Protective functions of methanol extract of *Cucumis Melo* (L. INODOROUS) seeds on Pyloric ligation induced gastric ulceration in male Wistar rats

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Abstract

Accumulation of gastric acid and Reactive oxygen species (ROS) have been associated with development of gastric ulceration in pyloric ligation, *Cucumis melo* Seeds is said to be possess a strong antioxidant. Thus this study evaluated the antiulcer activities of Methanol extract of *Cucumis melo* seeds on Pyloric ligation induced gastric ulceration in male Wistar rats. Thirty Male Wistar rats (n=5, 150-200g) rats were randomly groups in to five groups; A (control),B(Pyloric ligation induced gastric ulceration(PYL),C,D,E were administered with 50mg/kg,100mg/kg,200mg methanol extract of *Cucumis melo* seeds + PYL for 21 days prior to to pyloric ligation induction under ketamine anesthesia(60mg/kg). The animals were sacrifice after six hours, total gastric acidity was measured by titrimetric method, ulcer score and index was assessed, hematological parameters was measure using auto-analyzer while MDA,Catalase and protein was determined by spectrophotometry with histology analysis. Upon Pyloric ligation, the total gastric acidity, ulcer score index, lipid peroxidation(MDA level) was increased, while there was a decrease in the catalase, protein level and hematological parameters, with damages on the gastric mucosa. *Cucumis melo* seed extract reverses these changes induced by pyloric ligation. In conclusion, the gastric mucosa was disrupted as a result of accumulation acid, increases in oxidation after pyloric ligation with decrease in antioxidant and hematological parameters, methanol extract of *Cucumis Melo* provides a protection against the pyloric ligation induced gastric ulceration.

Keywords: Pyloric Ligation; Gastric Ulcer; Lipid Peroxidation; *Cucumis Melo*; Total Gastric Acidity.

1. Introduction

A number of physical and chemical barriers that stop auto digestion and gut erosion shield the gastrointestinal (GI) tract from a variety of endogenous insults[1]. The primary chemical barriers of the GI tract are mucus released by goblet cells, which are found throughout the gut length, as well as bicarbonates, which neutralize stomach acid[2]. The primary physical barrier sections of the GI tract are the epithelial tight, which are mostly junctions. A stomach ulcer develops when there is an imbalance between these barriers and the elements driving gut erosion. Pylorus ligation is a potent stimulator of acid secretion in rats. Although, the exact mechanism underlying this reaction is not entirely understood, available data points to the involvement of a vago-vagal reflex, a nervous control. In addition to stimulating the gastric acid output through an intramural reflex and a prolonged vago-vagal response in animals with innervation, pylorus ligation results in the accumulation of acid and pepsin, which promotes ulceration and the auto-digestive breakdown of the gastric mucosa.

Reactive oxygen species (ROS) have been linked to the pathogenesis of a number of human disorders, including peptic ulcers, according to several studies [3,4,5]. Platelets, macrophages, and smooth muscle cells, produces a byproducts of the metabolism of arachidonic acid, which are powerful ulcerogens in the stomach mucosa.

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The ability of numerous substances to scavenge free radicals has been demonstrated, and as a result, these substances are thought to be effective in preventing oxidative damage to the gastrointestinal mucosa. In addition to their capacity to neutralize free radicals, these substances are classified as antioxidants because they also prevent lipid peroxidation[3,6]. There are traditional medical practices using herbal medicines everywhere in the world. The three main traditions are Chinese, Indian, and European, as well as African. These ancient medicines' concepts share some similarities with one another but diverge significantly from those of contemporary Western medicine. The availability of herbal medications for patients in the geographic area of the particular traditional medicine is a benefit[7].

