Therapeutic strategies of *Moringa oleifera* Lam. (Moringaceae) for stomach and forestomach ulceration induced by HCl/EtOH in rat model

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1. Introduction

Stomach ulcer is a chronic disease featured with unexpected complications, including bleeding, stenosis and perforation, as well as a high incidence of recurrence (Kangwan et al., 2014). Their etiology is multifactorial and occurs when the balance between offensive and protective factors of the mucosa is disturbed (Serafin et al., 2020). Two major damaging causes implicated in peptic ulcer diseases and ulcer recurrence are infection with *Helicobacter pylori* (H. pylori), alcohol intake, stress, indomethacin, bile acids, ischemia, long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Kangwan et al., 2014), aging, gender, smoking, education level, income, obesity and abdominal adiposity, nutrients, blood parameters, and lifestyle (Lee et al., 2018). Already, drug classes such as proton pump inhibitors, histamine (H2) inhibitors, and antacids have been used to treat this disease for the past decades (Arman et al., 2021). The combination of omeprazole and rebamipide accelerated the quality of ulcer healing through an increasing level of prostaglandin E2 (PGE2) and a decreasing level of Interleukin-8 (IL-8) and malondialdehyde (MDA) in the gastric mucosa, but not omeprazole alone (Kangwan et al., 2014). However, the drugs used produce many adverse effects and are less effective than they ought to be. Therefore, there is a growing interest in alternative therapies and the use of natural products (Klein-Junior et al., 2012). There is a revival of interest in herbal products (botanicals) at a global level and the conventional medicine is now beginning to accept the use of botanicals once they are scientifically validated (Gilani and Attar-ur Rahman, 2005). Plants with antiulcerogenic activity were used either as raw materials which...
obtained by extraction with solvents or as individual isolated compounds (Awaad et al., 2013). Flavonoids are among the molecules of greatest interest in biological assays due to their anti-inflammatory and antioxidant properties (Serafim et al., 2020).

Moringa oleifera Lam. (Syn Moringa pterygosperma Gaertn), a fast-growing drought-resistant, deciduous, perennial softwood tree, is native to the Himalayan foothills (India/Bangladesh) and it become naturalized and widely cultivated in many countries (Adleye et al., 2021; Paliwal et al., 2011) and is found in the Chinese herbal medicine dictionary in China (Meireles et al., 2020). The trees serve as windbreaks and reduce soil erosion. It is used in lumber production as well as for light construction work in many parts of the developing world. The coarse fiber is often used for the production of ropes or mats. It is also used for contaminant flocculation as well as water purification (Alegbeleye, 2018). All parts of Moringa tree are edible and have long been consumed by humans (Paliwal et al., 2011) and used in Bakery products (Milla et al., 2021).

The variation of Moringa biocompounds depend on climatic conditions. It has abundant deposits of compounds containing simple sugar, rhamnose as well as a somewhat distinctive group of compounds in their radicle and crust called glucosinolates such as 4-[(a-L-rhamnopyranosyloxy) benzyl glucosinate and isothiocyanates, including 4-[(a-O-acetyl-a-L-rhamnopyranosyloxy)benzyl isothiocyanate, 4-[(a-L-rhamnopyranosyloxy) benzyl isothiocyanate and benzyl isothiocyanate (Alegbeleye, 2018; Bhattacharya et al., 2018). Additionally, its leaves contain high quantities of nutrients: vitamin A, vitamin C, calcium and potassium (Paliwal et al., 2011). Meanwhile, M. oleifera has been and continues to be used by folk medicine practitioners to prevent, mitigate, or treat many ailments and to prevent and cure several diseases such as inflammation, ulcer, cardiovascular diseases, diabetes, anemia, stress, skin, arthritis and hypertension (Meireles et al., 2020; Alegbeleye, 2018). Further, an herbal mixture formulation containing Moringa oleifera possess SARS-CoV-2 inhibitory activity (Adleye et al., 2021). The natural active compounds, microRNAs (p-miRs), present in the plant microvesicles purified from Moringa oleifera seeds aqueous extract counteract tumorigenesis (Potestà et al., 2020) and used as antiretroviral therapy, in managing HIV infection and restore in immune system (Minutolo et al., 2021).

To investigate the gastroprotective effect of M. oleifera, stomach ulcer was induced by acidified ethanol, lesion index and gastric secretion parameters in ulcerated rats were then evaluated. we measured and determined the levels of lipid peroxides and antioxidant enzymes in the gastric tissues of HCl/EtOH-treated rats.

