Gastro-protective and anti-acidic effects of *Corchorus trilocularis* Linn against diclofenac-induced gastric ulcers.

Cyril G. Siringo, Paul M. Mbugua, Boniface M. Chege, Lincone L. Oluoch

ABSTRACT

**Ethnopharmacological Relevance:** *Corchorus trilocularis* Linn leaves are consumed as green leafy vegetables that are boiled and used as relish, or potherb by some rural communities in Eastern Africa for the treatment of stomach ulcers.**Aim:** This study investigated the gastro-protective effects of *Corchorus trilocularis* Linn (local name 'mrenda'), a popularly consumed vegetable in Kenya, on diclofenac-induced gastric ulcers and established its possible mechanisms of action.**Methods:** In the in-vivo study, thirty six Sprague dawley rats of either sex weighing 150-200 grams were randomly assigned into a normal control (distilled water), negative control (distilled water plus diclofenac sodium), treatment (200 and 400 mg/kg *Corchorus trilocularis* Linn plus diclofenac sodium), positive control group (omeprazole plus diclofenac sodium), or comparison group (400 mg/kg aqueous leaf extract of spinach). The ulcer index, total acidity, volume, pH of gastric secretions, and gastric morphology were assessed. In the in-vitro anti-acidity study, a rat stomach was perfused with Kreb's solution and the pH of the mucosal perfusate measured using a digital pH meter for 1 hour after exposure to histamine, acetylcholine, or pre-treatment with 400 mg/kg of the extract prior to addition the acid secretagogues.**Results:** The extract, significantly reduced the ulcer index and total acidity in comparison to the diclofenac group. The high dose extract also increased the gastric pH and had cytoprotective effects. However, it did not significantly affect the volume of gastric secretions. In the in-vitro study, the extract significantly inhibited histamine and acetylcholine stimulated gastric acid secretions as analyzed by the areas under curve for pH against time.**Conclusions:** The gastro-protective effects of *Corchorus trilocularis* Linn against diclofenac induced gastric ulcers are; therefore, mediated through preservation of the gastric mucosal barrier, increase in gastric pH, and inhibition of gastric acid secretion through the histamine H₄ and acetylcholine M₁ extracellular pathways on the parietal cell.

**Key words:** *Corchorus trilocularis*, ulcer index, cytoprotective, parietal cell, gastric acid.

**LIST OF ABBREVIATIONS**

NSAID: Non-Steroidal Anti-inflammatory Drug
CCK: Cholecystokinin
ECL: Enterochromaffin-Like Cell
cAMP: Cyclic Adenosine 5’-Monophosphate
CTLE: *Corchorus trilocularis* Linn Extract

INTRODUCTION

Gastric ulcers are caused by an imbalance between aggressive factors and gastric mucosal defense factors that maintain mucosal integrity. One of the aggressive factors is the chronic or high dose usage of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), which are among the most commonly used drugs worldwide. The use of NSAIDs such as diclofenac is commonly associated with the development of gastric ulcers. Despite progress in conventional pharmacology in production of effective treatments against diclofenac-induced gastric ulcers, herbs and vegetables still provide an important source of alternative therapy in the prevention of ulcers.

*Corchorus trilocularis* Linn is a plant species belonging to the genus *Corchorus* in the Family Malvaceae (formerly Tiliaceae). The whole plant is used for treatment of diseases of the abdominal viscera and seeds for the treatment of stomach ache by some rural communities in India and parts of Eastern Africa. The leaves and seeds are also administered in cases of fever, and rheumatism since they are boiled and used as relish, or potherb. Similarly, other species within the genus *Corchorus* such as *Corchorus olitorius* and *Corchorus depressus* have been shown to...
possess gastro-protective effects against gastric ulceration\(^1\) and in-vitro anti-acidic effects\(^2\) respectively.

This study investigated the gastro-protective and anti-acidic effects of *Corchorus trilocularis* Linn methanolic leaf extract on diclofenac-induced gastric ulcers. The findings show that the extract possessed gastro-protective effects against gastric ulceration through the preservation of the gastric mucosal barrier, increase in gastric pH, and inhibition of gastric acid secretion through the histamine \(H_2\) and acetylcholine \(M_3\) extracellular signaling pathways on the parietal cell.