Medicinal herbs are essential in the fight against many ailments. Animals exhibit substantial antiulcer action when exposed to several herbal plants and plant extracts. Numerous plant compounds have been said to have antiulcer properties due to constituents such as compounds, including flavonoids, tannins, alkaloids, glycosides, terpenoids, steroids, saponin, and many more, , which is particularly important in terms of medicine. *Cucumis melo seed* is not an exception. The cucurbit (*cucumis melo*) is a member of the cucurbitaceae family. Numerous experimental investigations have been conducted to assess *citrullus lanatus*’s antiulcer and gastroprotective properties[8,9,10]. This study evaluated the effectiveness and protective functions of *Cucumis melo* against stomach ulcers caused by pyloric ligation in male Wistar rats.

2. Materials and method

2.1. Reagents

Normal saline, Distilled water, 10% formalin, Ketamine, Xylazine, 0.1M NAOH, Phenolphthalein, N-Hexane, Methanol, phenolphtaline, NaOH, Na₂HPO₄ (BDH Chemical Limited, England), of NaH₂PO₄·5H₂O (BDH Chemicals, England), potassium iodide, KI (BDH Chemicals, England), 30% Trichloroacetic acid (TCA), 0.75% Thiobarbituric acid (TBA), 0.15M Tris-KCl buffer, 5% K₂Cr₂O₇ (Dichromate Solution), 0.2M H₂O₂ (Hydrogen peroxide), Dichromate/acetic acid, 0.05M Carbonate buffer (pH 10.2), Phosphate buffer (0.01M, pH 7.0), 0.3mM Adrenaline

2.2. Preparation of methanolic extract of *cucumis melo*

*Cucumis Melo* fruits were purchased from Vendors in Nassarawa state Nigeria. It was taken to National Institute for Pharmaceutical Research and Development(NIPRID) for authentication. Mr. Akeem A. Lateef, a Taxonomist at the Herbarium and Ethnobotany Unit Department of Medicinal Plant Research and Traditional Medicine, NIPRD, identified the fruit. It was assigned Voucher Specimen Number NIPRD/H/7216, and documented in the laboratory. Peeling, slicing, and collecting the seeds from the fruits were done. After being ground in a blender, the seeds were dried at room temperature (25 ±2 °C). N-hexane was used to defat 4 kg of the powdered seeds for 24 hours at a temperature of 40 to 60 °C. It was repackaged after extracted with methanol from the dried, defatted marc. In order to completely release the volatile methanol, the concentrated methanol extract was exposed to air after being dilute with distilled water to double its capacity and then sieved to separate the shaft.

2.3. Animals

Thirty Male Wistar rats (n=5, 150-200g) in total were acquired from the Physiology Department of the College of Medicine at Baze University in Abuja. Prior to the creation of ulcers, the animals received a 21-days pretreatment of methanolic extract of *Cucumis Melo*.

2.4. Animal grouping

Group 1- Control (no ulcer);
Group 2- Pylorus Ligation Ulcerated untreated (PL);
Group 3- Pylorus ligation ulcer +50mg/kg of *Cucumis melo* seed extract.
Group 4- pylorus ligation Ulcer + 100mg/kg of *Cucumis melo* seed extract.
Group 5- pylorus ligation Ulcer + 200mg/kg of *Cucumis melo* seed extract.

2.5. Pyloric ligation induction

The animals underwent a 21- days pretreatment period before having their pylori tied based on the procedure outlined by Manowar et al[11]. Their abdomen was gently dilated and the pyloric sphincter was tied while they were under a little ketamine anesthetic (60mg/kg/), to protects any form of vascular tissue. Following ligation, the stomachs were promptly inserted into the abdomen. The animals were killed via cervical dislocation four hours after the ligation. Gastric juice was extracted from the stomach and centrifuged at 2000 rpm for 10 minutes. After centrifuging, 0.5 ml of
the supernatant was used to measure the amount of stomach acid secreted. Normal saline was used to rinse the stomachs, and the ulcer severity was graded.

