Ulcer Healing Mechanism of Ethanolic Extract of *Talinium triangulare* in Male Wistar Rats

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Abstract

*Talinium triangulare* have been proven to offer promising antiulcer properties which could validate its ‘folkloric’ use for the treatment of gastric ulcer in various part of Nigeria. This study investigates the role of methanolic extract of *Talinium triangulare* (METT) in gastric ulcer healing and the possible mechanisms involved. Twenty-eight male wistar rats (160-180g, n=7) were grouped into; A-control, B-ulcerated untreated, C-ulcerated treated with Omeprazole (20 mg/kg b.w.) and D-ulcerated treated with METT (100 mg/kg b.w.). Gastric ulcer was induced by injecting 0.2 MLs of 40% acetic acid into the glandular part of the stomach for 45 seconds after which it was withdrawn, and the stomach surface cleaned with normal saline. Stomach samples were collected by day 14 post ulceration and assessed for ulcer score; a section of it was fixed for histological evaluation and immunohistochemical (Avidin-Biotin Immunoperoxidase method) analysis. Stomach tissue homogenates were used for enzymatic activities. Results were subjected to statistical analysis using analysis of variance method. There was a significant reduction in the ulcer area of the METT treated group compared with other ulcerated treated and untreated groups. Malondialdehyde concentration of the gastric tissue homogenate was significantly lower in METT treated group compared to other groups. The METT treated group significantly increased the level of superoxide dismutase and catalase compared to other test groups. Histological study showed that only METT treated rats produces predominantly normal mucosa, METT treated rats also expressed CD31 (a marker of angiogenesis) and EGFR (a marker of proliferation) more than the other rats, while the expressions of Ki67 (proliferation) and p53 (Apoptosis) by METT group were not different from other groups.

Methanolic extracts of *Talinum triangulare* accelerated the healing of gastric ulcers in rats probably through reduced oxidative stress, increased cell proliferation, and angiogenesis.

1 Introduction

Peptic ulcer occurs due to an imbalance of the gastric mucous membrane milieu. This causes injury as a result of unevenness between aggressive factors (acid, pepsin, *Helicobacter pylori* and non-steroidal anti-inflammatory drugs) and mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). Gastric ulcer healing is a complex gastric tissue regenerative process comprising of cell migration, proliferation, re-epithelialization, gland reconstruction, angiogenesis, vasculogenesis and matrix deposition, all finally resulting in scar formation. The above-mentioned processes are controlled by growth factors, cytokines, hormones and transcription factors.

These different classes of anti-ulcer drugs namely; antacids, proton pump inhibitors, histamine receptor antagonists, anticholinergics, cytoprotective agents, and antimicrobials.
have been found effective in the treatment of peptic ulcer with different variations and mechanisms in the rate of gastric ulcer healing. Various adverse drug reactions and resistance to synthetic gastric ulcer drugs have been highlighted during the course of treatment not to over stress its cost constraint on the populace involved. As a result, it has been discovered that most patients resolve to herbal formulation from folkloric medicine which have recorded low cost of purchases, less adverse drug reactions and are ready available.

Anti-ulcer potentials of some botanicals like plants, herbs, fruits and vegetables are investigated to replace conventional drugs probably due to their effectiveness as a result of notable inherent active phytochemicals (such as flavonoids, terpenoids, and saponins) as well as their easy availability with low cost. The vegetables, in particular, are used partly as condiments or spices in human diets offering varied useful trait, micro, and macronutrients to the body. *Talinium triangulare* (*Portulacaceae*) popularly known as ‘water leaf’, is a non-conventional vegetable originating from tropical Africa, but grown extensively in West Africa, Asia, and South America. In Nigeria, the indigenous Efiks, Ibibios, and Yorubas, use it in the preparations of ‘Afang’, Edikaiko and ‘Gbure’ soups, respectively. The leaves are rich in vitamins, β-carotene, proteins and minerals (such as calcium, potassium, and magnesium). It has also been documented to contain an appreciable amount of flavonoids, alkaloids, and saponins.