**MATERIALS AND METHODS**

**Plant material and extract preparation**

The leaves of *Corchorus trilocularis* Linn were obtained from Kakamega, Kenya and identified by the herbarian at the University of Nairobi, department of Botany with the batch number SGC/2017/01. The leaves were washed with tap water to remove adherent dust particles, dried under shade for 7 days then ground to powder. 20g of powdered plant material was added to 100ml of methanol in a conical flask. The flask was then covered with aluminium foil and kept in reciprocating shaker for 24 hours with continuous agitation at 150 revolutions/minute. The extract was then filtered and the solvent from the extract removed by using a rotary vacuum evaporator. The residues were collected, measured and used for the study.

**Study animals**

Forty two (42) Sprague dawley rats of either sex weighing 150-200 grams were housed, 6 animals per cage, in cages measuring 40 by 65 by 20 cm. The animals were acclimatized for two weeks at free access to water and standard rat pellets ad libitum during the acclimatization period. This study was approved by the Bioethics, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (FVM/BAUEC/2018/140).

**In-vivo investigation of gastroprotective effects**

The method used was previously described by Devi et al. (2007). Probability sampling through a simple random sampling technique was used to divide the rats into six groups with six animals each (n=6): normal control (distilled water), negative control (20 mg/kg Diclofenac sodium), low dose treatment (20 mg/kg Diclofenac sodium + 200 mg/kg CTLE), high dose treatment (20 mg/kg Diclofenac sodium +400 mg/kg CTLE), positive control (20 mg/kg Diclofenac sodium + omeprazole 10 mg/kg), and comparison group (20 mg/kg Diclofenac sodium + aqueous leaf extract of *Spinacea oleracea* 400 mg/kg).

The doses of omeprazole (Reddy's Lab. Ltd.) and diclofenac sodium (Avanstra Inc.) were selected based on toxicity studies done in the laboratory.

All animal groups were fasted for 12 hours prior to each administration with free access to water until 2 hours before experimentation. Omeprazole and CTLE were dissolved in distilled water and administered at their respective doses via oral gavage in volumes not exceeding 0.5 ml once daily for five days, one hour prior to the induction of ulceration with 20 mg/kg diclofenac sodium. Four hours after induction of ulceration on the fifth day, the animals were sacrificed, the abdomen opened by a midline incision, and the stomach extracted from the gastro-esophageal junction to the pyloric end. The ulcer index, total acidity, histological studies, volume, and pH of gastric secretions were assessed.

**Ulcer index determination**

After the animals were sacrificed, the stomach was harvested, opened along the greater curvature, and the mucosa exposed for macroscopic evaluation. The ulcer Index was calculated based on the protocol by Takagi and Okabe (1968); where 0 = no lesion, 1 = mucosal oedema, 2 = one to five small lesions (1-2 mm), 3 = more than five small lesions or one intermediate lesion (3-4 mm), 4 = two or more intermediate lesions, or one gross lesion, and 5 = perforated ulcers.

**Biochemical analyses**

The harvested stomachs were opened along the greater curvature. The stomach contents were poured into a centrifuge tube and centrifuged at 3000 rpm for 10 minutes. The supernatant was used to determine total acidity by titration with 0.01 N NaOH against phenolphthalein indicator. The pH of the supernatant was determined by use of a digital pH meter (MRC PL 600, USA).

**Histological analysis**

Samples of gastric tissue from all groups were fixed in 10% buffered formalin for 24 hours. The tissues were then processed and embedded in paraffin blocks. Five-micrometer thick sections were cut out and stained using Haematoxylin and Eosin. The slides were then examined microscopically for histopathological changes such as hemorrhage, erosions, neutrophil infiltration, sub-mucosal edema, mucosal regeneration and congestion.\(^3\)

**In vitro anti-acidity effect of the extract**

The method used was previously described.\(^4\) Six male Sprague Dawley rats weighing 150-200 grams
were sacrificed. The abdomen was opened and the stomach carefully isolated by making incisions at the gastro-esophageal end and the gastro-duodenal end. The stomach contents were washed out using warm Kreb’s solution, and polyethylene canulæ inserted and tied into the stomach via the incisions at the fundic and pyloric end. The isolated stomach was immediately placed in an organ bath containing 10ml of Kreb’s solution at 37º Celsius gassed with 95% CO₂ and 5% O₂. The lumen of the stomach was perfused using modified Kreb’s solution at similar temperature, CO₂, and O₂ concentrations but at a pH of 7.25. The pH of the effluent perfusate of the stomach was continuously recorded by use of a digital pH meter (MRC PL 600, USA) at 5-minute intervals.

