in absorbance per min for sample}}{\text{Increase in absorbance per min for blank}} \right) \times 100}{\text{UI (Indomethacin untreated) - UI (treated)}} \times 100
\]

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the autooxidation of epinephrine.

Determination of catalase (CAT) activity

CAT activity was determined according to the method of Claiborne [32] by measuring change in absorbance at 240 nm via spectrophotometer. An extinction coefficient of 0.0436 mM\(^{-1}\)cm\(^{-1}\) [33] was used. 1 in 50 dilution was done for the sample. The reaction mixture contains 2 ml of H\(_2\)O\(_2\) solution and 2.5 ml phosphate buffer. 1.5 ml of the assay mixture was dispensed into 3 ml of dichromate acetic acid reagent at 60s intervals and then measured spectrophotometrically.

Calculation

\[
\text{Catalase activity} = \frac{\Delta A_{240}/\text{min} \times \text{reaction volume} \times \text{dilution factor}}{0.0436 \times \text{sample volume} \times \text{protein/mg}} \times \text{mg protein/mg}
\]

Estimation of reduced glutathione (GSH) level

The method of Beutler et al. [34] was followed in estimating GSH level. The absorbance of the yellow color formed upon the addition of Ellman’s reagent was read within 30 minutes at 412 nm with the use of spectrophotometer.

Estimation of glutathione s-transferase (GST) activity

GST activity was assayed according to the method of Habig et al. [35]. The decrease in absorbance was measured spectrophotometrically at 340 nm.
at interval of 30 seconds for 4 minutes. GST activity was calculated as unit per mg protein based on a molar extinction coefficient of 9.6 × 10^3 L/mol/cm. One unit of GST was defined as the amount of enzyme that catalyzes the conjugation of 1 nmol of GSH-CDNB per minute.

**Assay for glutathione peroxidase (GPx) activity**

GPx activity was measured according to the procedure of Rotruck et al. [36] with little modifications. Absorbance was measured at 412 nm spectrophotometrically.

**Histopathological investigation**

Stomach samples were fixed with 10% formalin solution and embedded in paraffin wax which were cut to 5 microns thickness. They were then stained with Hematoxylin and Eosin stain, and observed under light microscope [37].

**Assessment of lipid peroxidation**

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale [38].

**Statistical analysis**

All data were represented as Mean ± Standard Deviation. Statistical significant difference was accessed by one way analysis of variance (ANOVA), with Duncan’s multiple range test carried out using Graph Pad Prism 8 (GraphPad Software, San Diego, USA). P < 0.05 was considered as statistically significant.

**Results**

As shown in (Figure 1(a)) (Plates 1(a-g, b,c)), indomethacin causes an ulcerative effect on the rats. However, pre-treatment with doses of methanol leaves fraction (MEF) significantly ameliorated the observed effect to normal because there was similarity in their ulcer score values compared to control. Meanwhile, honey and its combination with MEF partially abrogated the ulcerative condition in the rats.

In indomethacin-ulcerative rats, there was reduced pH, denoting increasing acidity. Whereas, this effect was effectively prevented by pre-treatment with MEF doses, as observed in (Figure 2), while honey and its combined form to some extent prevented the excessive elevation of acidic state of the stomach.

SOD and CAT activities were significantly low in untreated control compared to control as shown in (Figure 3(a,b)). Also, pre-treatment with honey exhibited similar effect as untreated control. However, pre-treatment with others groups showed considerable improvement in the SOD and CAT activities in comparison to the untreated control, while highest dose of MEF and its combined form with honey completely ameliorated the observed effect.

(Figures 4(a,b)) and 5(a,b)) successively showed results of the stomach GSH, total thiol concentrations and, GST and GPx activities. Vast significant decrease (P < 0.001) occurred in the GSH level of the untreated group compared to control. Additionally, there was significant elevation (P < 0.05) in the GSH status of the groups administered 100 mg/kg MEF/2.5 g/kg H and 2.5 g/kg H but not as much as the control group. Whereas there was no significant difference among MEF (100 mg/kg and 400 mg/kg), 400 mg/kg MEF/2.5 g/kg H and the control group.