*Talinium triangulare* leaves are documented to be beneficial in the management of the cardiovascular diseases, act as an antibacterial, and as a tonic in the relief of dystocia. The macerated leaves are applied locally to treat a wound, scabies, and cuts. It is also used as a laxative, purgative, anti-diarrhea, and most especially as an anti-ulcer in the gastrointestinal tract probably as a result of its major phytoconstituents which include: alkaloids, flavonoids, saponins, and tannins, known for medicinal and nutritional values.

Despite the significant and promising anti-ulcer properties of *Talinium triangulare*, there is still paucity of information on the mechanism by which this plant exhibit gastric ulcer healing. Therefore, this present work was aimed to evaluate its role in the healing of gastric ulcer induced by acetic acid in male wistar rats as well as the possible mechanisms involved.

2 Materials and Methods

2.1 Collection and identification of plant materials

The leaves of *Talinium Triangulare* were obtained during the peak of a dry season from a waterlogged area near the Faculty of Agricultural sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The fresh leaves of *Talinium triangulare* were identified by Dr. A. T. Ogunkunle, plant taxonomist in the Department of Pure and Applied Biology, Faculty of Pure and Applied sciences, Ladoke Akintola University of Technology, Ogbomoso. A departmental specimen was deposited at the herbarium with a voucher number LHO 354 given.

2.2 Preparation of extracts

The collected identified leaves were air dried and constantly checked in a shade for 20 days to prevent rotting after which it was milled to powder using Philips Electric blender (made by Philips Electronics Ltd., Japan). 500gm of the coarse powder were weighed into glass jars and well macerated in 4000 ml of ethanol, by soaking the sample for 48 hrs and constantly turning with a glass rod for even extraction. The extracted sample was filtered using Whart manns’ filter paper of 9 cm diameter and a pore size of 11μ. The extracted sample was concentrated using a steam bath set at a temperature of 35 °C, so as to preserve the active phytochemicals and compounds present in the extract. After concentrating the filtrate, the percentage yield was 6.4% and a residue of 109.2gm was obtained. The formula for calculating the amount of extract administered to each experimental animal in millilitres is given below.

\[
\text{Weight of extract per 200 g of body weight} = \frac{\text{Percentage yield}}{100} \times \frac{\text{Extract concentration}}{\text{Concentration of extract}}
\]

2.3 Experimental animals

Twenty-eight male Wistar rats, weighing between 160-180gm was purchased from the animal house, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were allowed to acclimatize to the laboratory condition (temperature 25 °C and environmental 12 hours light-dark cycle) for two weeks before the experiment, with free access to solid pellet diet and water ad libitum (throughout the acclimatization and experiment period). All animals received humane care in compliance with the institution’s guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. Animal ethical clearance was obtained from the Oyo state research ethical review committee, Ministry of Health secretariat Ibadan, Nigeria. The reference Number of the ethical approval for this research is AD13/479/134.

2.4 Experimental design

The animals were randomly allocated into 4 groups, with each group consisting of seven rats: Group A- control group (received distilled water), Group B- Ulcerated untreated group (were not given any treatment after ulcer induction), Group C- Ulcerated treated with 20 mg/kg b.w. Omeprazole group and Group D- Ulcerated treated with 100 mg/kg b.w. METT group. The distilled water and extracts were given orally with an orogastric tube for 14 days after which the animals were sacrificed by cervical dislocation. Stomach samples were collected on ice and transferred into cold buffer saline part of which was cut and homogenized for enzymatic activities and part also cut and...
stored in 10% formalin for histological and immunohistochemical staining.