The Kreb’s solution in the organ bath contained the following salts in Millimolar concentrations: NaCl-119, KCl-4.7, CaCl₂-2.5, MgSO₄·7H₂O-1.2, NaHCO₃-25, KH₂PO₄-1.03, and glucose-5.6. The modified Kreb’s solution was similar in composition but lacking NaHCO₃ and KH₂PO₄. All drugs were added in volumes not exceeding 0.5ml to the 35 ml complete Kreb’s solution bathing the serosal surface of the stomach. To terminate the action of the drugs, the Kreb’s solution perfusing the serosal surface of the stomach was drained and replaced with fresh solution.

Histamine and acetylcholine acid suppressant effects of extract
After setting up the stomach preparation, the basal hydrogen ion output was allowed to stabilize for 15 minutes then the pH was measured after every 5 minutes for 1 hour. The serosal solution was then drained and replaced by a fresh solution. After stabilization of the pH, 10 μg/ml histamine or 1 mM acetylcholine was added to the serosal solution and the pH of the mucosal solution measured for an hour at 5-minute intervals.

To investigate the anti-acidic effects, the serosal Kreb’s solution was pre-treated with 400 mg/kg CTLE and the pH measured for 1 hour prior to addition of 10 μg/ml histamine or 1 mM acetylcholine sulphate to stimulate acid secretion. The pH was then measured after every 5 minutes for 1 hour by use of a digital pH meter.

A graph of pH against time was plotted afterwards for the basal pH, histamine or acetylcholine mediated pH changes, and 400 mg/kg CTLE plus histamine, or 400 mg/kg CTLE plus acetylcholine mediated pH changes over a duration of 1 hour.

Data and statistical analysis
The data was expressed as mean ± SEM. Data analysis was done using one way ANOVA followed by Bonferroni post hoc test for the determination of total acidity, pH and volume of gastric secretions, and one way ANOVA followed by Tukey HSD post hoc for the areas under curve in the in-vitro anti-acidity pH changes. The ulcer index was analyzed using Kruskal Wallis followed Dunn’s post hoc test. The level of significance was set at p < 0.05. Statistical software Graphpad prism 6.0 (Graphpad, USA) was used in the analysis.

RESULTS
Effect on ulcer index
Ulcer index was determined by grading the severity of the gastric mucosal ulcerations. Omeprazole (0.20 ± 0.12 Arbitrary units), 400 mg/kg CTLE (0.20 ± 0.12) and the normal control (0.30 ± 0.20) groups showed a significant reduction in ulcer index as compared to the Diclofenac group (3.80 ± 0.51) (P < 0.05, < 0.05 and 0.0028 respectively). The 200 mg/kg CTLE (2.10 ± 0.73) and Spinach (0.3 ± 0.20) groups also exhibited a reduction in ulcer index caused by administration of diclofenac, but the reduction was not significant (P > 0.99 and 0.08 respectively) as shown in figure 1 and 2.

Effect on total acidity
400 mg/kg CTLE and normal control groups demonstrated a significant reduction in total acidity in comparison to the Diclofenac (negative control) group [68.60 ± 15.65 mEq/l (Diclofenac) vs. 17.40 ± 4.65 (400 mg CTLE) vs. 20.20 ± 6.59 (Normal control); 400 mg vs. Diclofenac (P < 0.0001) and Normal control (P = 0.0002)]. Omeprazole (41.2 ±

Figure 1 Bar graph showing ulcer indices of the various groups within the study. *p < 0.05, **p < 0.01
5.05), 200 mg/kg CTLE (40.00 ± 2.61) and Spinach (17.40 ± 4.65) groups also elicited a reduction in total acidity in comparison to the negative control group, but the change was not significant (P = 0.0757, 0.0546 and 0.1592 respectively) as presented in figure 3.

