Anti Ulceration Efficacy of Boiled Aqueous Leaf Extract of *Morinda lucida* on Ethanol Induced Gastric Ulceration Rats

Nkiruka Millicent Amadi¹, Peter Uwadiegwu Achukwu², S. O. Onwukwe², Emmanuel Ifeanyi Obeagu³, Nonyelum V. Anoh¹ and Victoria I. Okpokwu⁴

¹Department of Medical Laboratory Science, ESUT College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria.
²Department of Medical Laboratory Science, University of Nigeria, Enugu Campus, Enugu State, Nigeria.
³Department of Medical Laboratory Science, Imo State University, Owerri, Imo State, Nigeria.
⁴Department of Medical Laboratory Science, Enugu State University of Science and Technology Teaching Hospital, Parklane, Enugu, Enugu State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i47A33000

Editors:
(1) Dr. Sirigireddy Sivajothi, Sri Venkateswara Veterinary University, India.

Reviewers:
(1) Shaimaa Mahmoud Mohamed Saleh, Assiut University, Egypt.
(2) Jigar Goswami, RK University, India.

Complete Peer review History: [https://www.sdiarticle4.com/review-history/75142](https://www.sdiarticle4.com/review-history/75142)

Received 15 August 2021
Accepted 21 October 2021
Published 25 October 2021

ABSTRACT

Stomach ulceration study was carried on 25 groups (5 rats each of the groups), groups (E, E₁, E₂, E₃ and E₄), placed on 24 hours fasting before the single dose of intra peritoneal dose administration of 5ml/kg body weight of 99% ethanol and after one hour, received daily extract of dilution doses of ((500, 1000, 1500) mg /kg) body weight and 100 mg /kg body weight cimetidine (standard drug) respectively for 7 days. Groups (EA₁, EA₂, EA₃ and EA₄) were treated with the same extract doses and drug concentration for 7 days before the dose ethanol administration. Group E and 0 served as a positive control and a negative control respectively. On day 9, target organs; stomach and intestines were harvested under anaesthetize weighed, gross macroscopically and

*Corresponding author: E-mail: emmanuelobeagu@yahoo.com;
histomorphological studied. Result of the study showed plant inhibition on ethanol induced gastric ulceration; the standard drug (cimetidine) and the mapped extract doses of ((500, 1000, 1500) mg /kg) body weight respectively showed normal organ architecture. Ulcer index study activities 70% indicating evidence of curative and preventive index range 35 to 43% showed some inhibition as seen on the gastric mucosa of the treated group. It was observed that the treatment and anti-ulceration effect of boiled aqueous leaf extract metabolites showed reduction on the histomorphological changes in the gastric mucosa and provided inhibition effectiveness of ethanol induced injury.

Keywords: Anti ulceration; efficacy; boiled aqueous leaf extract; Morinda lucida; ethanol gastric ulceration; rats.

1. INTRODUCTION

The result of the phytochemical screening on the powered leaves sample showed evidence of alkaloids, anthraquinones, flavonoids, saponins, tannins, cardiac glycosides and phlobatannins [1]. Oshiobugie et al. [2] report on phytochemical analysis of Morinda lucida methanol leaf extract revealed presences of five bioactive compounds and mineral constituents; Iron, Magnesium, Potassium, Phosphorus and Copper; while the phytochemical screening showed significant presences of secondary metabolites such as tannins, anthocyanin, steroid, terpenoids, saponins, phenolic compound, reducing sugar and alkaloids. Phytochemical analysis of Morinda lucida leaf extracts reveals presence of alkaloid, flavonoids, saponins, tannins, glycosides, anthraquinones, phenolic compound and absence of oxalates [3-5].

Gastrointestinal tracts are a connection sphincter muscular tube situated between the oesophagus and the rectum; stomach lies in the upper part of the abdomen just below the ribcage and extends to intestines [6]. Ingested substances are pushed down through hollow to mix with enzymes and chemical substances such as Hydrochloric acid in the stomach to aid food churning and break down into chemical substances; its main function is absorption of nutrients into blood capillaries and lymphatic drainages to nourish the body [6].