2.4.1 Induction of ulcer

Gastric ulcer was induced using acetic acid with little modifications. Briefly, animals were fasted for 24 hours, anesthetized with a mixture of xylazine (0.005 ml/g b.w.) and ketamine (0.0015 ml/g b.w.) and their stomach exposed. The outer wall of the exposed stomach was held with a pair of forceps ring and fastened for firm grip (to prevent leakage) while 0.2ml of 40% acetic acid was injected into the intra-luminal glandular portion of the stomach using a syringe. The injected acetic acid was later withdrawn after 45 seconds. A cotton wool soaked in normal saline was used to clean the stomach before the abdomen was sutured back and animals were allowed to recover. Animals were then given free access to feed and water throughout the experiment.

2.4.2 Ulcer area

The inner surface of the stomach was observed with a dissecting magnifying glass to evaluate gastric lesion in form of macroscopic ulcer area (mm²) by the method of Tsukimi and Okabe.

2.4.3 Measurement of protein content

The protein content of the gastric tissue collected was measured using method developed by Bradford, as reported by Dewi et al.

2.4.4 Determination of lipid peroxidation status and antioxidant enzyme activities

On sacrificing the animal after the treatment period, stomach was carefully excised, cut along greater curvature and gently rinsed in cold phosphate buffered saline to eliminate the gastric contents. The stomach tissues were then homogenized in a phosphate buffer (pH 7.0) with the aid of a homogenizer after which it was centrifuged at 4000 revolutions/minute for ten (10) min. The supernatant layer was then collected for the biochemical analyses. Malondialdehyde (MDA) concentration, Superoxide dismutase (SOD), Catalase (CAT) and Glucose Oxidase Activities were determined as described in previous studies.

2.4.5 Histological processing and examination

A histological study was carried out as described by Ogihara and Okabe. A small section of a stomach was taken from two distinct areas of each stomach and placed in 10% formalin for histological examination. This excised stomach piece was fixed, cut into 5 μm sections, stained with Hematoxylin and Eosin.

2.5 Immunohistochemistry study

Immunohistochemistry study was carried out by using the Avidin-Biotin peroxidase Complex (ABC) in immunoperoxidase techniques. Serial section 3 micron thick were cut from the paraffin embedded tissue block and allowed to heat on a hot plate for 25 min at 60°C, the tissue sections were deparaffinized in xylene and dried with ethanol. The antigens were retrieved in 10 mmol Sodium Citrate buffer after boiling in the microwave. Endogenous Peroxidase activity was blocked with peroxidase (0.3% H₂O₂) for 15 minutes, then washes with excess water and stabilizes with Phosphate Buffer Solution mixed with tween 20 for 2 minutes. The non-specific binding proteins were blocked with egg Avidin protein in the humidified chamber for 15 minutes and remove gently by washing for 2 minutes with PBS. The sections were then incubated with primary antibody (p53 Ab-6, EGFR Ab-l, Ki67 Ab-1, CD31 Endothelial marker NCL-END, Novacastro laboratories, UK) in a humid chamber for 45 minutes, after which the sections were washed extensively with PBS for 3 minutes. Secondary biotinylated antibodies were used for Incubation for 45 minutes at room temperature followed by washing in PBS thrice, polymerization was initiated by incubating with the Streptavidin-horseradish peroxidase system for 15 minutes, then wash twice with PBS. Adding peroxidase substrate DAB for 15 minutes developed brown colour precipitate indicating positive reaction. The sections were washed with water and counterstained for 2 minutes with haematoxylin, followed by dehydration in graded ethanol, cleared in xylene, and then mounted with DPX and examined under a light microscope at magnification X10.

2.5.1 Evaluation of immunohistochemistry results

To analyze the expression of p53, EGFR, Ki67 and CD31 protein, the intensities of staining and quantity of cells stained were evaluated in proportion to the ulcerated areas. Brown staining of the cytoplasm and nucleus of cells were viewed under X10 magnification. Positive control slides were prepared from a cell known to express the protein a control negative prepared by omitting the incubation state with the primary antibody. An expression index was also created to evaluate the expression as described earlier. Protein expression was classified into four categories based on the number of cells with positive expression.