2. Material and methods

2.1. Reagents

Chlorohydric acid (HCl); Ethanol (EtOH); 4-nitro blue tetrazolium chloride (NBT); sodium carbonate (Na2CO3); sodium tartrate (C4H4Na2O6); Copper sulfate (CuSO4); Folin-Ciocalteu (F-C) reagent (Ethylenediaminetetraacetic Acid, Disodium Salt); Na2EDTA; sodium hydroxide (NaOH); 2-thiobarbituric acid (TBA); sodium nitrite (NaNO2); Tris (HOCH2)3CNH2; sodium chloride (NaCl); 2,2-Diphenyl-1-picrylhydrazyl (DPPH); Trichloroacetic acid (TCA); ferric chloride (FeCl3); Potassium ferricyanide (K3Fe(CN)6); 2,2-Diphenyl-1-picrylhydrazyl (DPPH); Trichloroacetic acid (TCA); ferric chloride (FeCl3); Potassium ferricyanide (K3Fe(CN)6); Butylated hydroxytoluene (BHT); ascorbic acid (VIT C); aluminum chloride (AlCl3); hydrogen peroxide (H2O2); 5, 5′-dithiobis (2-nitrobenzoic acid (DTNB), gallic acid, quercetin, vanillic acid, catechin and bacterial growth medium (Mueller Hinton agar) were obtained from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). Bovine Serum Albumin (BSA) was purchased from Atlanta Biologicals (Norcross, GA, USA). Omeprazole® Zentiva 40 mg was purchased from Tunisian pharmacy (Tunisia).

2.2. Plant material

The dried leaves of M. oleifera (500 mg) were extracted by maceration with methanol and distilled water for 24 h. The extracts were then filtered with wattman paper (11 µm, Merck), centrifuged (4500 g/10 min) and then kept in vials at –20 °C. The yield of methanolic, aqueous and infusion extracts of M. oleifera was found to be 14.3, 21 and 28.4%, respectively (Fig. 1B).

2.3. Bacteria

The tested bacteria used in this study, Escherichia coli (E. coli ATCC 35218), Pseudomonas aeruginosa (P. aeruginosa ATCC 27855) and Staphylococcus aureus (S. aureus ATCC 25923) were purchased from the ATCC.

2.4. Antibiotics

Vancoumycin (VA) (36 µg/µL); Penicillin (P) (10 µg/µL); Bacitracin (B) (10 µg/µL); Ampicillin (AM) (10 µg/µL); Streptomycin (S) (100 µg/µL) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.5. Polysaccharides extraction and FTIR analysis of M. Oleifera

Liposoluble fractions were removed from M. oleifera leaf by mixing its powder with EtOH. Afterwards, the sample was soaked in water bath at 80 °C for 3 h, filtered and concentrated to extract polysaccharides. Sevag reagent was used to remove protein fractions from the plant powder. Extracted polysaccharides were then analysed by FTIR spectra that was evaluated in the range 4000–400 cm⁻¹ at room temperature with a resolution of 4 cm⁻¹ by FTIR spectrometer (Shimadzu 8400 s, France). Experiment has been repeated three times.

2.6. Phenolic content

The phenolic content was evaluated using Folin-Ciocalteu (FC) reagent. Aliquots of plant samples was mixed with FC (10%) and distilled water. Then, Na2CO3 (7.5%) was added. The solution was kept for 1 h until the development of blue color. Gallic acid is the reference standard used to evaluate phenol content. The absorbance was recorded at 725 nm using spectrophotometer (Spectronic 200). Total phenolic values were expressed as milligram gallic acid equivalent per gram dry weight of plant extract (mg GAE/g DW).

For flavonoid content, aliquots of plant extract was mixed with NaNO2 (5%). Six minutes later, AlCl3 (10%) solution was added. The solution was kept for 1 h until the development of blue color. Then, NaOH (1 M) and distilled water were added to the solution. The absorbance was measured after 15 min at 510 nm. The flavonoid content was expressed as milligram quercetin equivalent per gram dry weight of plant extract (mg QE/g DW).

To evaluate plant condensed tannins, aliquots of M. oleifera leaf extracts were mixed with vanilline (4%). After 15 min, concentrated HCl was added and the absorbance was read at 500 nm. The condensed tannin content was expressed as milligram catechin equivalent per gram dry weight of plant extract (mg CE/g DW). Experiment have been repeated three times.

2.7. Antioxidant activity

2.7.1. DPPH assay

To estimate the ability of the M. oleifera leaf extracts to reduce the DPPH Effect, we prepared different sample and synthetic antioxidant (ascorbic acid) concentrations (100 to 500 µg/ml). The decrease in the absorbance of DPPH in ethanol at 517 nm
mediated by radical scavengers was detected after 30 min. Briefly, aliquots with increasing doses of prepared extracts were mixed with DPPH ethanolic solution. For blank, the ethanol was used instead of extracts. Test has been repeated three times.

\[
\text{DPPH inhibition (\%)} = \frac{A_b - A_s}{A_b} \times 100
\]

Where \( A_b \) is the absorbance of the blank and \( A_s \) is the absorbance in the presence of plant sample.

