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ORIGINAL ARTICLE

Anti-ulcerant Activity of an Aqueous Fruit Extract of *Musa x paradisiaca* on Acetic Acid-Induced Gastric Ulceration in ICR Mice

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Peptic ulcer disease has and continues to cause high mortality in Ghana and other countries worldwide. This study investigates the anti-ulcerant effect of an aqueous fruit extract of *Musa x paradisiaca* and its possible receptor site of action to verify and ascertain its traditional use. Phytochemical analyses on the extract revealed the presence of alkaloid, tannins, saponin, glycosides and flavonoids. Thin layer and high performance liquid chromatography analyses performed on the extract to establish fingerprint chromatograms showed four spots and three peaks respectively. Acetic acid–induced (0.2 ml; 8%) gastric ulceration in ICR mice treated with 0.2, 0.4, and 0.8 mg/kg of the extract and 0.3 mg/kg Esomeprazole significantly decreased the ulcerative index ($P \leq 0.001$) and the number of ulcers formed per stomach and increased curative ratio ($P \leq 0.01 - 0.001$). Histopathological studies of gastric mucosa showed corrections in the architectural distortions caused by the acetic acid-induced ulceration. Contractile effect of histamine on the isolated guinea-pig ileum was significantly inhibited ($P \leq 0.001$) by Mepyramine and the extract. The aqueous fruit extract of *Musa x paradisiaca* has anti-ulcerant property in ICR mice and possibly works as an antagonist to histamine receptors.
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Keywords*: Ulcer, traditional medicine,* rodent, Musa x paradisiaca, acetic acid.

INTRODUCTION

Peptic ulcer is a disease of the gastro-intestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion and it usually occurs in the stomach and proximal duodenum (Ramakrishnan and Salinas, 2007). Peptic ulcer may also result from *Helicobacter pylori* which survive the acidic environment of the stomach (Baako and Darko, 1996; Goodman *et al.,* 1997; Peach *et al.,* 1997). Infections and co-morbidities that are associated with peptic ulcer disease e.g., cytomegalovirus, tuberculosis, Crohn's disease, hepatic cirrhosis, chronic renal failure, sarcoidosis and myeloproliferative disorders increases the risk of patients having more complications (Ramakrishnan and Salinas, 2007; Huang *et al.,* 2012). The use of non-steroidal anti-inflammatory drugs (NSAIDs) could also lead to peptic ulcer development (Bytzer and Teglbjaerg, 2001; Hamid *et al.,* 2006) while burns and trauma, acute illness, multi-organ failure and ventilator support can cause physiologic stress ulcers (Ramakrishnan and Salinas, 2007; Wachirawat *et al.,* 2003). The lifetime risk for developing peptic ulcer is approximately 10% (Snowden, 2008).

Peptic ulcers are associated with several signs and symptoms which include, abdominal and epigastric pain, indigestion, loss of appetite, weight loss, vomiting, heart burns and reflux disease (Spiegelhalter *et al.,* 1987). In very severe conditions, certain complications like gastrointestinal bleeding, perforation and gastric outlet obstruction may set in (Hilton *et al.,* 2001).

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A number of drugs including proton pump inhibitors, prostaglandin analogues, histamine receptor antagonists and cyto-protective agents are available for the treatment of peptic ulcer. Most of these drugs produce several adverse reactions including toxicities and may even alter biochemical mechanisms of the body upon chronic usage (Ariyphisi *et al.,* 1986).Although there are several effective orthodox medications for the treatment of ulcer on the market, research into plant extracts with potent medicinal activities have been on the rise as they are readily available and reported to be comparatively safer,. Most African societies depend on medicinal activities of plants as their orthodox congeners are expensive and not readily accessible. Ethno-medicinal remedies are being adopted in disease treatment as herbs are staging a comeback and herbal renaissance is happening world-wide (Chinthana and Ananthi, 2012).