Results were reported as positive when a complete or incomplete circumferential membrane staining was observed in at least 1% of the ulcerated area. Staining was defined as immunostaining of the ulcerated mucosa above background level and scored as follows:

1+ = weak/mild expression: when a complete or incomplete circumferential membrane staining was observed in at least 1-10% of the ulcerated area,

2+ = moderate expression: when a complete or incomplete circumferential membrane staining was observed in at least 10-50% of the ulcerated area,
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3+ = strong expression: when a complete or incomplete circumferential membrane staining was observed in more than 50% of the ulcerated area.

The absence of membrane staining or cytoplasmic staining was reported as negative.

2.6 Statistical analysis

All values are presented as Mean ± SEM (standard error of the mean). The statistical significance of differences among groups was assessed using one-way ANOVA and value of p<0.05 was considered significant.

3 Results

3.1 Effect of methanolic extract of Talinum triangulare treatment on ulcer area of gastric mucosa after induction of ulcer

There was a significant decrease in ulcer area (by day 14 post-ulcer induction) of gastric mucosa in METT treated group (0.10±0.02 mm²) compared with the ulcerated untreated (0.25±0.01 mm²) and Omeprazole treated (0.15±0.02 mm²) groups (Figure 1).

3.2 Effect of methanolic extract of Talinum triangulare treatment on oxidative stress parameters

Table 1 summarized the effect of methanolic extract of T. triangulare on markers of oxidative stress. The treatments produced no significant effect on glucose oxidase activity and total protein levels. The anti-oxidant factors like SOD and Catalase activities were significantly higher with METT treatment compared to other groups while MDA concentration significantly reduced with METT treatment when compared to other groups.

3.3 Effect of Methanolic extract of Talinum triangulare on the histology of gastric mucosa

Figure 2 below shows the histology of gastric mucosa of experimental animals on the 14th day of ulcer healing. The METT treated animals showed predominantly normal mucosa compared to the deep ulceration and oedema with mild inflammation seen in ulcerated untreated and Omeprazole treated animals, respectively.

3.4 Immunohistochemical Analysis

3.4.1 Effect of Methanolic extract of Talinum triangulare on the Ki67 expression

Figure 3 below shows the expression of Ki67 by day 14 post ulceration; all other groups of animals showed mild expression of Ki67, except the Omeprazole treated animals that were negative for the expression of Ki67.

3.4.2 Effect of methanolic extract of Talinum triangulare on the epidermal growth factor receptor (EGFR) expression

Figure 4 below shows the expression of Epidermal Growth Factor Receptor (EGFR) by day 14 post ulceration; all other groups of animals showed mild expression of EGFR, except the group of ulcerated animals treated with METT that has a moderate expression of EGFR on the mucosa.

3.4.3 Effect of methanolic extract of Talinum triangulare on the expression of CD31

Figure 5 below shows the expression of CD31 by day 14 post ulceration; the ulcerated untreated rats showed mild expression of CD31, the control rats and ulcerated treated with Omeprazole rats showed moderate expression while the ulcerated animals treated with METT has High expression of CD31 on the mucosa.

3.4.4 Effect of methanolic extract of Talinum triangulare on the expression of p53

Figure 6 below shows the expression of p53 by day 14 post ulceration; all the groups of rats showed mild expression of p53 on the gastric mucosa.

3.4.5 Effect of methanolic extract of Talinum triangulare on the intensity of expression of Ki67, EGFR, CD31 and p53

Table 2 below showed the intensity of expression of Ki67, EGFR, CD31 and p53 by day 14 post ulceration.

4 Discussions

The form of gastric ulcer produced for this study has been established to resemble human ulcers in terms of pathological features and similar healing processes and or mechanisms. This study sought to investigate the probable roles methanolic extract of Talinum triangulare (METT) would have on healing of acetic acid-induced experimental gastric ulcers.