2.6. Determination of Ulcer score and ulcer index

The ulcer score was assessed by independent two observers, and was assessed as follows; 0 - no macroscopic changes, (1) mucosal erythema only, (2) mild mucosal edema, slight bleeding or small erosions, (3) moderate edema, bleeding ulcers or erosions, and (4) severe ulceration, erosions, edema and tissue necrosis[12]. Each animal’s average ulcer score was reported as an ulcer index Utilizing the following formula, the ulcer index (UI) was calculated: UI = 101 UN + US + UP Where: UN (Average Number of Ulcers Per Animal); US (Average Number of Severity Score); UI (Ulcer Index); UP (Percentage of Animals With Ulcers).

2.7. Determination of total gastric acidity

Gastric juice was diluted with 1 ml of distilled water and then transferred to a conical flask (10 ml) with the addition of 2 drops of phenolphthalein indicator. Titration was then performed using 0.01 NaOH until a persistent pink hue resulted, and the amount consumed was calculated. Using Reddy et al.’s formula[13], the total acidity is calculated and given as mEq/l.

\[
\text{Gastric Acidity} = \frac{\text{volume of } \text{NaOH} \times \text{Normality}}{0.1} \times \frac{\text{mEq}}{\text{L}} \times \frac{100}{\text{g}}
\]

2.8. Determination of Lipid peroxidation

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale [14].

2.9. Assessment of Antioxidants

The protein concentration of the homogenate was determined using the modified version of Gornal et al. [15] description of the Biuret reaction. The catalase activity was measured using the Claiborne technique [16].

2.10. Hematological Analysis

Using an auto-analyzer device (SFRI blood cell Counter, H18 light, France), the hemoglobin, PCV, red blood cell (RBC), white blood cell (WBC), platelet count was assessed.

2.11. Histological analysis

A part of the ulcerated stomach was removed, fixed in 10% buffered neutral formalin solution, and then the tissues were embedded in paraffin. Solid sections were then cut at 5 m using a LICA microtome, stained with common hematoxlin and eosin (H&E), and then the section was solidly divided into smaller sections. Lesions were evaluated accordingly.

2.12. Statistical analysis

All data is presented as mean ±SEM, Comparison between mean was done by using one-way ANOVA and data considered statistically significant with p<0.05 with Bonferroni Post Hoc. Graphpad Version 5.0 was employed in this study.

3. Result

3.1. Methanolic extract of Cucumis melo seed on weight changes during Pyloric ligated induced gastric ulceration

Cucumis Melo caused a significant increase in the weight changes of animals in group C and D compared to group B,P<0.05. A significant decrease was also observed in group E compared group A and group B, P<0.05 as shown in figure 1.
3.2. Methanol extract of *cucumis melo* seeds decreases the relative stomach weight during Pyloric ligation induced gastric ulceration

*Cucumis melo* methanol seed extract significantly decrease the relative stomach weight in group C, D as compared to group B, P<0.05 however a significant increase in relative weight of the stomach compared to group A, P<0.05.

3.3. Methanol extract of *Cucumis melo* seeds decrease the Total gastric acidity in Pyloric Ligation induced gastric ulceration

Methanol extract of *Cucumis melo* significantly decrease the total gastric acidity after pyloric ligation in group C,D,E compared to group B, P<0.05 as seen in figure 3.

![Graph 1](image1.png)

All data are Mean ±SEM, P<0.05.*significant compared to A;** significant compared to B

**Figure 1** Effect of methanol extract of *cucumis melo* seed on weight changes of Male wistar rats.

![Graph 2](image2.png)

All data are Mean ±SEM, P<0.05.*significant compared to A;** significant compared to B

**Figure 2** Effect of methanol extract of *cucumis melo* seed on Relative stomach weight.
Figure 3 Effect of methanol extract of *cucumis melo* seed on total gastric acidity during Pyloric ligation induced gastric ulceration in male Wistar rats

3.4. Methanol extract of *Cucumis melo* seeds decreases the ulcer score and ulcer index in Pyloric ligation induced gastric ulceration

Treatment with methanol extract of *Cucumis melo* seeds significantly decrease the ulcer score and ulcer index in groups C,D,E compared to group A and B, P<0.05 as shown in table 1.