In the Ashanti Region of Ghana, the freshly chopped fruits of *Musa x paradisiaca* are steeped in hot water and the decoction taken orally for the treatment of peptic ulcer. The aim of the study was to determine the anti-ulcerant activity of the aqueous fruit extract of *Musa x paradisiaca* (Family Musaceae) as used traditionally in the management of peptic ulcer and the possible receptor site(s) of action.

MATERIALS AND METHODS

Collection, Identification and Authentication of Plant Material

The unripe fruit of *Musa x paradisiaca* (Family Musaceae) was obtained in January, 2013, at Ayeduase, a suburb in the Kumasi Metropolis of the Ashanti Region. It was authenticated at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. A specimen of the plant sample (voucher number: KNUST/HM1/2012/S010) has been kept in the Department of Herbal Medicine, KNUST, Herbarium.

Preparation of Aqueous Fruit Extract of *Musa x paradisiaca*

The unripe fruit of *Musa x paradisiaca* was washed thoroughly and chopped into small pieces. Eight kilograms of the chopped pieces was soaked in 11 litres of distilled water and allowed to stand for at least 8 hours and strained. The strained solution was then dried in a hot air oven (Gallenkamp Oven 300 Plus series, Weiss Technik, UK) at 40°C to obtain 15 g of a solid mass which was labeled aqueous fruit extract of *Musa x paradisiaca* (AEMP) for use in this study. AEMP was then reconstituted in distilled water and administered at different doses to experimental animals.

Phytochemical Screening

Phytochemical analysis was done to ascertain the presence of secondary metabolites such as flavonoids, phytosterols, alkaloids, glycosides (saponin glycosides, anthracene glycosides, cyanogenetic glycosides), tannins and terpenoids on AEMP using standard procedure as described by Wagner and Bladt, (1996); Harborne (1998) and Kujur *et al.* (2010).

Thin Layer Chromatography

Aluminium pre-coated silica gel plates 60 F_{254} (0.25 mm thick) was cut to an appropriate size so as to fit in a chromatank. AEMP (5 mg) was constituted in ethanol (95%) and applied onto the TLC plates as spots with the aid of capillary tubes at one end of the plate in a straight line of about 2 cm above the edge and 1.5 cm away from the margins. Using the one way ascending technique of TLC development, the plates bearing the dried spots were placed in a chromatank saturated with a chloroform, ethanol and water (ratio: 7:3:0.5) solvent system as the mobile phase (Praha *et al.,* 2011). The zones on the TLC plates corresponding to separated components were detected under UV light 254 nm and 366 nm by spraying with anisaldehyde (0.5 % w/v) in an acetic acid/sulphuric acid/methanol mixture (ratios: 10:5:85) and heating for 5-10 minutes at 105°C.

High Performance Liquid Chromatography (Qualitative Analysis)

Approximately 2 ml of a 0.1% w/v ethanol solution of AEMP was transferred into 1cm square cuvette and placed in a double beam UV machine (T90 +

UV/Visible Spectrophotometer, PG Instruments Ltd., UK). A quantity (20 µl) of the sample was analyzed isocratically at a wavelength of 230 nm (flow rate of 1 ml/min) to obtain a chromatogram.

Ethical and Biosafety Considerations
Laboratory studies were carried out in a level 2 biosafety laboratory. Protocols for the study were approved by the Departmental Ethics Committee. All activities during the studies conformed to accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC). Biosafety guidelines for protection of personnel in the laboratory were observed.

Experimental Animals
ICR mice (22-26 g) were obtained and maintained in the animal house of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi. The animals were housed in plastic cages with soft wood shavings as bedding. They were fed with normal commercial animal food pellet from Ghana Agro Food Company Limited (GHAFCO), Tema, Ghana (with water *ad libitum*) and kept under ambient conditions of temperature, relative humidity, and light/dark cycle throughout the experiment.

Drugs and Chemicals Used
Acetic acid (BDH Limited, Poole England) was used to induce ulcer in the mice while Esomeprazole (AstraZeneca Pharmaceuticals, USA), a proton pump inhibitor, was used as the reference antiulcerant.