The previous study has confirmed the leaves to contain flavonoid reported to have gastric cytoprotective effect as well as essential nutrient like β-carotene, minerals, vitamins, omega 3-fatty acid which have been identified to be responsible for antioxidant as well as anti-inflammatory activities of the leaf.
Saponin, especially triterpenes type mediate its effect by the potentiating formation of mucus on the gastric mucosa thus protecting the mucosa from the adverse effect of gastric acid by inhibiting prostaglandin F₂ alpha \(^{35,36}\).

**Table 1: Effect of methanolic extract of *Talinum triangulare* treatment on oxidative stress parameters and protein content of the stomach**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group(A)</th>
<th>Ulcerated untreated(B)</th>
<th>Ulcerated treated with omeprazole(C)</th>
<th>Ulcerated treated with METT(D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>17.91 ±2.17 (^a)</td>
<td>20.93 ±2.71 (^a)</td>
<td>17.62±1.75 (^a)</td>
<td>14.41±1.01 (^b)</td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>2.20 ±0.35 (^a)</td>
<td>2.91 ±0.64 (^a)</td>
<td>2.74±0.60 (^a)</td>
<td>2.43±0.28 (^a)</td>
</tr>
<tr>
<td>SOD (unit/g)</td>
<td>8.54 ±0.30 (^a)</td>
<td>8.24 ±0.58 (^b)</td>
<td>8.26±0.24 (^b)</td>
<td>9.78±0.30 (^b)</td>
</tr>
<tr>
<td>Catalase (unit/g)</td>
<td>26.10 ±6.34 (^a)</td>
<td>25.67 ±7.00 (^a)</td>
<td>28.12±5.81 (^a)</td>
<td>30.66±6.96 (^b)</td>
</tr>
<tr>
<td>Total Protein</td>
<td>69.00±15.00 (^a)</td>
<td>67.62±17.84 (^a)</td>
<td>74.06±12.19 (^a)</td>
<td>94.30±5.70 (^b)</td>
</tr>
</tbody>
</table>

\(^a\) means significantly different at P<0.05 when compared with \(^b\), Values are expressed as Mean ± SEM

**Table 2: Shows the intensity expression of Ki67, EGFR, CD31 and p53**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ki 67 Expression</th>
<th>EGFR Expression</th>
<th>CD31Expression</th>
<th>p53 Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>10%</td>
<td>10%</td>
<td>50%</td>
<td>10%</td>
</tr>
<tr>
<td>B (Ulcerated untreated)</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>C (Ulcerated treated with Omeprazole)</td>
<td>0%</td>
<td>10%</td>
<td>50%</td>
<td>10%</td>
</tr>
<tr>
<td>D (Ulcerated treated with METT)</td>
<td>10%</td>
<td>50%</td>
<td>90%</td>
<td>10%</td>
</tr>
</tbody>
</table>

**Figure 2: Photomicrography of the histology of gastric mucosa of experimental rats (H&E staining MAGX10).** (A) Control (Arrow indicating a normal gastric mucosa); (B) ulcerated untreated (Arrow showing area of deep ulceration); (C) ulcerated treated with omeprazole (20 mg/kg) (Arrows indicating oedema and mild inflammation); and (D) ulcerated treated with METT (100 mg/kg) (Arrow indicating a predominantly normal mucosa).

Administration of METT to animals (with experimental gastric ulcers) significantly reduced gastric ulcer area compared to the untreated and omeprazole treated groups. This observed enhanced healing rate might be as a result of earlier identified phytochemicals (flavonoids and saponins) presence in the leaf extract. These phytochemicals might have facilitated the rate at which healing occurred via investigated immunohistochemical receptor mechanisms in this study.

During gastric ulcer formation, there is a constant imbalance between aggressive and cytoprotective factors\(^{37}\); this ultimately leads to the generation of free radicals (increased oxidative stress) that prevents or slows down the rate of healing\(^{38}\).