2.7.2. Ferric reducing antioxidant power

The reducing of Fe\(^{3+}\) into Fe\(^{2+}\) ions in the presence of \( M. \) oleifera leaf extracts indicated its potent antioxidant effect. Briefly, aliquots with different doses (100 to 500 \( \mu \)g/ml) of plant extracts or synthetic standard (BHT) were mixed with \( K_2 Fe(CN)_{6} \) (1%) and phosphate buffer (0.1 M; pH 6.6) and incubated in water bath (50 °C/20 min). Afterwards, TCA (10%) was added to the solution. The mixture was centrifuged (3000 rpm/10 min) and then mixed with FeCl\(_3\) (0.1%) and distilled water. Optical density was measured at 700 nm using spectrophotometer. Increase in absorbance of the reaction mixture shows the reducing power of the samples (Jayaprakasha et al., 2001). Test has been repeated three times.

2.8. In vitro antitumoral effects of \( M. \) Oleifera

The method used in this report to investigate the antibacterial effect of \( M. \) oleifera is the agar diffusion assay. Briefly, discs (6 mm) of Whatman filter paper N°1 were impregnated with methanolic and aqueous extracts and infusion of Moringa and placed in petri dishes that contain Mueller-Hinton agar and bacteria (10° CFU/mL). Antibiotics were considered as positive controls and DMSO were used as negative controls. Plates were incubated (24 h/37 °C) and inhibition diameter (mm) were measured. Experiment has been repeated three times.

2.9. In vivo experimental design

2.9.1. Animal experiment

A total of 30 Sprague –Dawley male rats (8-week-old, 230 ± 3, 6 g) were obtained from the Experimental Animal Laboratory (Sfax, Tunisia), housed in the animal research center of the department of Biology, Faculty of Sciences of Gafsa (FSG) (Gafsa, Tunisia), maintained in propylene cages under normal conditions (12/12 h light/dark cycles, 25 ± 5 °C, 50% relative humidity) and provided with standard pellets diet (SNA, Sfax) and water. The experimental protocols were approved by Animal Ethics Committee of Gafsa University (G/A/SV; Approval Number GU-2016–001).

2.9.2. Experimental protocol and gastric lesion assessment

After an overnight fasts with food starvation and free access to water, rats were divided into six groups (n = 5) (Table 1):

- Group 1 (sham group, water consumption);
- Group 2 (ulcerated group) orally treated with 0.1 ml HCl/EtOH (60% ethanol in 40% 150 mmol/L hydrochloric acid) mixture per 10 g body weight (Zhang et al., 2020);
- Group 3 (Omeprazole) orally pretreated with the standard stomach ulcer drugs, Omeprazole (20 mg/kg bw);
- Group 4 (Omeprazole + HCl/EtOH Plant extract 100 mg/kg + 150 mM HCl in 60% EtOH)
- Group 5 (Omeprazole + HCl/EtOH Plant, 100 mg/kg + 150 mM HCl in 60% EtOH)
- Group 6 (Omeprazole + HCl/EtOH Omeprazole 20 mg/kg)

| Animal Groups | Treatment |
|---------------|-----------|
| Sham          | Distilled water |
| Ulcer Control | Distilled water, 150 mmol/L HCl in 60% EtOH (0.1 ml/10 g) |
| Omeprazole    | 20 mg/kg |
| Plant extract | 100 mg/kg |
| Plant + HCl/EtOH | Plant, 100 mg/kg + 150 mM HCl in 60% EtOH |
| Omeprazole + HCl/EtOH | 20 mg/kg + 150 mM HCl in 60% EtOH |

Fig. 1. A. Plant image (i), FTIR bands of biocompounds (ii) and polysaccharides (iii) of \( M. \) oleifera leaves. B. Yield of \( M. \) oleifera extracts. C. Total phenolic content of \( M. \) oleifera leaf extracts (i). Phenolic content values were expressed as gallic acid equivalents (GAE) mg/g dry weight (DW). Flavonoid content (ii). Flavonoid content values were expressed as catechin equivalents (CE) mg/g dry weight (DW). AE: aqueous extract; ME: Methanolic extract; INF: infusion. Each test has been repeated three times.
Group 4 (M. oleifera) orally pretreated with M. oleifera leaf infusions (100 mg/kg);
Group 5 (M. oleifera, 150 mmol/L HCl/60% EtOH) orally pretreated with M. oleifera leaf infusions (100 mg/kg bw) 1 h before oral intubation of the mixture of HCl/EtOH to induce gastric mucosa damage;
Group 6 (Omeprazole, 150 mmol/L HCl/60% EtOH) orally pretreated with Omeprazole (20 mg/kg bw) 1 h before oral intubation of the mixture of HCl/EtOH.

For the preparation of M. oleifera leaf infusions, dried leaf parts (500 mg) were dissolved in distilled water (5 ml) and extracted at the day of experiments.

One hour after treatment with acidified ethanol, all rodents were euthanized using Ether, sacrificed and stomach were collected (Fig. 3A), opened along the greater curvature, washed with normal saline solution. Mucus in the stomach mucosa of each rat was scraped with glass slide and transferred to conical tubes and weighed using an electronic balance. Stomach lesions were photographed using LP VEYRON (LP_N-50) digital camera and quantified.