Induction of Gastric Ulceration
The protocol for the induction of gastric ulceration was as described by Wang *et al.,* (1989). Experimental animals were starved for 24 hours but were provided with water *ad libitum*. Mice were given 0.2 ml of 8% acetic acid orally. After 5 hours they were sacrificed, dissected and their stomachs removed. The stomachs were dissected along the greater curvature, the contents removed and washed with normal saline to enable observation for ulcerative lesions.

Experimental Procedure
Gastric ulceration was induced in 50 ICR mice. After 6 hours, the animals were put into five groups (A-E) of 10 animals each. Group A was treated with 1 ml/kg distilled water only (vehicle group). Groups B, C, and D were treated with 0.2, 0.4, and 0.8 mg/kg AEMP respectively and Group E was treated with 0.3 mg/kg Esomeprazole. Another group, F, in which ulcerations were not induced were also kept and given 1 ml/kg distilled water. All treatments were conducted for 7 days after which the mice were sacrificed and their stomach removed and examined by count for ulcerative lesions. The maximum length of each lesion in millimetres was determined and the sum of the lengths of all lesions in each stomach expressed as the Ulcerative Index (UI) (Sivaraman and Muralidharan, 2010). The Curative Ratio (CR), expressed in percentage, was determined for each group using the formula:

$$CR\,\% = \frac{(UI\ of\ Control - UI\ of\ treatment)}{UI\ of\ Control}\ x\ 100$$

The stomachs were then fixed in 10% buffered paraformaldehyde for histopathological evaluation.

Determination of Site of Action of AEMP
A 2 cm long guinea-pig ileum was mounted in 20 ml of Tyrode solution in a Harvard tissue bath (Harvard Apparatus Ltd, Kent, UK) maintained at 32^{o}C as described by Koffuor *et al.,* (2012). The tissue was constantly aerated and allowed to stabilize in the bath for 15 minutes. With a contact time of 30 seconds, a time cycle of 1 minutes and a Harvard kymograph (Harvard Apparatus Ltd, Kent, UK) speed of 4 mm/min, a complete dose-response tracing was generated for Acetylcholine ($2.0 \times 10^{-3} - 2.56 \times 10^{-1}$ µg/ml). A sub-maximal response of about 75% of the maximum response given by a dose of 6.4×10^{-2} µg/ml of Acetylcholine, was selected. Equipotent doses (doses that gave similar responses to the submaximal response selected for Acetylcholine) of Nicotine (9.2×10^{-2} µg/ml) and Histamine (1.28×10^{-1} µg/ml) were obtained and responses matched.

A dose of Hexamethonium (0.05 mg/ml) was added to the organ bath and left in contact with the tissue for 30 seconds after which the equipotent dose of Nicotine was added to the bath and response recorded. The tissue was then washed free of the drugs and the step was repeated for AEMP (0.16 mg/ml) and the matched dose of Nicotine. The procedures were performed for Atropine (5.0×10^{-6} mg/ml)/ Acetylcholine, AEMP/Acetylcholine, Mepyramine (0.2 mg/ml)/Histamine and AEMP/Histamine.

Data analysis

All graphs and statistical evaluations were done using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). Data were presented as mean ± SD. Significant differences between percentage inhibitions of agonists (comparing to "zero inhibition") were determined using One-Way Analysis of Variance followed by Dunnett's Multiple Comparisons Test *post hoc*. $P \leq 0.05$ was considered statistically significant.

RESULTS

Phytochemical Screening

The phytochemical screening of the aqueous extract showed the presence of alkaloid, tannins, saponins, glycosides and flavonoids.

Thin Layer Chromatography

The developed TLC plate showed the presence of four different components with the following retention factors (Table 1).

High Performance Liquid Chromatography

HPLC analysis showed the development of 4 peaks which represent the presence of four groups of compounds absorbing ultraviolet radiation at 280 nm in the AEMP (Figure 1).