Gastrointestinal disorders are mostly caused by infection, drugs, ingestion of alcohol, irritant or corrosive substances and other predisposing factors among which Gastritis, ulceration, pernicious anaemia and tumour are common complications of lesion [7]. There are several varieties of diseases such as mal-absorption, ulceration and intestinal haemorrhage due to infections and other predisposing factors that can lead to damage of intestinal villi such infections include; Tuberculosis, Typhoid and amoebic dysentery [8]. Ulceration disorders are mostly common and occur as sores or erosion of muscular tube of the gastrointestinal tracts which may lead to necrotic lesion of the gastric mucosa by distortion of the wall [9].

Pharmacokinetics is a process of drug administration which binds to circulate mainly in the wall of blood vessels through diffusion; the plasma proteins will increase the rate of passive absorption by maintaining the concentration gradient of free drug which may be influenced by routes of administration [10,11]. In oral drugs administration, the bioavailability of gastrointestinal absorption rate is reduced by the presence of food in the gut while some drugs are enhanced by food bile secretion by liver in response to food in the tract to increase drug absorption [12]. During gastrointestinal drug absorption, some drugs are irritating and may cause adverse effects by producing free radicals, intracellular oxidative stress, mitochondrial permeability changes and depolarization protein [13]. Mucus secretion is important in defense mechanisms and protection of the gastric mucosa against necrotic lesions [14]. These necrotic lesions result from damage due to imbalance between the gastric defense factor and the aggressive factor thus generating radical scavengers that affect the tract [15].

2. MATERIALS AND METHODS

The effect of the boiled aqueous leaf (BL) of Morinda lucida extracts on ethanol induced gastric ulceration were studied using two (2) stages (curative and anti-ulcer) analysis.

2.1 Animals

Twenty five (25) albino rats of mixed sexes, body weighing between 130-250 g were used in the study.
2.2 Drugs and Chemicals

Ethanol 99.7-100% v/v GPR re-packaged in Nigeria by NAAFCO scientific supplies LTD was purchased from Mekason chemicals Ltd Ogbete Enugu metropolis of Enugu state while Cimetidine tablet B.P 400 mg manufactured by Krishat Pharmaceutical Industry Ltd Ibadan, Nigeria was purchased from Arluc pharmaceutical shops Trans- Ekulu Enugu, Enugu State, Nigeria.

2.3 Boiled Aqueous Leaf crude (BL) Extracts Preparation

Extractions were prepared by immersing 250 g of respective plant parts powder to 1000 ml of boiled water respectively following with the model:

1. The boiled aqueous extractions were placed on hot plate heat for 30 mins on a shaker to ensure maximum extractions.

2. Resultant crude extractions were obtained by first filtration through the muslin cloth; then further filtrations through Whatman No. 1 filter paper.

3. The filtrates were placed in an oven set at 60°C to get rid of water. Each jelly concentrate obtained was placed in a well labeled plastic container and stored in a refrigerator at 4°C until required.

2.4 Experimental Drug Treatment Design

The ulceration activities were conducted by grouping the animals into E, E₁, E₂, E₃, E₄, EA₁, EA₂, EA₃, EA₄, & E₀ groups of 5 rats each.

2.4.1 Curative analysis

1. Group E, E₁, E₂, E₃, E₄ were placed on 24 hours fasting before receiving a single dose of intra peritoneal administration of 5 ml/kg b. wt of 99% Ethanol.

2. After one hour, E₁, E₂, E₃ and E₄ groups commence daily boiled extract of doses ((500, 1000, 1500) mg/kg) b. wt and 100 mg/kg body weight cimetidine respectively for 7 days.

3. Group E serve as a positive control and received no extract or drug treatment for 7 days [16] modifications.

2.4.2 Anti-ulcer analysis

1. Group EA₁, EA₂, EA₃ and EA₄ seven received (7) days daily administration of different concentrations of boiled aqueous leaf extract ((500, 1000, 1500) mg/kg) body weight and 100 mg/kg body weight cimetidine respectively.

2. On the 8th day were placed on 24 hours fasting before receiving a single dose of intra peritoneal administration of 5 ml/kg of 99% Ethanol.