3.5. Gross morphology

Methanol extract of *cucumis melo* seeds inhibit the damage to the gastric mucosa during the Pyloric ligation induced gastric ulceration.

The gross morphology of the stomach mucosa in group shows intact gastric mucosa. In group B, an erosion above 1mm was observed, however in the group C-D, hemorrhage streaks was observed as shown in figure 4.

Table 1 Effect of methanol extract of *Cucumis melo* seeds on Ulcer score and ulcer Index

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer score</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0±0.0</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td>B</td>
<td>3.33±0.17</td>
<td>0.56±0.28</td>
</tr>
<tr>
<td>C</td>
<td>1.33±0.17**</td>
<td>0.22±0.28**</td>
</tr>
<tr>
<td>D</td>
<td>1.17±0.33**</td>
<td>0.14±0.56**</td>
</tr>
<tr>
<td>E</td>
<td>2.83±0.33**</td>
<td>0.94±0.11**</td>
</tr>
</tbody>
</table>

All values are expressed as SEM, P<0.05 is considered to be statistically significant.
Figure 4: Gross morphology showing the effect of methanol extract of *Cucumis melo* on the stomach mucosa of animal induced with pyloric induced gastric ulceration

3.6. Histological analysis

Administration of *Cucumis melo* extract inhibit the disruption of the epithelial layer of the gastric mucosa in the group C,D,E compared to the group B which was not treat with the extract.

A: The gastric rugae/mucosa (star) is thick. There are locally extensive foci of mild loss of covering epithelium (black arrow). The parietal (green arrow) and mucous (red arrow) cells of the gastric glands appear normal. Other tunics appear normal. Vascular changes are unremarkable. H&E; Left: 100X; Right: 400X
**B:** The gastric rugae is thick and intact (star). There is preponderance of the parietal (red arrows) and some mucous (green arrows) cells. There is marked congestion of the submucosal blood vessels (thick arrow). H&E; Left: 100X; Right: 400X

**C:** There is severe extensive ulceration of the gastric rugae/mucosa (thick arrow). There is dense accumulation of inflammatory cells in the submucosa (black arrows). There is moderate congestion of the blood vessels (red arrows). H&E; Left: 100X; Right: 400X

**D:** The gastric rugae (star) is thick and intact. There is preponderance of mucous cells in the gastric glands (red arrows). Parietal cells (black arrow) are very few. There is accumulation of moderate numbers of inflammatory cells at the base of the tunic mucosa and submucosa (thick arrow). H&E; Left: 100X; Right: 400X
E: The gastric ruggae/mucosa (star) is thick. There is no visible lesion. The parietal (green arrow) and mucous (red arrow) cells of the gastric glands appear normal. Other tunics appear normal. There is mild congestion of the mucosal blood vessels (black arrow). H&E; Left: 100X; Right: 400X

**Figure 5** Photomicrograph showing the effect of methanol extract of *cucumis melo* seeds on gastric tissue of the animal with pylorus ligated induced gastric ulceration

### 3.7. Methanol extract of *Cucumis melo* seeds on hematological parameters during Pyloric ligation induced gastric ulceration

Pretreatment with methanol extract of *cucumis melo* seeds significantly increase RBC count in groups in group C,D,E compared to B, P<0.05. The packed cell volume and hemoglobin level was significantly increase in group C,D,E compared to group A and B, P<0.05 while the platelet count was significantly increase in group C and D compared to group A and B, P<0.05 as shown in table 2. The white blood cell count significantly increase in the group B compared to group A, P<0.05. In group C and D does not show any significant increase compared to group B but significantly increase compared to group A, P<0.05.