The Effect of AEMP on Acetic Acid-Induced Gastric Ulcer

It was observed that the vehicle-treated group had the highest lesion lengths. AEMP-treated groups (0.2-0.8 mg/kg) showed significant ($P \leq 0.001$) reduction in gastric ulceration similar to the esomeprazole-treated group (Table 2). Photographs taken after acetic-acid treatment of ICR mice showed gastric erosion and ulceration (Plate 1B) which were healed on treatment with 0.3 mg/kg esomeprazole and AEMP (Plate 1C and 1D). Histopathological studies showed gross appearances of hemorrhagic gastric mucosal lesions during the ulceration (Plate 2F), which was healed and regenerated during treatment with 0.3 mg/kg esomeprazole and 0.4 mg/kg

Table 1: The number of spots obtained on the

Spots	Retention Factor
1	0.725
2	0.875
3	0.925
4	0.975

Developed plate was viewed under ultraviolet light at 254 nm and 366 nm.

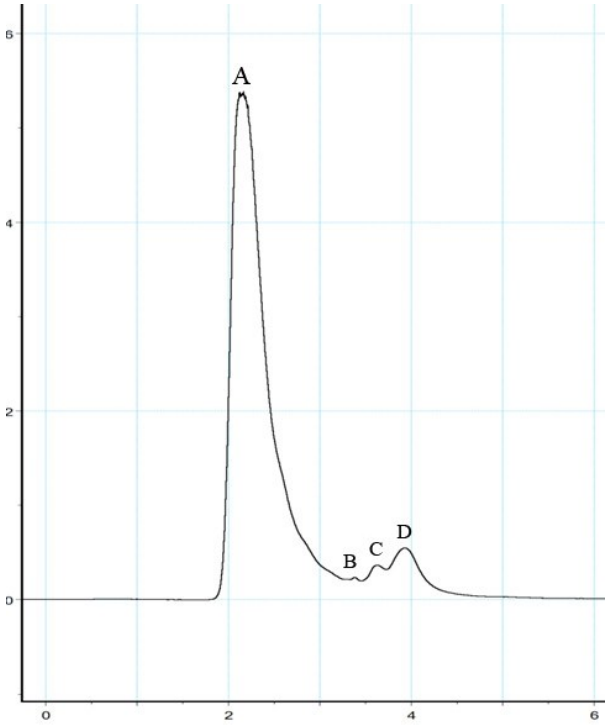

Figure 1: A chromatogram of AEMP showing peaks A, B, C, and D of different heights and area.

Table 2: The number of ulcers formed, the Ulcerative Index (UI) and the Curative Ratio (CR) for 7-day AEMP and Esomeprazole (ESO) treated ulcerated mice and ulcerated but untreated mice

Parameters	Control	ESO	AEMP		
		0.3 mg/kg	**0.2 mg/kg**	**0.4 mg/kg**	**0.8 mg/kg**
Number of ulcers/ stomachs/group	1.50 ± 0.4	1.05 ± 0.33 ns	1.00 ± 0.41 ns	1.00 ± 0.41 ns	0.75 ± 0.48 *
UI (mm)	7.50 ± 0.87	2.25 ± 1.32***	1.50 ± 0.50***	1.25 ± 0.48***	1.00 ± 0.58***
CR (%)	0.00 ± 0.00	65.47 ± 19.96**	79.52 ± 7.36***	80.95 ± 7.53***	85.71 ± 8.25***

Values are mean ± SD (n = 10), ulceration was induced in all groups. Significant differences between the ulcerated but treated groups and the control were determined using One-Way Analysis of variance followed by Dunnet's Multiple Comparison's Test. ns implies P > 0.05; * implies P ≤ 0.05** implies P ≤ 0.01; *** implies P≤ 0.001.

AEMP as seen in photomicrographs in Plate 2G and 2H.

Determination of Site of Action of AEMP

Acetylcholine, nicotine, histamine showed contractions of the guinea-pig ileum. AEMP inhibited significantly the responses of acetylcholine, nicotine and histamine by 4.7 ± 1.56 %, 12.44 ± 4.9 % and 54.2 ± 7.2 % respectively compared to zero inhibition (Figure 2). This shows that the extract has significant (P ≤ 0.001) anti-histaminic activity with minimal anti-nicotinic and anti-muscarinic activities.