**Effect on gastric pH**

The Diclofenac group had the lowest gastric pH in comparison to the rest of the groups [2.96 ± 0.08 (Diclofenac) vs. 5.59 ± 0.83 (Omeprazole) vs. 4.36 ± 0.79 (200 mg CTLE) vs. 4.24 ± 0.50 (400 mg CTLE) vs. 4.14 ± 0.42 (Spinach) vs. 3.31 ± 0.14 (normal control)]. However, only the Omeprazole group showed a significantly higher increase in gastric pH as compared to the Diclofenac (negative control) group in-vivo (P < 0.05). These results are summarized in figure 4.

**Gastric morphology**

Histological sections of the gastric mucosa showed extensive mucosal erosions and epithelial disruption in the Diclofenac (negative control) group. There was also presence of numerous mucus-secreting surface mucosal cells within the Diclofenac...
group. The Omeprazole and 200 mg/kg CTLE groups showed relatively intact gastric epithelia whereas the 400 mg/kg group had preserved gastric epithelium with a mild degree of sub-mucosal oedema. The normal control (distilled water) group had normal gastric histological appearance as shown in figure 5.

Figure 6  Bar graph showing the volumes of gastric secretion for the groups within the study

**Effect on volume**

![Bar graph showing the volumes of gastric secretion for the groups within the study](image)

Figure 7  Time course and pH of basal, histamine and acetylcholine-stimulated gastric acid secretion. Values expressed as mean ± SEM

The change in the volume of gastric secretions was not significant across all test groups (P > 0.99 in each case) as demonstrated in figure 6.

**Effect on volume**

![Bar graph showing the volumes of gastric secretion for the groups within the study](image)

Figure 8  The time course and pH of histamine, and histamine plus 400 mg/kg *Corchorus trilocularis* Linn. Values expressed as mean ± SEM

**In-vitro anti-acidic effects of CTLE**

To study the effects of CTLE on basal, histamine, and acetylcholine-stimulated gastric acid output, the areas under curve (AUC) of pH against time were analyzed using pH 5.70 as the baseline, since it was the lowest recorded value. 400 mg/kg of the extract was the dose selected since it elicited a higher ulcer-inhibiting effect *in-vivo*.

**Effect on volume**

![Bar graph showing the volumes of gastric secretion for the groups within the study](image)

Figure 9  The time course and pH of acetylcholine and acetylcholine plus 400 mg/kg *Corchorus trilocularis* Linn. Values expressed as mean ± SEM

**Effect on volume**

![Bar graph showing the volumes of gastric secretion for the groups within the study](image)

Figure 10  The time course and pH of basal and *Corchorus trilocularis* Linn mediated gastric acid secretion

In-vitro anti-acidic effects of CTLE

To study the effects of CTLE on basal, histamine, and acetylcholine-stimulated gastric acid output, the areas under curve (AUC) of pH against time were analyzed using pH 5.70 as the baseline, since it was the lowest recorded value. 400 mg/kg of the extract was the dose selected since it elicited a higher ulcer-inhibiting effect *in-vivo*.
Histamine and acetylcholine stimulated gastric acid secretion
Stimulation of acid secretion using histamine caused a significant decrease in gastric pH as compared to the basal pH of acid secretion when analyzed using AUC [58.81 ± 0.98 (Basal pH) vs. 29.56 ± 0.95 (Histamine-stimulated); P < 0.0001]. This corresponds to a significant increase in gastric acid secretion. Similarly, acetylcholine significantly increased acid secretion in comparison to the basal pH of acid output [58.81 ± 0.98 (Basal pH) vs 31.18 ± 0.31 (Ach-stimulated); P < 0.0001]. This demonstrates that histamine and acetylcholine significantly increased gastric acid secretion as shown in figure 7.

CTLE inhibition of histamine stimulated gastric acid secretion
Pre-treatment with the extract elicited a significant increase in gastric pH corresponding to a decreased level of gastric acid secretion in comparison to histamine-stimulated acid secretion [29.56 ± 0.95 (Histamine-stimulated) vs. 72.00 ± 0.60 (CTLE + Histamine); P < 0.0001]. These results are summarized in figure 8.