**Table 2** Effect of methanolic extraof *Cucumis melo* seeds seeds on hematological Parameters in pylorus Ligation Induced ulcer.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>9.66±0.58</td>
<td>2.433±0.99*</td>
<td>12.26±0.17**</td>
<td>11.85±0.32**</td>
<td>8.50±0.08**</td>
</tr>
<tr>
<td>PCV</td>
<td>55.13±2.81</td>
<td>22.47±2.46*</td>
<td>73.40±1.36**</td>
<td>64.37±1.48**</td>
<td>36.97±5.59*</td>
</tr>
<tr>
<td>HB</td>
<td>179.3±10.14</td>
<td>30.67±0.88*</td>
<td>227.0±3.79**</td>
<td>219.3±8.57**</td>
<td>139.0±12.70**</td>
</tr>
<tr>
<td>PL</td>
<td>207.3±26.03</td>
<td>376.7±69.23</td>
<td>3142±248.3**</td>
<td>859.7±85.25**</td>
<td>354.0±45.61*</td>
</tr>
<tr>
<td>WBC</td>
<td>2.690±0.70</td>
<td>13.19±0.93**</td>
<td>13.56±1.23*</td>
<td>12.96±1.83*</td>
<td>1.967±1.23**</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM, *P<0.05 *significant compared to group A, **significant compared to group B

### 3.8. Methanol extract of *Cucumis melo* seeds seeds enhances the antioxidants in pylorus ligation induced ulcer

The protein level increases in all the pretreated groups but not statistically significant compared to group A and B, P>0.05 as shown in figure 6. In figure 7, the catalase increased significantly in all the groups(C-E) pretreated with methanol extract of *Cucumis melo* seeds in compared to the group A and B, P<0.05.
Figure 6 Effect of methanol extract of *Cucumis melo* seeds on protein level in pylorus ligation induced ulcer. All values are mean ± SEM.

Figure 7 Effect of methanol extract of *Cucumis melo* seeds on activities of catalase in pylorus ligation induced ulcer. All values are mean ± SEM. * significant compared to group A, ** significant compared to group A

3.9. Effect of methanol extract of *Cucumis melo* seeds on Malondialdehyde (MDA) protein in pylorus ligation induced ulcer

Figure 8 Effect of methanol extract of *Cucumis melo* seeds on MDA level in pylorus ligation induced ulcer. All values are expressed as SEM±P<0.05, * significant as compared to group A, ** significant as compared to group B.
Pyloric induction increases the lipid peroxidation in the gastric mucosa, upon administration of methanol extract of *Cucumis melo* seeds, a significant decrease was observed in the MDA level as seen in group C-E compared to group B, *P*<0.05. No significant difference in group C-E compared to group A, *P*<0.05.

4. Discussion

Most of the times, the cause of peptic ulcers is unknown, but it is widely acknowledged that it is caused by an imbalance between aggressive factors and the endogenous defense mechanisms' maintenance of mucosal integrity[17].

To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilising the surface epithelial cells or interfering with the prostaglandin synthesis[18]. In this study, the anti-ulcerative activity of methanol extract of *Cucumis melo* seeds was investigated using pylorus ligation. The findings of this study, also suggests that *Cucumis melo seeds* causes a significant increase in animal's weight. When compared individually with the control group (pylorus ligation), treatment groups (50mg, 100mg) *Cucumis melo seeds* pretreatment exhibited a significant increase in weight changes except for the group pre-treated with 200mg which decreased in weight changes possibly because of dose dependent.

This study more also shows that relative stomach weight is significantly increased in pylorus ligation group. Normal control relative stomach weight was decreased as compared to group administered 200mg of *Cucumis melo seeds*. Relative stomach weight significantly decreased in groups pre-treated with 50mg, 100mg of *Cucumis melo seeds* seed extract suggesting that ulceration plays a role in inflammation of the stomach and increasing the stomach relative weight.

The RBC count was significantly increased as compared to the pylorus ligation group in groups pre-treated with 50mg, 100mg of the extract except 200mg which had a decrease. This suggests that ulceration without treatment decreased the RBC count, this might be attested to Anemia. Also the WBC count significantly decreased in pylorus ligation group as compared to groups pre-treated with the *Cucumis melo seeds* extract indicating a disease condition in pyloric ligation. More also the PCV, HB and Platelets decreased in the pylorus ligation group as compared to pretreatment group and normal control. *Cucumis melo* seed extract was able to enhance the hematology parameters.