A significant decline (P < 0.001) in total thiol status was observed in the ulcerative control group relative to control. However, the reduction was significantly (P < 0.05) prevented by administration of varying doses of MEF, the combination of treatments and 2.5 g/kg H but not the same as the control group.

Significant diminution (P < 0.001) was noticed in the GST activity of the untreated group compared to control. Also, groups pre-treated with different doses of MEF, 100 mg/kg
MEF/2.5 g/kg H and 2.5 g/kg H were observed to exhibit significant ($P < 0.01$) elevation of GST activity but not up to the control group. Conversely, there was no significant difference between 400 mg/kg MEF/2.5 g/kg H pre-treated group and the control.

There was no significant difference in GPx activity of groups pre-treated with 100 mg/kg
MEF, 100 mg/kg MEF/2.5 g/kg H, and 2.5 g/kg H relative to control. Statistically considerable increase \((P < 0.05)\) was evident in 400 mg/kg MEF and 400 mg/kg MEF/2.5 g/kg H compared to control \((\text{Figure 5})\). Meanwhile, when comparing ulcerative untreated to control, significant \((P < 0.01)\) decrease was observed.

\((\text{Figure 6})\) reveals that there was no significant difference in the MDA level of all the pre-treated groups except for ulcerative untreated which was significantly elevated \((P < 0.001)\) relative to control.

\((\text{Plate 2(a)})\) shows the photomicrograph of a stomach section stained by Hematoxylin and Eosin stain showing moderate architecture. The parietal \((\text{orange arrow})\), chief cells \((\text{red arrow})\) and pits \((\text{green arrow})\) were normal. There was moderately preserved mucosa epithelial cells layer, the mucosa layer show no infiltration of the gastric glands and lamina propria \((\text{slender arrow})\). The submucosal layers appear normal and not infiltrated by inflammatory cells, the circular muscle layer appears normal.

The architecture of the stomach section of untreated rats \((\text{Plate 2(b)})\) was moderate. There was fairly preserved mucosa epithelial cells layer, the mucosa layer shows mild infiltration of the gastric glands with widened pit \((\text{green arrow})\) and lamina propria \((\text{slender arrow})\). The submucosal layers appear severely infiltrated by inflammatory cells \((\text{blue arrow})\), the circular muscle layer appears normal.

In \((\text{Plate 2(c)})\), animals pre-treated with 100 mg/kg MEF pretreated showed moderate architecture, moderately preserved mucosa epithelial cells layer, the mucosa layer show very mild infiltration of the gastric glands and lamina propria \((\text{slender arrow})\). The submucosal layers appear normal and not infiltrated by inflammatory cells, the circular muscle layer appears normal.
arrow). The submucosal layers appear moderately infiltrated by inflammatory cells (blue arrow), the circular muscle layer appears normal.

The photomicrograph of a stomach section of 400 mg/kg MEF pre-treated rat (Plate 2(d)) revealed normal architecture, well preserved mucosa epithelial cells layer (white arrow), no infiltration of the gastric glands and lamina propria into the mucosa layer (slender arrow). The submucosal layers appear normal and not infiltrated by inflammatory cells. The circular muscle layer also presented normal.

(Plate 2(e)) depicts that photomicrograph of a stomach section pretreated with 100 mg/kg MEF/2.5 g/kg H showed moderate architecture, mildly preserved mucosa epithelial cells layer, the mucosa layer show no infiltration of the gastric glands and lamina propria (slender arrow). The submucosal layers show few infiltrates and mild vascular congestion (blue arrow), the circular muscle layer appears normal.

(Plate 2(f)) shows that there was moderate architecture as well as fairly preserved mucosa epithelial cells layer, with the mucosa layer displaying no infiltration of the gastric glands and lamina propria (slender arrow) in the stomach section of rats pre-administered 400 mg/kg MEF/2.5 g/kg H. The submucosal layers appear mildly infiltrated by inflammatory cells (blue arrow) while the circular muscle layer appears normal.