The ulcer index (UI) was measured as follows:

\[
UI = \frac{\text{Average number of lesions per rat} + \text{Average number of severity score}}{\% \text{ of rats with ulcers}}
\]

2.9.3. Microscopic observations
For histopathological investigation, the stomach sections undergoing all administrations were fixed in buffered formalin (10%), embedded in paraffin and thick slices (4 µm) were obtained after section of gastric tissues by Leica microtome. Obtained sections were stained with Hematoxylin and Eosin (H&E) and then observed with light microscopy.

2.9.4. Protein quantification and oxidative stress levels of damaged stomach
2.9.4.1. Gastric sample preparation. The stomach was excised from all animals and homogenized in potassium phosphate (pH 7.4) buffer solution. Then, the homogenates were centrifuged (3,000 rpm/15 min) and kept at -20 °C until use (antioxidant enzymes and lipid proides analysis).

2.9.4.2. Protein content. The quantification of the stomach tissue proteins was performed according to the method of Lowry et al. (1951) using BSA as standard for the calibration curve. Briefly, stomach sample was mixed with distilled water. Afterwards, the mixture (Na2CO3 + C4H4Na2O6 + CuSO4) was added to the diluted homogenate. Then, Folin-Ciocalteu reagent was added to the solution and absorbance (A500 nm) was measured.

The ulcer index (UI) was measured as follows:

\[
UI = \frac{A_{\text{average number of lesions per rat}} + A_{\text{average number of severity score}}}{\% \text{ of rats with ulcers}}
\]

**Fig. 2.** A. DPPH radical scavenging activity (i) and ferric reducing power (ii) of different concentrations (100, 200, 300, 400, 500) of M. oleifera leaf extracts. (B) Antibacterial effects of M. oleifera extracts using disc diffusion assay. Whatman filter disc (6 mm) impregnated with extracts were placed onto the petri dishes seeded with bacteria on the agar Mueller-Hinton. DMSO was used as negative controls and extracts were tested with positive controls (vacoumycin 36, penicillin 10, bacitracin 10, ampicillin 10, and streptomycin 100). The petri dishes were incubated (37 °C/24 h), and inhibition zones (mm) of pathogens in the presence of natural compounds and antibiotics were evaluated. AE: aqueous extract; ME: Methanolic extract; INF: infusion. Each experiment has been repeated three times.
tion and the mixture were kept in the dark at ambient temperature/30 min. Optic density (OD) was measured at 490 nm using spectrophotometer. Assays were carried out in triplicate.

2.9.4.3. Assessment of antioxidant enzymes. To assess the SOD enzyme level in stomach mucosa of all animals, the supernatant was mixed with Na2EDTA-Methionine and phosphate buffer (7.8, 50 mM). Then, NBT and riboflavin were added to the solution and kept for 20 min in the light, except for the blank, which was maintained in the dark. The absorbance was assayed at 580 nm. SOD enzyme level was determined as follows:

\[
\text{SOD activity (Y)} = \frac{\% \text{Inhibition}}{mg \text{ protein}} = \frac{OD \text{ Sample} - OD \text{ Blank}}{OD \text{ Blank}} \times 100 \times 1 \text{/ Protein mg/ml x FD}
\]

SOD Unit correspond to the quantity of protein that induce 50 % inhibition:

\[
\text{SOD specific activity} = Y/50 \text{ Unit SOD/mg protein}
\]

CAT activity (IU) = \(\Delta \text{ OD} / \varepsilon \text{ L X Fd}\)

For CAT assay, tissue homogenate was mixed with phosphate buffer (pH 7, 100 mM) and H2O2. Absorbance was measured at 240 nm using spectrophotometer. The enzyme activity was evaluated by reading the change in absorbance between the fifteenth and sixty second [17].

With CAT activity (IU): Catalase activity expressed in International Unit (\(\mu\)moles H2O2/min/mg protein); \(\Delta \text{ OD}\): variation of the optic density per min \((A_1-A_2)\); \(\varepsilon\): Molar extinction Coefficient \((0.043 \text{ mM}^{-1} \cdot \text{ cm}^{-1})\); \(L\): Length of optic curve = 1 cm or 0.776 cm; \(X\): concentration of protein in the homogenate (mg. Ml\(^{-1}\)) ; \(F_d\): dilution factor (0.02).