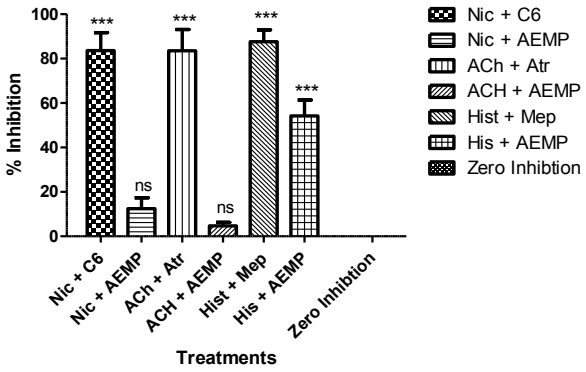

Figure 2: Percentage inhibition of Nicotine (Nic), Acetylcholine (Ach), and Histamine (Hist) by Hexamethonium (C6), Atropine (Atr), Mepyramine (Mep), and AEMP. Values plotted are means SD, n=3. Significant differences between percentage inhibitions were determined (comparing to zero inhibition) using One-Way Analysis of Variance followed by Dunnett's Multiple Comparisons Test *post hoc*. ** implies P≤ 0.01; * implies P≤ 0.001.**

DISCUSSION

Acetic acid-induced gastric ulceration has been noted to be a suitable model for investigating anti-ulcerant effect as it causes round haemorrhagic lesions which resemble human ulcers. The ulcers do not heal spontaneously and are simple and reproducible (Okabe *et al.,* 2010). Acetic acid ulcer induction is due to the embolization of blood vessels in the gastric mucosa leading to a blockade in mucosal blood flow. The imbalance between mucosal oxygen supply and demand leads to ischemia and necrosis of the mucosal tissue (Okabe *et al.,* 2010).

AEMP increased curative ratio and significantly decreased the number of ulcers formed per stomach and ulcerative index thus indicating anti-ulcerant effects. The anti-ulcerant activity could have been due to the collective effects of glycosides, flavonoids, alkaloid, tannins and saponins which are phytochemicals present in the extract. These classes of phytochemicals conform to the presence of more specific phytochemicals reported by Ahlborn

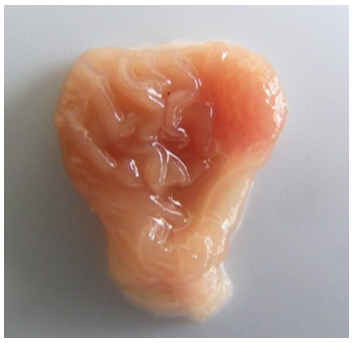

Plate 1A: Normal stomach of an ICR mouse

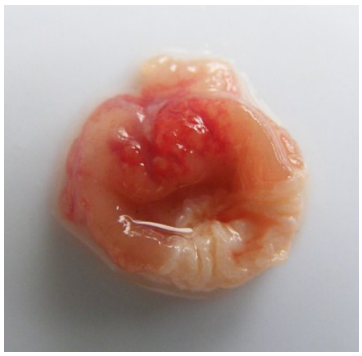

Plate 1B: Acetic acid-induced gastric ulceration in an ICR mouse

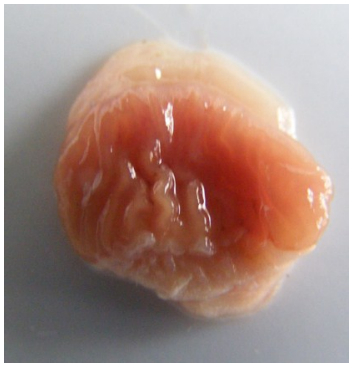

Plate 1C: 0.3 mg/kg Esomeprazole treatment after acetic acid-induced gastric ulceration in an ICR mouse