3. Group E₀ serve as negative control and was not be treated while E₀₁, E₀₂ and E₀₃ received daily boiled extract of doses ((500, 1000, 1500) mg/kg) b. wt [17] Nworgu et al., 2019 modifications).

2.5 Ulcerative Index

Ulceration lesions were counted using dissecting microscope and scored based on grading scale as: Normal stomach (0); Red colouration (0.5); Spot ulceration (1); Haemorrhagic streaks (1.5); Deep ulcer (2); Perforation (3). Mean ulcer scores of each animal were expressed as ulcer index (Raju et al., 2009).

2.6 Histopathological Study

On Day 9 of various treatments, the animals were anaesthetized and sacrificed. Gastric emptying was done, target organs (stomach) were dissected out for gross macroscopic architectural studies and processed for microscopic examinations using Haematoxylin and Eosin staining methods.

3. RESULTS

3.1 Ulcer Index

E₄, E₁, E₂ and E₃ stomach ulcer index shows significant increase (P < 0.001) compared to Positive control group E as shown in table 1. EA₄, EA₁, EA₂ and EA₃ stomach ulcer index shows significant increase (P < 0.001) compared to Positive control group EA₀ as shown in Table 2.

3.2 Histopathological Findings

Stomach section from group E shows an area of epithelial erosion and mild infiltration of the
submucosal region by inflammatory cells while the stomach section from E_C shows an area of epithelia erosion with mild infiltration by inflammatory cells. E_1 shows an area of erosion extending into the submucosa while E_2 and E_3 appear normal with intact epithelia and submucosa.

Stomach section from EA_0 shows an area of erosion extending into the submucosa (arrows) with mild infiltration by inflammatory cells while EA_C sub mucosa appears normal with slight sloughing of the gastric epithelia. Group EA_1, EA_2 and EA_3 shows intact stomach histoarchitecture without erosion or epithelia disruption.

Fig. 1. Macrography of stomachs from rats treated in Group E_1 (A) and E_2 (B)

Fig. 2. Photomacrography of stomachs empties from rats treated in Groups E_3 (C) and E_C (D)
Fig. 3. Photomacrography of stomachs gastric empties from rats in Groups EA₁ (E) and EA₂ (F)

Fig. 4. Photomacrography of stomachs gastric empties from rats in Groups EA₃ (G) and EA₄ (H)

MICROGRAPHS
ULCER CURATIVE STUDY

Fig. 5. Stomach section from group E₀, E₀₁, E₀₂ and E₀₃ appears normal with intact epithelia (E) and submucosa (S).
Stain: H&E Magnification: X100

Fig. 6. Stomach section from E – positive control shows an area of epithelial erosion (arrow) and mild infiltration of the submucosal region (*) by inflammatory cells.
Stain: H&E Magnification: X100

Fig. 7. Stomach section Eₐ – standard drug control group shows an area of epithelia erosion (arrows) with mild infiltration by inflammatory cells.
Stain: H&E. Magnification: X100
Fig. 8. Stomach section from $E_1$ shows an area of erosion extending into the submucosa (arrows)
Stain: H&E Magnification: X100

Fig. 9. Stomach section from $E_2$ appears normal with intact epithelia (E), submucosa (S)
Stain: H&E Magnification: X100

Fig. 10. Stomach section from $E_3$ appears normal with intact epithelia (E), submucosa (S).
Stain: H&E. Magnification: X100
Fig. 11. Stomach section from EA₀ shows an area of erosion extending into the submucosa (arrows) with mild infiltration by inflammatory cells

*Stain: H&E Magnification: X100*

Fig. 12. Stomach section from EA₀ appears normal with slight sloughing of the gastric epithelia (arrow), submucosa (S) appears normal

*Stain: H&E Magnification: X100*

Fig. 13. Stomach section from EA₁ appears normal with intact epithelia (E), submucosa (S)

*Stain: H&E Magnification: X100*
Fig. 14. Stomach section from EA₂ appears normal with intact epithelia (E), submucosa (S)
Stain: H&E. Magnification: X100