For the estimation of GPx activity, homogenate was mixed with reduced GSH (0.1 mM) and phosphate buffer (pH 7.8). The reaction mixture was incubated at 25 °C/5 min. Then, H2O2 (1.3 mM/l) was added to initiate the reaction and kept to react for 10 min. The reaction was stopped by the addition of TCA (1%) (30 min in the ice). Afterwards, we centrifuged the mixture at 3000 rpm/min for 10 min. The supernatant was mixed with Na2HPO4 (320 mM) and DTNB (1 mM). OD was recorded at 412 nm over an interval of 5 min. One unit of GPx activity was defined as a decrease of 1 \(\mu\)mol/L of [GSH] (37 °C; pH 6.5). GPx activity was evaluated as follows:

\[
\text{GPx activity (Y)} = \frac{[\text{OD}_{50} - \text{OD}_{0}] \times 0.045 \times 1000}{10 \text{ min x X (mg/ml)}}
\]

With GPx activity: Glutathion peroxydase activity expressed in \(\mu\)moles of consumed or oxydized GSH /min / g protein; \(\text{OD}_{50}\): optic density of the sample; \(\text{OD}_{0}\): optic density of the blank; \(X\): concentration of protein in the homogenate (mg. Ml\(^{-1}\)).

2.9.4.4. TBARS level in ulcerated mucosa. Stomach mucosal tissue MDA was assessed by the reaction with TBA. Briefly, gastric homogenate was mixed with TCA-BHT and TBS (Tris + NaCl) (pH 7.4) and then centrifuged for 10 min. Afterwards, hydrochloric acid and Tris-TBA were added to the solution and the mixture was incubated in water bath (80 °C/10 min). OD was measured at 530 nm. The [TBARS] was expressed as nmol MDA/mg protein and calculated as follows:

\[
[\text{TBARS}] = \frac{OD^{10^6}/\varepsilon \cdot L \cdot X \cdot F_d}{F_d}
\]

With \(\varepsilon\): Molar extinction Coefficient of MDA \((\varepsilon \text{ MDA} = 1.56 \cdot 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1})\); \(L\): Length of optic curve = 0.779 cm; \(X\): concentration of protein in the homogenate (mg. Ml\(^{-1}\)) ; \(F_d\): dilution factor.
2.10. Statistical analysis

Data were analysed by GraphPad Prism 4.02. The values were reported as mean ± S.E.M and One-Way ANOVA and Tukey’s test were used to compare the groups. Values of P < 0.05 were considered statistically significant.

3. Results

3.1. FTIR analysis of M. Oleifera

As seen in Fig. 1A, FTIR chromatogram of M. oleifera (Fig. 1Aii) leaf extract revealed the presence of several bands ([Fig. 1Aii]). The bands at 3447 cm⁻¹ and at 3417 cm⁻¹ would be due to O–H bending vibration. The bands at 2970 cm⁻¹, 2921 cm⁻¹, 2847 cm⁻¹ and 2752 cm⁻¹ correspond to CH₂, CH₃ and C–H stretching vibrations. The bands at 1674 cm⁻¹, 1635 cm⁻¹ and 1618 cm⁻¹ would be related to C = C and C = O stretching vibrations. The bands at 1379 cm⁻¹ and 1314 cm⁻¹ were assigned to C–H stretching and to O–H bending vibrations, respectively. The band in the region 1074 cm⁻¹ would be related to –CH₂OH and CO bending vibration and the band at 861 cm⁻¹ would be due to C-C stretching vibrations. Fig. 1Aiii showed the FT-IR spectrum for polysaccharides present in M. oleifera leaves. Bands observed in 3400 and 2800 cm⁻¹ are attributed to the group –OH and C–H stretching that correspond to asymmetric aliphatic polysaccharides (methyl and methylene). The bands at 1500 cm⁻¹, 1600 cm⁻¹ and 1700 cm⁻¹ would be due to the tannic compounds, the carbonyl group (C = O) and aliphatic aldehydes (CHO).

3.2. Total phenolic content

Phenols are plant active phytocompounds and act as antioxidative scavengers. The total phenolic content (phenolic acids, flavonoids, condensed tannins) of M. oleifera leaf extracts were evaluated in the Fig. 1C. The results presented in Fig. 1C showed that M. oleifera methanolic extract (ME) displayed higher total phenolic content, amounting to 106 ± 0.56 mg GAE/g dry weight of extract. For phe-

3.3. DPPH Reducing ability

Radical scavengers are popular because they scavenge free radicals that cause oxidative stress, cell damage and inflammation (Milla et al., 2021). Exposure to antioxidants that donate electron or hydrogen atom reduce the DPPH free radical with deep violet color into its reduced product (DPPH-H) with yellowish color. The DPPH radical scavenging activity of the 3 M. oleifera extracts at various tested concentrations are summarized in Fig. 2Ai. The highest dose (500 μg/ml) of the ME markedly exhibit the greatest DPPH reducing capacity with an estimated value of 91.69%, followed by AE (87.54%), and INF (80.58%). Therefore, the synthetic antioxidant ascorbic acid used for the preparation of calibration graphs showed similar activity with the methanolic extract.

The order of reducing activity was: ascorbic acid ≈ ME > AE > INF.

These data suggest that the potent DPPH scavenging activity may be related to plant-derived biocompounds and the position of hydroxy group in its structures.