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Lipid peroxidation as a biomaker of oxidative stress is caused by aggravated reactive oxygen species (ROS). It had been confirmed that endogenous antioxidants (superoxide dismutase and catalase) are also involved in mopping up these free radicals produced during healing processes. The reaction of superoxide dismutase with these ROS leads to the production of hydrogen peroxide within itself is also toxic and hinders healing. Catalase, on the other hand, reacts with this hydrogen peroxide produced to form water and oxygen to the healing tissue. The rate at which these endogenous antioxidant enzymes are produced to mop up the ROS produced invariably leads to being enhanced or facilitated ulcer healing.

Figure 3: Photomicrograph showing the expression of Ki67 on the stomach section of experimental rats on day 14 post ulceration (MAG.X10). (A) Control (Arrow indicates mild surface expression) (+1); (B) Ulcerated untreated (Arrow indicates area of mild surface expression) (+1); (C) Ulcerated treated with Omeprazole (Arrow indicates negative expression) (0) and (D) Ulcerated treated with METT (Arrow indicates mild expression) (+1) of Ki67 on the gastric mucosa.

Figure 4: Photomicrograph showing the expression of EGFR on the stomach section of experimental rats on day 14 post ulceration (MAG.X10). (A) Control (Arrow indicates area of mild surface expression) (+1); (B) Ulcerated untreated (Arrow indicates area of mild surface expression) (+1); (C) Ulcerated treated with Omeprazole (Arrow indicates area of mild surface and deep mucosa expression) (+1) and (D) Ulcerated treated with METT (Arrows indicate area of moderate expression) (+2) of EGFR on the gastric mucosa.

The methanolic extract of *Talinium triangulare* leaves was able to mitigate against the oxidative stress produced during ulcer formation and healing by significantly reducing lipid peroxidation (MDA) values with a concomitant increased endogenous antioxidant (SOD and catalase) activities. This is suggestive of a probable mechanism by which the leaves extracts exerts its anti-ulcer property thus promoting healing of the ulcer. Studies on extracts of *Zingiber officinale* and *Althaea officinalis* also
reduced MDA with increased level of SOD and GSH on Pyloric Ligation-Induced Gastric Ulcer\cite{41}.

Figure 5: Photomicrograph showing the expression of CD31 on the stomach section of experimental rats on day 14 post ulceration (MAG.X10). (A) Control (Arrow indicates area of moderate expression) (+2); (B) Ulcerated untreated (Arrow indicates area of mild expression) (+1); (C) Ulcerated treated with Omeprazole (Arrow indicates area of moderate mucosa expression) (+2) and (D) Ulcerated treated with METT (Arrows indicate area of high expression) (+3) of CD31 on the gastric mucosa.

Figure 6: Photomicrograph showing the expression of p53 on the stomach section of experimental rats on day 14 post ulceration (MAG.X10). A) Control (Arrow indicates area of mild surface mucosa expression) (+1); (B) Ulcerated untreated (Arrow indicates area of mild expression) (+1); (C) Ulcerated treated with Omeprazole (Arrow indicates area of mild surface and deep mucosa expression) (+1) and (D) Ulcerated treated with METT (Arrows indicate area of mild expression) (+1) of p53 on the gastric mucosa.

Gastric ulcer healing is a genetically programmed cell repair process which includes inflammation, cell proliferation, re-epithelization, the formation of granulation tissue, angiogenesis, an interaction between various cells and the matrix and tissue remodelling, all resulting in scar formation\cite{2,42}. These events are controlled by programmed cytokines, growth and transcription factors activated by tissue injury in spatially as well as temporarily coordinated manner\cite{43,44}. The molecular mechanism by which METT promotes gastric ulcer healing was investigated immunohistochemically in this study by the evaluation of proliferative (Ki67 and EGFR), apoptosis (p53) and angiogenesis (CD31) markers.