Photomicrograph of a stomach section of honey pretreated group (Plate 2(g)) displays moderately normal architecture, well preserved mucosa epithelial cells layer. The mucosa layer show scanty infiltration of the gastric glands and lamina propria (slender arrow), the submucosal layers appear mildly infiltrated by inflammatory cells (blue arrow).

Discussion

The molecular basis of NSAID-induced gastropathy is widely ascribed to their inhibitory activity against cyclooxygenases, which causes them to block the prostaglandin synthesis leading to increased gastric acid, reduced gastric mucosal blood flow, disturbance of microcirculation, increased leukocyte adherence, lipid peroxidation and neutrophil infiltration [6].

The results observed in (Figure 1(a-c)) showed that pre-treatment with doses of Og leaves extracts was able to effectively prevent ulceration as there was no lesion on the stomach of the rats (Plates 1(a, c, d)). It could however be revealed that 2.5 g/kg H was not capable of abrogating the onset of the ulcer as
evident in the numerous physical lesions seen (Plate G). This could be associated with the natural acidic nature of honey which can exacerbate gastric acidic medium of the stomach, thus, leading to inflammation and corrosion of the protective gastrointestinal mucosa. This was corroborated by the study of Supijona et al. [39], who reported that excessive intake of honey could precipitate irritation of the gastric mucosa of the stomach as honey has low pH of 3.8. Previous research also showed that indomethacin has ulcer-inducing aggressive factors resulting from its ability to elevate gastric juice volume, free and total acidity and reduce gastric pH [39]. Combined use of MEF and honey was able to reduce the ulceration level in dose-dependent manner but did not effectively prevent it. This may be that MEF does not contain sufficient alkaline secondary metabolites to help checkmate the highly reduced honey pH.

When the oxygen radicals overwhelm the antioxidant defense system, a condition called oxidative stress is created [40]. Significant production of free radicals causes damage to the cell and cellular membrane due to excessive oxidative stress. The generation of reactive oxygen species (ROS), for example, superoxide anion, hydrogen peroxide, and hydroxyl radicals, may cause lipid peroxidation, especially in membranes, and results in tissue injury [41]. This observation may emphasize the role of oxidative damage in ulcer induction, and development. Indomethacin has previously been reported to decrease antioxidant enzymes (SOD, CAT and GST) activity in rat stomach thereby inducing gastric ulceration [42].

However, it has been reported that the first line of defense against oxidative damage caused by injury like ulcers involves the migration of free radical scavenging enzymes such as SOD, CAT, and GPx, to eliminate first O₂ and H₂O₂ before forming harmful hydroxyl (OH⁻) radical [43].

The evident SOD activity (Figure 3(a)) decline in the ulcerative untreated group depicts the generation of free radicals which could not be prevented by 2.5 g/kg H. Meanwhile, the reduction in the enzyme activities which was not as much as untreated group shows that 100 mg/kg MEF, 100 mg/kg MEF/2.5 g/kg H and 400 mg/kg MEF/2.5 g/kg H did not possess the requisite antioxidant capacity to completely abrogate the free radical generation. This may
be alluded to the fact that low dose MEF as well as differing doses MEF combined with honey was not high enough to scavenge free radicals efficiently. MEF (400 mg/kg) was capable of preventing the debilitating effect of oxidants generated by indomethacin probably due to the presence of higher dose of the extract. This may be one of the mechanism by which this plant prevents ulcerogenesis.