3.4. M. Oleifera ferric-reducing power evaluation

Ferric reducing antioxidant power is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe³⁺) to form potassium ferrocyanide (Fe²⁺), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm (Bhalodia et al., 2013). The best reducing power effect was detected with ME (Fig. 2Aii). In fact, the reduction of a colorless Fe³⁺/ferricyanide (Fe(CN)₆³⁻) complex into blue-colored Fe²⁺/ferrous (Fe(CN)₆²⁻) complex that binds the ferricyanide is dose-dependent manner.

3.5. Antibacterial activity

Phenolic compounds have been associated with antimicrobial and antifungal activities of Moringa oleifera extracts (Milla et al., 2021). The methanolic and aqueous extracts and infusion of M. oleifera mediate growth inhibition of bacteria (Fig. 2B), with an inhibition diameter between 13 and 25 mm, detected in E. coli strains and between 8 and 16 mm for P. aeruginosa. Our data sug-

3.6. Ulceroprotective effect of M. Oleifera

Macroscopic observation of the gastric damage induced by HCl/EtOH was shown in the left panel of Fig. 3B. Administration of acidified ethanol to animals induced high stomach mucosal damage (red bands), whereas, oral gavage of the M. oleifera leaf infusion (500 mg/kg bw) reduced the gastric lesions to a markedly extent (79%) and protected the rats from ulceration compared to omeprazole-treated rats. Gastric gross appearance of vehicle-administered sham rats was normal compared to acidified ethanol group.

As seen in the right panel of Fig. 3B, the histological gastric injuries induced by HCl/EtOH were reversed by the M. oleifera pretreat-

As seen in Fig. 7, oral treatment of animals with acidified ethanol attenuated antioxidant enzyme levels and increased MDA (21.54 ± 0.105 mmol MDA/mg protein, P < 0.05) in gastric tis-

3.7. Effect of M. Oleifera on antioxidant status and MDA levels into ulcerated tissues

As shown in Fig. 7, oral treatment of animals with acidified ethanol attenuated antioxidant enzyme levels and increased MDA (21.54 ± 0.105 mmol MDA/mg protein, P < 0.05) in gastric tis-

6
mg protein, $P < 0.05$) and exhibited highly synthesis of SOD (3.54 ± 0.115 U/mg protein, $P < 0.05$), CAT (7.54 ± 0.122 μmoles $H_2O_2$ consumed/min/mg protein, $P < 0.05$) and GPx (2.45 ± 0.112 μmoles of consumed or oxidized GSH/min/g protein, $P < 0.05$) into ulcerated mucosa tissue. Indeed, proton pump inhibitor, omeprazole, slowly reduced lipid peroxides (12.55 ± 0.124 mmol MDA/mg protein) and highly elevated the level of CAT (4.58 ± 0.172 μmoles $H_2O_2$ consumed/min/mg protein), SOD (2.58 ± 0.111 U/mg protein) and GPx (1.74 ± 0.012 μmoles of consumed or oxidized GSH/min/g protein) in the stomach mucosa of ulcerated rats as compared to sham group.

### 4. Discussion

Gastric ulcer affect many people in Tunisia, and this may be due to the traditional Tunisian diet, because of lack of natural healthy diet, including vegetables and fruits with high level of carbohydrates, phenols and fibers (pomegranate, apple, curcumin, green tea, grape). Natural diets have antacid and anti-secretory effects mediated by reducing $H^+$, $K^+$/ATPase activity and antihistaminic function, along with the enhancement of mucosal defensive agents (mucin and hexosamine) (Farzaei et al., 2015). In view of previous reports regarding minerals and antioxidant compounds found in M.
oleifera, many researchers noted that this species serves as an extremely valuable food source of highly digestible protein, calcium, iron, fiber, lipids, carbohydrates, fatty acids, potassium, vitamins (A, B1, B2, B3, C, E), magnesium, sodium, phosphorus, sulfur, zinc, copper, manganese, iron, selenium, amino acids (arginine and histidine—2), antioxidants (phenols, flavonols), and carotenoids suitable for combating malnutrition in many developing nations of the world where malnourishment is a major concern (Alegbeley, 2018). It is considered one of the world’s most useful trees, as almost every part of the Moringa tree can be used for food or has some other beneficial property (Palival et al., 2011).

The current study demonstrates that the methanolic extract M. oleifera possessed relatively the highest phenolic (106 mg GAE/g DW), flavonoids (80 mg QE/g DW), and condensed tannins (3.6 mg CE/g DW) values along with the strongest antiradical activity, followed by aqueous extract and leaf infusion; as evidenced by the FTIR biocompound profiling that indicate the presence of phenols, polysaccharides, hydrocarbon and esters. Interestingly, the greater antiradical scavenging activity of M. oleifera was investigated. Among various methods of extraction, extracts obtained by maceration of the dried leaves with ethanol (70%) provided the highest yield (40.50%, w/w) with the important phenolics (13.23 g CAE/100 g extract) and flavonoids content (6.20 g IQE/100 g extract) and displayed high antioxidant potential using DPPH assay (EC50 62.94 g/mL) and ferric reducing power (FRAP) total reducing power activities with inhibitory concentration at 50 percent (IC50) values of 1.02 ± 0.13 mg/mL and 0.99 ± 0.06 mM Fe2+/g, respectively (Xu et al., 2019).