Fig. 15. Stomach section from EA₃ appears normal with intact epithelia (E), submucosa (S)
Stain: H&E. Magnification: X100

Table 1. Ulcer Treatment effects of extracts

<table>
<thead>
<tr>
<th>Treatment group (dose)</th>
<th>Ulcer index</th>
<th>Ulcer curative index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E  Positive control (ethanol only)</td>
<td>1.33 ± 0.15</td>
<td>-</td>
</tr>
<tr>
<td>E₉ (Drug control/cimetidine group)</td>
<td>0.47 ± 0.07**</td>
<td>64.99</td>
</tr>
<tr>
<td>E₁ (low dose extract)</td>
<td>0.37 ± 0.09**</td>
<td>72.49</td>
</tr>
<tr>
<td>E₂ (medium dose extract)</td>
<td>0.30 ± 0.06**</td>
<td>77.49</td>
</tr>
<tr>
<td>E₃ (high dose extract)</td>
<td>0.37 ± 0.07**</td>
<td>72.49</td>
</tr>
</tbody>
</table>

Values for ulcer index are expressed as mean ± S.E.M. Statistical significance was set at P < 0.05. ** - P < 0.001 compared to the Positive control group

Table 2. Antiulcer effects of extracts

<table>
<thead>
<tr>
<th>Treatment group (dose)</th>
<th>Ulcer index</th>
<th>Ulcer preventive index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA₀ Negative control (ethanol only)</td>
<td>1.33 ± 0.15</td>
<td>-</td>
</tr>
<tr>
<td>EA₉ (Positive control/cimetidine group)</td>
<td>0.77 ± 0.07**</td>
<td>42.48</td>
</tr>
<tr>
<td>EA₁ (low dose extract)</td>
<td>0.87 ± 0.14**</td>
<td>34.98</td>
</tr>
<tr>
<td>EA₂ (medium dose extract)</td>
<td>0.87 ± 0.03**</td>
<td>34.98</td>
</tr>
<tr>
<td>EA₃ (high dose extract)</td>
<td>0.77 ± 0.07**</td>
<td>42.48</td>
</tr>
</tbody>
</table>

Values for ulcer index are expressed as mean ± S.E.M. Statistical significance was set at P < 0.05. ** - P < 0.001 compared to the negative control group
4. DISCUSSION

Oral administration of alcohol in animals has a strong diffusion effect into the gastric mucosa thus producing lesions [18]. Study revealed that alcohol-mediated inflammation signals are caused by the increased production of pro-inflammatory cytokines [19]. Necrotic lesions occur from damage due to an imbalance between the gastric defensive and toxic factor which can be due to physical, chemical or psychological action on the mucosal epithelia [20]. Ulceration results in an increase of total acid secretion of the gastric contents present in the stomach [21]. Radical scavenger produced by disruption of mucus-bicarbonate barrier, sufficient mucus secretion lead to ischemia causing blood vessel wall rupture and depolarization that result to cell death play very crucial role in stomach ulcer formation [22]. Excessive alcohol ingestion results in coagulopathy process in the stomach lesion to produce hemorrhage, severe congestion in the sub mucosa inflammatory cell infiltration, epithelial cell loss [23].

Findings from the ulcer index study activities indicated evidence of aqueous boiled leaf curative index above 70% and anti-ulceration index range 35 to 43 %. Ulcer index evaluation on the degree of percentage inhibition against stomach ulceration lesions plays an important role in healing and protective assessment [24]. Treatment and protective ulceration reduced histomorphological changes in the gastric mucosa and provided inhibition effectiveness of ethanol induced injury. Anti-inflammatory plays a crucial role in gastric ulceration inhibition activities by stimulating neutrophil infiltration in gastric inflamed areas [25]. Flavonoids are among the most metabolites that poses anti-inflammatory activities [26-29].

5. CONCLUSION

The study on Morinda lucida aqueous boiled leaf treatment and protective ulceration may be attributed to flavonoid constituents in the extract. Research had shown that plant rich flavonoids have inhibitory effect on induced gastric injury or ulceration.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


© 2021 Amadi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/75142