Figure 5. Effect of methanol fraction of *O. gratissimum* leaves extract and honey on stomach GST and GPx activities in indomethacin-induced ulcer in male Wistar rats. * P < 0.05; ** P < 0.01; *** P < 0.001 (Control vs. test groups); H: Honey

Figure 6. Effect of methanol fraction of *O. gratissimum* leaves extract and honey on stomach MDA level in indomethacin-induced ulcer in male Wistar rats. *** P < 0.01 (Control vs. Control_Ulcerative), H: Honey
Significant enhancement of catalase activity in the animals administered 100 mg/kg MEF and 100 mg/kg/2.5 g/kg H could result from the individual or joint effect of MEF and honey in combating the damaging effect of ROS. Also, the antioxidant potency of 400 mg/kg MEF and 400 mg/kg MEF/2.5 g/kg H was so overwhelming to prevent depletion of the antioxidant reserve of the rat stomach. This is consistent with the research conducted by Nwachukwu and Okwousa [44] who reported that gastroprotective potentials of plants may result from the improved antioxidant defense system known to be effective pro-oxidant scavenger.

The observed drastic dwindling in the levels of the non-enzymic antioxidants such as GSH and total thiol (Figure 4(a,b)) in the ulcerative untreated group revealed that indomethacin could produce overpowering free radicals that would result in abrupt depletion of the antioxidant reserve in the system. However, there was a dose-dependent increase in the GSH and total thiol status for both MEF and MEF/H combined as well as 2.5 mg/kg H. This is an indication that both MEF and honey can boost and maintain the antioxidant level of the biological system.

Dose-dependent increase in GST activity (Figure 5(a)) of MEF and MEF/H is an indication of the efficacy of Og alone and enhanced improvement in combination with honey. However, honey alone could not exhibit sufficient antioxidant potency to strongly prevent the depletion of GST activity observed in ulcerative untreated group. This result could be corroborated by the report that Og leaves extract enhanced antioxidant enzymes in indomethacin-induced ulcer in Wistar rat [45].

The observed significant boost of GPx activity (Figure 5(b)) by pre-treatment with both MEF and honey depicts their antioxidant-stabilizing potentials both singly and additively.

Indomethacin showed great free radical generating capacity, lipid peroxidation precipitation with resultant gastric irritant substances production and damage to the gastrointestinal epithelial mucosa (Figure 6). The extract as well as its combined form was efficient in fully impeding the synthesis of malondialdehyde (MDA) in the stomach of Wistar rats. These results suggest that MEF and its combination with honey may be exhibiting their anti-necrotizing capability by fortifying the antioxidant reserve of the system and scavenging the free radicals generated in the body [46].

The observed normal histological structures in all gland cells, gastric epithelial cells, parietal and the chief cells in stomach of the control group is a suggestion that there was no gastrocellular damage in the animals. The mild infiltration of the gastric glands, lamina propria and the severe infiltration by inflammatory cells in the submucosal layers of the untreated group indicated that indomethacin induced inflammation in the stomach. This is supported by the study that indomethacin is known to induce the reactive oxygen metabolites in animal models which may contribute to mucosal injury. The ability of MEF in dose-dependent manner as well as its combination with honey to prevent ulceration could suggest that the plant and honey contain some bioactive principles which were capable of impeding the onset of gastric ulcer, thus exhibiting a gastro-intestinal protective effects. This may be through preservation of mucosa membrane and stimulation of prostaglandin synthesis necessary for gastro-protection. Decreased prostaglandin level has been attributed to impaired gastro-protection and increased gastric secretion which are important events in the etiology of mucosal ulceration [47–49].

**Conclusion**

From the data generated above, the study revealed that MEF and honey demonstrated gastro-protective capacity as observed in their ability to increase the status of various antioxidant enzymes and reduce MDA level in the stomach which help to prevent damaging...
effect of free radicals and consequently reduction of ulceration.

Also, the honey may contribute significantly to increase gastric acidity which could overwhelm the alkali reserve of the gastrointestinal tract mucosa thereby making the epithelia lining more prone to corrosion. Although increased acidity is an advantage in a condition where *H. pylori* is the causative, in which case it uses its urease activity to evade the acidic microenvironment in the stomach. Therefore, it would be better to ascertain the causative agent before embarking on honey for the treatment or management of gastric ulcer.

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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