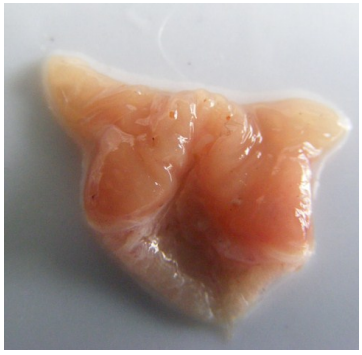

Plate 1D: A 0.4 mg/kg AEMP treatment after acetic acid-induced gastric ulceration in an ICR mouse

Plate 1: Photographs of the gastric mucosa of normal, ulcerated and ulcerated but treated with AEMP and Esomeprazole for 7 days in ICR mice

(2013) in *Musa x paradisiaca*. *Musa x paradisiaca* contains a glycoside named aucubin which has anti-histaminic activity (Ahlborn, 2013) thus supporting the anti-histaminic activity observed. Research by Lewis and Shawb, (2001) reported the presence of leucocyanidin, a flavonoid, which has the ability to increase mucus and mucosal protein production. Lectin, a protein with a high affinity for carbohydrates, is said to be present (Ahlborn, 2013) and this binds a mannose oligosaccharide in the cell wall of normal commensals to the gastric and intestinal lining. The presence of these normal commensals prevents the colonization of the gastric lining by *H. pylori* hence reducing their ulcerative potency.

Tannins, specifically allantoin, present in *Musa x paradisiaca* (Ahlborn, 2013), has astringent and protein coagulation properties. Baicalein (a flavonoid) and iridoid (a glycoside) have anti-inflammatory activity (Ahlborn, 2013). The characteristics of these phytochemicals can be associated with the wound healing (anti-ulcerant) and mucosal healing ability of AEMP. Gastritis is one of the signs of gastric ulceration and may be caused by erosion of the mucosal defenses. *H. pylori* have the ability to cause mucosal damage by immune/inflammatory response alteration in the host (Suerbaum and Michetti, 2002).

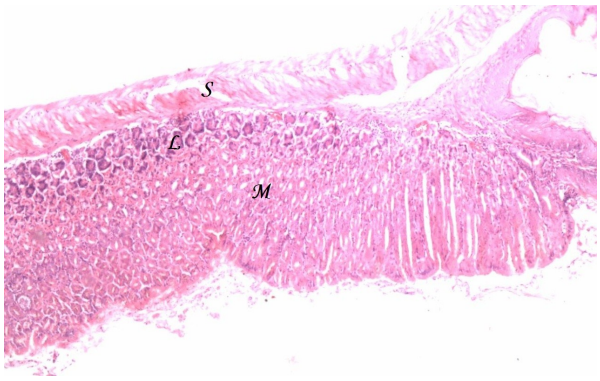

Plate 2E: Normal arrangement of cells in the gastric mucosa and sub-mucosa of an ICR mouse

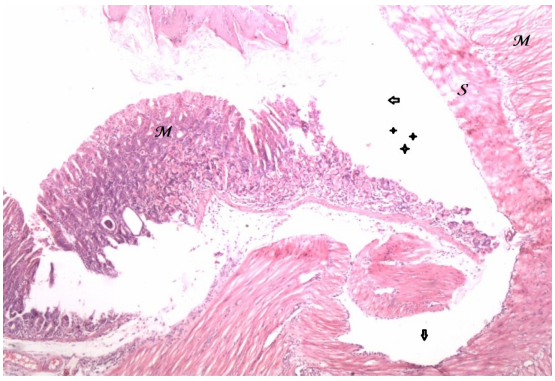

Plate 2F: Gross appearances of hemorrhagic gastric mucosal lesions (shown by arrows) in an ulcerated ICR mouse