This report investigates also the antibacterial effect of M. oleifera that penetrates deeply into the body’s tissues and particularly into the bone marrow itself cleaning all impurities, toxins, parasites and metabolic wastes (Meireles et al., 2020). Moreover, Alegbeley (2018) revealed that M. oleifera extracts may replace chemicals and antibiotics for waste treatment and disease prevention and control, since it is an abundant source of antioxidants, and coagulating substances. In addition, M. oleifera seed and leaf extracts have exhibited antimicrobial activity against human pathogens such as Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, E. coli, Salmonella typhi, and V. cholerae. Ethanolic extracts of bark and Root of the Moringa species possessed antifungal activity against Microsporum gypseum, Aspergillus niger, Rhizopus stolonifer and Neurospora crassa and also promoted inhibitory effect against Leishmania donovani (Bhattacharya et al., 2018).

This report indicates that M. oleifera infusion was efficient in protecting stomach mucosa of rats against oxidation induced by acidified ethanol and the secretion of mucus; however omeprazole was somewhat less effective in healing ulcerated area. Other studies have demonstrated that Omeprazole was effective in alleviating oxidative stress in the HCl/Ethanol model (Guesmi et al., 2014). The comparative drug, omeprazole (20 mg/kg) and Kaempferol (40, 80 and 160 mg/kg, p.o.) protects stomach against ulcers mediated by ethanol in mice, suppressing neutrophils accumulation myeloperoxidase activity and decreasing the levels of pro-inflammatory cytokines (TNF-α, interleukin-6 (IL-6), IL-1β), improving gastric mucus and NO (Serafim et al., 2020). Interestingly, the main factor implicated in the development and progression of peptic ulceration was an hypersecretory acidic environment and together with dietary factors and/or stress was thought to cause most of peptic ulcer disease (Périco et al., 2020). The mucus and the bicarbonate are the first line of defense against acid because they cover the

**Fig. 6.** Forestomach ulceration (H & E stain); a: sham group with normal forestomach architecture; b: M. oleifera treated group with normal forestomach; c: Forestomach of ulcerated group with absence of a portion of the epithelium, loss of epithelial cell layers of the mucosa extending through to the submucosa, submucosal edema (arrow) and congested mucosal blood vessel (arrow head); d: Stomach and forestomach of M. oleifera-pretreated rat with mild ulceration, congestion of the blood capillaries (arrow head) and submucosal edema (arrow); e: Mild-ulceration of the forestomach of omeprazole-pretreated rats.
entire stomach mucosa and protects against the colonization of bacteria and mechanical forces of proteolytic digestion (Caldas et al., 2014). Other studies have shown the ulceroprotective effect of *M. oleifera*. The ethanolic extract of *M. oleifera* root-bark markedly decreased the free acidity, ulcer index and the total acidity and increased the gastric content pH compared with the control group (Choudhary et al., 2013). The results of the present study are similar to the finding of Devaraj et al. (2007) who found that extracts of *M. oleifera* prepared with acetone and methanol reduced the secretion of gastric acid showing their antisecretory activity. This study is in accordance with recent studies (Almuzafar, 2018), demonstrated that *M. oleifera* ethanolic leaf extract possesses significant gastroprotective effect by reducing ulcer index compared to control (*P* < 0.01), and this effect may be due to its direct action on the mucus secretion or by increasing prostaglandins. Furthermore, *M. oleifera* leaf extracts reduced ulcer index in ibuprofen-induced gastric ulcer model and in pyloric ligation test and a significant reduction in cysteinamine-induced duodenal ulcers and stress ulcers was also observed (Bhattacharya et al., 2018). Oral treatment with HCl/EtOH induced a significant increase of the lesion numbers in the gastric mucosa, gastric juice volume, acidity, reduced the pepsin activity, increased the level of lipid peroxides and diminished tissue antioxidant enzymes (Ganesan et al., 2010). In another report, oral administration of acidified ethanol significantly increase the nuclear translocation factor (NF-κB), also it elevate pro-inflammatory cytokine mRNA (IL-1β and TNF-α), and reduce IkB-α protein expression (Shin et al., 2020).