CTLE inhibition of acetylcholine stimulated gastric acid secretion
Pre-treatment with the extract significantly decreased acetylcholine-stimulated gastric acid output causing an increase in gastric pH. [Acetylcholine (31.18 ± 0.31) vs. CTLE + acetylcholine group (68.47 ± 0.84) (P < 0.0001)] as presented in figure 9.

Effect of CTLE on basal acid output
Pre-treatment with the extract significantly reduced basal acid secretion and increased gastric pH (76.45 ± 1.21 AUC) in comparison to the basal acid output (58.81 ± 0.98) (P < 0.0001) as presented in figure 10.

DISCUSSION
The severity of gastric lesions in the study was determined by grading the ulcers within the various groups. *Corchorus trilocularis Linn* exhibited a dose-dependent reduction in the severity of gastric mucosal ulceration in comparison to the diclofenac group, with the 400 mg/kg dose showing more reduction. In addition, there was relatively equal potency between the reduction in ulceration exhibited by omeprazole and 400 mg/kg dose of the extract since there was no significant difference between the two groups. The spinach group also showed a reduction in the ulcer index induced by the administration of diclofenac. However, the reduction in ulcer index exhibited by spinach was lower in comparison to that of 400 mg/kg of the extract.

The reduction in ulceration by *Corchorus trilocularis Linn* extract may be due to a significantly preserved gastric wall mucus (Du Plessis, 1965), reduction in oxidative stress, and apoptosis as demonstrated in other Corchorus species such as *Corchorus olitorius* 200 and 400 mg/kg *Corchorus trilocularis Linn* extract increased the gastric pH as compared to the Diclofenac (negative control) group. The increase in pH was neither dose-dependent nor significant in-vivo. However, the Omeprazole group showed a significant increase in pH in comparison to the Diclofenac group. Similar elevations in gastric pH have been shown in previous studies using *Corchorus olitorius*.11

The increase in pH is attributed to a decrease in gastric acid secretion by the parietal cells, or an increase in mucus and bicarbonate secretion by the gastric surface mucosal cells. Omeprazole, a proton pump inhibitor that inhibits the gastric H+/K+ ATPase, causes an increase in gastric pH by inhibiting gastric acid secretion by the parietal cells.

Gastric total acidity is the total amount of free and bound acid within gastric contents. In this study *Corchorus trilocularis Linn* extract also showed a dose-dependent decrease in gastric total acidity in comparison to the Diclofenac group, with the high dose group eliciting more reduction. The results are consistent and confirm the increase in pH of gastric contents by the extract groups in comparison to the Diclofenac group. *Corchorus olitorius* and *Corchorus depressus* have also been shown to reduce gastric total acidity.11,12 The reduction in gastric total acidity results from inhibition of gastric acid secretion by the parietal cells.

There was no significant difference in the volumes of gastric secretion among all groups in the present study. The results contradict those of which showed a significant dose-dependent decrease in the volume of gastric secretions using 500 mg/kg and 1000 mg/kg spinach extracts in comparison to the ulcerated negative control group. The difference in results may be due to methodological differences between the two studies.15 Used a pylorus ligation model to collect gastric secretions; whereas, in the present study, the pylorus was not ligated. In addition, the doses of spinach used in the other study were higher than the 400 mg/kg dose used in the present study. There may therefore be a dose-dependent relationship between the volume of gastric acid secretion and the doses of herbal extracts used.

The effect of *Corchorus trilocularis Linn* extract in causing a decrease in gastric total acidity and...
corresponding increase in gastric pH suggest that the extract also works by decreasing gastric acid secretion. The decrease in gastric acid secretion may be mediated by any of the three final pathways that regulate gastric acid secretion by the parietal cells including histamine H$_2$, acetylcholine M$_3$ or gastrin (CCK$_2$) pathway.\textsuperscript{21}

To investigate the effect of \textit{Corchorus trilocularis Linn} extract on gastric acid secretion, an in-vitro isolated whole stomach preparation perfused with Kreb's solution was used. The histamine H2 and acetylcholine M$_3$ pathways were assessed using histamine and acetylcholine respectively to stimulate gastric acid secretion prior to, or after incubation of the stomach with 400 mg/kg dose of the extract.