The number of ulcers formed was significantly lower in animals pre-treated with *Cucumis melo seeds* methanol seed extract. Animals receiving *Cucumis melo seeds* seed extract at 50mg, 100mg showed a significant reduction in Ulcer score and ulcer index with exception of the group treated with 200mg which showed a reduction in ulcer score and ulcer index. This coincides with the work of Adebayo-Gege et al[10].

The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid[18]. Pylorus ligation induced ulcer was used to study the effect of *Cucumis melo seeds* methanol seed extract on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 36 hours followed by ligation of pyloric end of the stomach.

In this study there was significant increase in gastric acidity in pyloric ligation group as in this and it coincides with study of Zaghlool et al.[18], and significant decrease in groups administered with 50mg, 100mg orally as compared to normal control respectively except for the groups administered with 200mg which had a slight increase in gastric acidity as shown in fig.3. This result conforms to the work performed by Adebayo-Gege et al.[9,10], where aqueous extract of *cucumis melo* decrease total gastric acidity during the NSAID induced gastric ulceration. The ability of *Cucumis melo extract* (CUM) to lessen overall stomach acidity accounts for its anti-secretory characteristics as well as its role in repairing the mucosal membrane's altered hydrophobicity and diminished protective activity[9,10]. This is further explained by the gross morphology and H&E to corroborate that the methanol extract of *Cucumis melo* inhibit the damage caused by pyloric ligation induced gastric ulceration.

According to research, the imbalance between oxidants and antioxidants promotes to inflammation, which might result in gastric mucosa ulceration [10,19]. Reactive oxygen species have been found to be a primary cause of oxidative stress, which can result in DNA damage, protein breakdown, and lipid oxidation, all of which can cause cell death. A spike in mucosal lipid peroxides, which are formed by the interaction of oxy-radicals and cellular polyunsaturated fatty acids, may be a sign of oxidative stress in the disease state. Malondialdehyde (MDA) is a byproduct of polyunsaturated fatty acid peroxidation and related esters in cell membranes, and its measurement provides a useful indicator of oxidative
tissue damage. In this study the MDA level significantly increased in pyloric ligation control group, and there was a drastic reduction in groups treated with 50mg, 100mg, 200mg as compared to pylorus ligation which had an elevated MDA level. Pyloric ligation’s damaging effects on the stomach was mitigated by Methanol extract of *Cucumis melo* seeds presumably because due to its contents mostly antioxidants such alkaloids, flavonoids, steroids, phenols, cardiac glycosides, and terpenoids.

In this study the protein levels significantly decrease in pylorus ligation group as compared to the control. Pretreatment with 50mg, 100mg, 200mg of methanol seed extract of *Cucumis melo* seeds significantly increased the protein level in pre-treated group. This suggests that the extract is rich in protein and also suggests that protein levels are reduced in pylorus ligation ulceration. Catalases significantly increase in the treatment groups. *Cucumis melo* seeds enhances the protein level and antioxidant level which is in line with Adebayo-gege et al.[10]

5. Conclusion

In conclusion, the results of this study have revealed that methanol extract of *Cucumis melo* seeds seed shows a gastroprotective, and antiulcerative effect on the stomach. The observed substantial reduction in ulcer score in this investigation could be linked to the anti-secretory effect of *Cucumis melo* seeds seed extract, which importantly reduced the formation of ulcers. It is also suggested that *Cucumis melo* seeds seed contains high antioxidant activity due to its ability to reduce the MDA level. This inhibition is conjectured to be due to its anti-inflammatory and antioxidant activity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declared no conflict of interest.

Statement of ethical approval

All Animals were handled according to the Guide for the Care and Use of Laboratory Animals by the National Research Council (US), 2011. The experiments were approved by the Ethics Committee of the Baze University, Abuja, Nigeria.

Reference