The re-growth of blood vessels into the ulcerated area, i.e., angiogenesis is essential for healing to take place as increased flow of nutrients and oxygen to the ulcerated or breached tissue accelerates the healing process/ rate\cite{45,46}. This initial step is of utmost importance for healing to occur. The cluster of...
differentiation 31 (CD31) is a protein molecule which is important for leukocyte migration, angiogenesis, and integrin activation. Previous studies have recently recognised CD31 for its angiogenic role as well as mediating endothelial cell migration in vitro. Animals treated with METT showed the highest expression of CD31 on the gastric mucosa surface following immunohistochemical staining compared to other treatment groups in this study. This is indicative that angiogenesis process was higher in the METT treated animals, which could be responsible for the observed faster healing activity recorded in this group of animals. Angiogenesis is critical for the improvement of the gastric mucosa as well as prevention of ulcer relapse.

In the process of gastric ulcer healing, Cell proliferation also plays a major role with Ki67 being an important protein marker during this process. Studies have observed and reported that removal of Ki67 by antisense nucleotide did prevent the proliferation of cells during healing. Immunohistochemical analysis of animals treated with METT showed positive mild surface expression in all groups except the omeprazole treated group where there is a negative expression. This finding is suggestive of *T. triangulare* methanolic leaves extract inducing or stimulating proliferative activity than the reference drug (Omeprazole) during the healing of the gastric ulcer.

The stimulus for increased epithelial cell proliferation in the mucosa of the ulcer margin is most likely initiated by Epithelial Growth Factors (EGF) and/or Tissue Growth Factor (TGF)-
alpha with the marked increase of EGF receptors and EGF-producing cells around experimental gastric ulcers in rats induced by acetic acid. The previous study also reported that EGFR plays an important role in apoptosis, proliferation, differentiation and activate cell migration via autocrine or paracrine action. In this study, the expression of EGFR was mild in other groups but moderate in the *Talinum triangulare leaf extract* treated group this might be suggestive of another probable mechanism by which it (METT) might have facilitated experimentally induced gastric ulcer healing.

P53 is also known as cellular tumor antigen p53 or phosphoprotein p53 or tumor suppressor; p53 is an apoptotic protein seen to be expressed in so many cancers, aging and some chronic inflammation cases. The expression of p53 was not significant for the healing rate since there was no disparity in the intensity of expression among the groups which might be suggestive that apoptosis may not have a role in this (form of experimental chronic gastric ulcer) healing process.

The histological observations show that there were no injuries to the gastric mucosa of the control group compared with the untreated group which showed the appearance of deep and surface at mucosal ulceration. There was mild disruption of the mucosa and edema with mild inflammation in the group treated with omeprazole while those treated with *Talinum triangulare* showed apparently normal gastric mucosa. The results implied that treatment with *Talinum triangulare* at a dose of 100 mg/kg improved healing of gastric ulcers better than omeprazole. These histological (H and E staining) evaluations revealed that rats treated with *Talinum triangulare* demonstrated the ability to regenerate breached or ulcerated gastric mucosa faster than other treatment groups.

**5 Conclusions**

In conclusion, healing was probably accelerated in METT treated group due to the appreciable amount or presence of flavonoids and saponins in the leaves. The observed suggestive mechanism by which *Talinum triangulare* accelerated gastric ulcer healing might be by increased proliferative and angiogenic activities via an antioxidant dependent pathway.

**6 Acknowledgements**

The corresponding author thanks Mr Jonathan Maduweke, a pathologist of The Immunohistopathological unit, National Hospital, Abuja, Nigeria for the technical assistance given during the Immunohistochemistry study.

**7 Conflict of interests**

There is no conflict of interests in this work.

**8 Author’s contributions**

AAF and SAT carried out literature review and draft the manuscript. AAF designed and monitored the experimental protocol. FMA collected the material and performed whole experimental procedures. All authors read and approved the final manuscript.

**9 References**


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