Histamine caused a significant increase in the level of gastric acid secretion as compared to the basal level of gastric acid secretion. This was evident through a time-dependent decrease in gastric pH. This finding is in agreement with the findings of\textsuperscript{22} Whereas Gastrin Does Not, Augment Maximal Histamine-Stimulated Acid Secretion. The increase in acid secretion by histamine is a result of its binding and activation of the H$_2$ receptors on the parietal cells coupled to the activation of adenylate cyclase and subsequent increase in in intracellular cAMP within the parietal cells.\textsuperscript{23} 400 mg/kg \textit{Corchorus trilocularis Linn} produced a significant time-dependent inhibition of histamine-stimulated gastric acid secretion. Similar effects have been observed using \textit{Aloe vera} in previous studies.\textsuperscript{24} The inhibition of histamine-stimulated gastric acid secretion may be due to the inhibition or antagonism of the H$_2$ receptors in the H$_2$-cAMP pathway or to a lesser extent the reduction in biosynthesis or release of histamine from the enterochromaffin-like (ECL) cells.\textsuperscript{25}

The final pathway of inhibition of gastric acid secretion by \textit{Corchorus trilocularis Linn} extract that was investigated, was the acetylcholine M$_3$-mediated gastric acid secretion pathway on the parietal cell. The results in the study showed that acetylcholine significantly stimulated gastric acid secretion in comparison to the basal acid secretion, as indicated by the time-dependent decrease in pH. Similar elevations in gastric acid secretion after stimulation by acetylcholine were obtained by.\textsuperscript{26} Acetylcholine stimulates gastric acid output through the direct activation of M$_3$ receptors on the parietal cells coupled to the activation of phospholipase C and subsequent increase in intracellular calcium. The stimulation of acid secretion may also be indirect, through the activation of M$_3$ receptors on the ECL cells coupled to the increase in intracellular levels of cAMP, with a resultant increase in histamine secretion.\textsuperscript{25}

\textit{Corchorus trilocularis Linn} extract showed a significant inhibition in acetylcholine-stimulated gastric acid secretion. This is in conformity to the results obtained by the reduction of acetylcholine stimulated gastric acid secretion using \textit{Solanum paniculatum}.\textsuperscript{27} The inhibition of acetylcholine-stimulated gastric acid secretion by the extract may, therefore, be attributed to antagonism of the M$_3$ receptors within the parietal or ECL cells.

There was also a time-dependent inhibition of acid secretion by \textit{Corchorus trilocularis Linn} extract on the basal acid secretion, without histamine or acetylcholine stimulation. This shows that the extract may work through inhibition of alternative signaling pathways of acid secretion other than the H$_2$ or M$_3$ extracellular pathways.

The findings in this study support the use of the use of \textit{Corchorus trilocularis Linn} by some communities in Eastern Africa for the relief of stomach pain and ulcers. In addition, it provides a novel finding that encourages its use as alternative therapy for those with pre-existing gastric ulcers, with the preferred consumption in its original state as a vegetable.

**CONCLUSION**

This study shows that \textit{Corchorus trilocularis Linn} methanolic leaf extract has gastro-protective effects against diclofenac-induced gastric ulcers. The effects can be attributed to its inhibition of gastric acid secretion through multiple signaling pathways, including the histamine H$_2$ and acetylcholine M$_3$ extracellular pathway within the parietal cell, and preservation of the gastric mucosal barrier.

**DATA AVAILABILITY**

The data on Gastro-protective and anti-acidic effects of \textit{Corchorus trilocularis Linn} against diclofenac-induced gastric ulcers used to support the findings of the study are included within the article.

**CONFLICT OF INTEREST**

The authors declare no competing interests.

This manuscript/data, or parts thereof, has not been submitted for possible publication to another journal or previously been published elsewhere.
Gastro-protective and anti-acidic effects...

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AUTHOR CONTRIBUTIONS
Cyril George Siringo and Paul Mungai Mbugua designed the study. Cyril George Siringo, Boniface Mwangi Chege and Lincone Linus Olouo did the experimental work. Cyril George Siringo, Paul Mungai Mbugua, Boniface Mwangi Chege and Lincone Linus Olouo analyzed the experimental data and wrote the paper. All authors reviewed the manuscript and approved its submission.

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