Ethanol destroys cells by causing mucosal disturbances in the microcirculation of free radicals in the mucosa, increased lipid peroxidation, decreased non-protein sulphydryl groups (GSH) and mucus production and inhibition of gastric prostaglandins (Caldas et al., 2014). Consumption of alcohol may interfere with metabolism and gastric motility (Shin et al., 2020). It occurs throughout the gastrointestinal tract. After absorption, the alcohol dehydrogenase enzyme transform ethanol to acetaldehyde and then to acetic acid, which is cytotoxic to gastric cells (Serafim et al., 2020). Ethanol directly penetrates the mucosa of the stomach, damaging the membrane, exfoliating the cells, and leading to the erosion of tissues via mechanisms particularly the reactive oxygen species (ROS) formation, a decrease in the SH concentrations, increase in the secretion of gastric acid, rupture of the mucus, and mucosal damage due to hemorrhagic lesions, lipid peroxidation induction, cellular apoptosis, and GSH decrease (Périco et al., 2020). In addition, polymorphonuclear cell infiltration that release ROS, increase the pro-oxidative substances and

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**Fig. 7.** Effect of *Moringa oleifera* and omeprazole treatments on the activities of catalase (CAT), glutathione peroxidase and superoxide dismutase (SOD) and the gastric level of malondialdehyde (MDA) in acidified ethanol ulcerated rats. Values are expressed as means ± SD (*n* = 5). Means marked with different letters are significantly different (*P* < 0.05); a: significant difference between sham group and the other groups (*P* < 0.05); b: significant difference between ulcer group and treated groups (*P* < 0.05); c: significant difference between group pre-treated with omeprazole and other groups (*P* < 0.05).
pro-inflammatory molecules formation was mediated after the contact of the gastric mucosa with ethanol (Serafim et al., 2020).

As expected, *M. oleifera* leaf infusion pretreatment protected gastric mucosal layers of rats from ulceration induced by HCl/EtOH. In a study previously reported by Ilijoma et al. (2018), the level of protection of stomach against aspirin-induced ulcer was sufficiently increased in animals treated with 800 mg/kg of Moringa extract as there was increased protection of surface epithelium with more mucus globules. Other reports showed a significant reduction in ulcer index and increase in regenerated glandular epithelium width and collagen content after treatment with *M. oleifera* flower and leaf extracts when compared with ethanol-treated animals (Patel and Lariya, 2019; Devaraj et al., 2007).

When gastric mucosa is exposed to damaging agents, it encompasses the disruption of the unstirred mucus bicarbonate/phospho lipid layer, exfoliation of the surface epithelium with loss of its barrier and the deeper gastric mucosal layers, including microvascular endothelial cells, progenitor, parietal and chief cells (Kangwan et al., 2014).

*M. oleifera* protects the gastric mucosa against oxidative stress through the increase of the antioxidant enzyme activities (SOD, CAT, GPx) and decrease the MDA level. Lipid peroxidation occurs when ROS attack cell membranes, allowing them to enter intracellular structures (Caldas et al., 2014). Our findings are consistent with the data obtained by Ganesan et al. (2010) indicated that HCl/EtOH reduce the levels of GSH dependent antioxidant enzymes (GPx and GST), GSH and antiperoxidative enzymes, such as SOD and CAT in ulcerated rats. Recently, the work done by Kim et al. (2020), demonstrated that the GSH levels and SOD activities were markedly elevated in the stomach tissues of HCl/EtOH-ulcerated rats. Further, it was observed that in acidified EtOH-administered rats there was increased ROS generation assessed by increased level of TBARS and attenuated levels of CAT, SOD and GPx activities and GST along with decreased mucus secretion (Guesmi et al., 2014). According to Shin et al. (2020), gastric cells promote mediation of several antioxidant enzymes to maintain the homeostasis of gastrointestinal tract through the ROS scavenging. The antioxidant system depletion enhances the susceptibility of the gastric mucosal cells to oxygen metabolites and acid mediated cell damage (Ganesan et al., 2010). ROS overproduction under oxidative stress results in stomach cellular damage (Shin et al., 2020). Myeloperoxidase (MPO), as the main markers to investigate antioxidant mechanisms, is an enzyme present in the malondialdehyde (MDA), a final product from the reaction between ROS and membrane proteins responsible for the colors of the leaves (Serafim et al., 2020). The phenolic compounds allylpyrocatechol demonstrated a protective effect on mucosa and submucosa through improvement of prostaglandin expression and activation, which is mediated by the stimulation of COX-1 (Farzaei et al., 2015). Further, 200 mg/kg, p.o. of rutin demonstrated gastroprotective activity against gastric lesions mediated with indomethacin in rats, suppression of the generation of oxidative stress (increasing GSH and SOD and reducing MPO), and inhibition of neutrophil infiltration (Serafim et al., 2020).

**5. Conclusion**

In conclusion, the present observations indicate the potent anti-ulcerogenic effects of *M. oleifera* in rat models. The overall cytoprotective effect of *M. oleifera* may be due to its hydrochloric acid secretion neutralization. In addition, it maintains the antioxidant enzyme levels under the normal conditions and diminish lipid peroxide levels induced by acidified ethanol-treated rats. Hence, the importance of *M. oleifera* requires other studies to explore the principle that act as gastroprotective agent and its mechanism of action. Further, why not to develop ulceroprotective drugs, food diet formulations and to apply the tree in clinical trials.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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