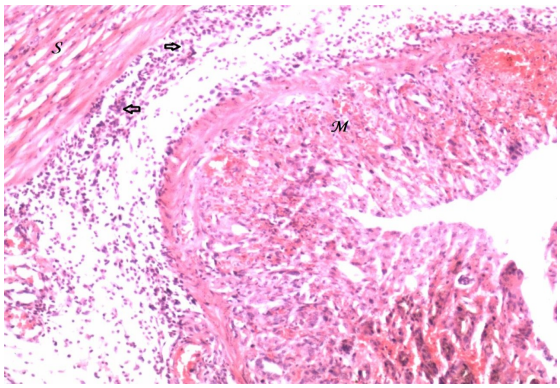

Plate 2G: Effect of the esomeprazole (0.3mg/kg) showing mild mucosal healing

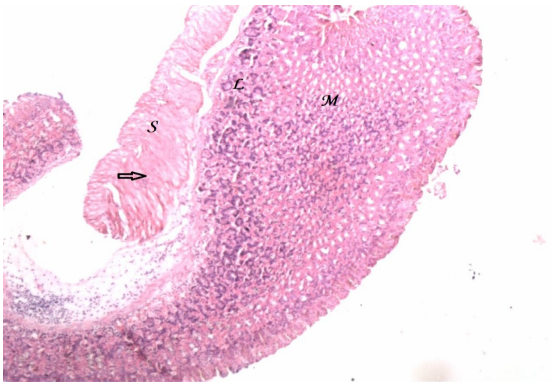

Plate 2H: Gastric mucosa of a 0.4 mg/kg AEMP-treated mouse showing mucosal regeneration

Plate 2: Photomicrographs of the gastric mucosa of normal, ulcerated and ulcerated with AEMP and Esomeprazole treatment for 7 days in ICR mice

In general, mucosal defense and repair mechanisms are important in protecting the integrity of the mucosal layer and resultant inhibition of these mechanisms could lead to necrosis. Examples of such defense mechanisms include pre-epithelial factors (mucus-bicarbonate-phospholipid barrier), surface epithelial cells connected by tight junctions, bicarbonate and mucus production, prostaglandins, heat shock proteins and blood flow through the mucosal vessels (Laine *et al.,* 2008).. . AEMP in effect, may directly protect the mucosal layer from noxious substances such as NSAIDs, acids and alcohol and enhances mucosal regeneration. In ulcer healing it is important that the distorted architecture of the mucosal and submucosal layers regain their normal arrangement through cell regrowth and protein coagulation. The findings of histopathological studies conducted from this study confirmed the mucosal cell regenerative property of the AEMP.

Parietal cells of the stomach bear receptors for three stimulators of acid secretion: Acetylcholine

(muscarinic type receptor), Gastrin and Histamine (H2 type receptor). Gastric acid is produced via the M2 receptors through the parasympathetic stimulation of neurons (e.g. vagus) which stimulates acetylcholine production and hence binding on the M2 receptors for the release of the gastric acid by the parietal cells. Gastrin, synthesized in the endocrine cells of the gastric mucosa also stimulates the parietal cell to produce acid (Rang *et al.,* 2007). Histamine from enterochromaffin-like cells is however thought to represent the final mediator of acid secretion but the magnitude of the stimulus appears to result from a complex additive or multiplicative interaction of signals of each type (Furutani *et al.,* 2003; Yao and Forte, 2003; Samuelson and Hinkle Forte, 2003; Zhu, 2010). From the results of this study, the AEMP extract had significant anti-histaminic effect, with minimal anti-nicotinic and anti-muscarinic effects. This reveals the possible suppressor effects of the AEMP on gastric acid secretion hence its subsequent healing of gastric ulcers as excessive gastric acid secretion is one of the factors promoting mucosal damage. Excess acid in the stomach, affects the integrity of the mucus membrane thereby leading to erosions and subsequent ulcerations.

The HPLC chromatogram run at a wavelength of 230 nm showed three peaks with one distinct peak. Evidence of these peaks signifies the presence of UV absorbing secondary metabolites. Some of these secondary metabolites have aromatic structure and show varying polarity according to their retention times. The most prominent peak if determined to be monocomponent may be used as a biomarker in the standardization of the extract.

CONCLUSION

The aqueous fruit extract of *Musa x paradisiaca* has anti-ulcerant effect in acetic acid-induced peptic ulcers in ICR mice. This fruit extract had mainly antihistaminic and gastric mucosal cell regenerative property. The chromatogram obtained should serve as a fingerprint or a standard to which another sample prepared under the same conditions may be compared to.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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