Protective Effect of *Mormodica balsamina* Leaf Extract on the Gastric Morphology of Experimental Rats Against Ethanol Induced Gastric Ulcers

**Efecto Protector de los Extractos de Hojas de *Mormodica balsamina* en la Morfología Gástrica de Ratas Experimentales Contra Úlceras Gástricas Inducidas por Etanol**

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**SUMMARY:** Mormodica balsamina is a valuable medicinal plant that is used to treat wounds and inflammation; its leaves are also used as an antibiotic and in the treatment of stomach pain. This study was conducted to determine the anti-ulcer activity of methanolic leaf extract of *Mormodica balsamina* on ethanol-induced ulcer in albino rats. A total of 32 rats were used for the study. Groups I and II served as the baseline and negative controls respectively, while groups III-VII served as the test groups. Group I was untreated, while group II received 1ml/kg body weight of the vehicle (2 % DMSO). Three test groups (III – V) received methanol extracts (75 mg, 150 mg, 300 mg/kg body weight respectively) while the other three test groups (VI – VIII) received aqueous extracts (75 mg, 150mg, 300 mg/kg body weight respectively) via oral gavage for seven days prior to ulcer induction. The rats were sacrificed, stomachs excised and ulcers scored. Histological sections were produced and examined. Findings revealed that *M. balsamina* extracts protected the rats’ gastric epithelia from ethanol induced ulceration to varying degree with the high dose (150 and 300 mg/kg) of both extracts offering the best preservation (42 % and 50 % ulcer protective index respectively) when compared to untreated animals. Histological findings correlated with calculated ulcer indices, with treated animals having less severe gastric mucosal lesions. In conclusion, extracts of *M. balsamina* may possess reasonable antiulcer activities in rats against ethanol induced gastric ulcer.

**KEY WORDS:** Stomach ulcers; Medicinal plants; Mormodica balsamina; Gastric mucosa.

**INTRODUCTION**

Gastric ulcer is a common human ailment with a high lifetime morbidity rate of 5-10% (Lanas & Chan, 2017). Invasive factors such as Non-steroidal Anti-inflammatory Drugs (NSAIDs), smoking, antibiotics, irritants, and alcohol frequently come in contact with the gastric mucosa lining; these factors can cause gastric ulcers by destroying the protective mucosal barrier through bicarbonate secretion, mucus, prostaglandins, cell regeneration, and endogenous toxicants (Ibrahim *et al.*, 2016).

Alcohol has been documented to cause cell damage and harm to the gastric mucosa by increasing reactive oxygen species generation and inhibiting mitochondrial electron transport (Simon *et al.*, 2022). Nigeria reported one of the highest rates of current alcohol use among persons 15 years and older in Sub-Saharan Africa (SSA), ranging from 40 to 59.9% at a population level, according to data from the 2016 Global Burden of Disease research. This suggests that roughly 40-60% of Nigerians are at risk of developing stomach ulcers as a result of excessive ethanol (alcohol) drinking (GBD 2016 Alcohol Collaborators, 2018).

Plants are a key source of medicines, and they are frequently utilized to cure a variety of ailments by humans (Gregory *et al.*, 2013). *Momordica balsamina* (African pumpkin), belongs to the family Cucurbitaceae. The tree is

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widely distributed in the Northern and Eastern part of Nigeria. The Kanuri-speaking people of Borno State, Nigeria, utilize the leaves of *M. balsamina* as an antibiotic and for the treatment of gastrointestinal pain (Sharma *et al.*, 2009). It is a tendril-bearing, wild climber with a wide range of medicinal and nutritional qualities that has been used as a traditional folk medicine in many Countries. Resins, alkaloids, flavonoids, glycosides, steroids, terpenes, cardiac glycoside, and saponins are found in the plant’s leaves, fruits, seeds, and bark, and they have varied medicinal properties (Thakur *et al.*, 2009).

The recent resurgence of herbal treatments has been attributed to consumers’ preference for natural therapies, as well as a heightened interest in alternative medicine. This present study was thus designed to assess the anti-ulcer potentials of *Mormodica balsamina* crude leaf extracts against ethanol induced gastric ulcer in experimental rats.

**MATERIAL AND METHOD**

**Experimental Animals.** Thirty two (32) apparently healthy disease-free Albino rats weighing (150-220g) were used for the studies. They were housed in metallic cages at the Animal House of the College of Medicine University of Nigeria Enugu Campus (UNEC). The animals were allowed to acclimatize to the laboratory’s ambient temperature (28-30 °C) and 12-hour light-dark cycle. They were weighed and divided into five groups of four rats each, based on their weights. They were given free access to clean water and commercial rat feed (Guinea Feed®, Enugu, Nigeria).

**Ethical consideration.** Ethical approval was sought and obtained from Animal Welfare and Ethics Committee, Department of Animal Science University of Nigeria Nsukka. International and institutional guidelines on animal experimenting and humane treatment of experimental animals (Sahni & Mark, 2000) were adhered to throughout the study.

**Plant Collection and Extraction and Phytochemical analysis.** Fresh leaves of *Mormodica balsamina* were collected from a farm settlement in Enugu, South Eastern Nigeria in the month of July 2020. The leaves were identified at the Herbarium Unit, Department Plant Science and Biotechnology, University of Nigeria Nsukka (Voucher number UNH/257). The leaves were washed thoroughly, dried under the shade and then grinded into fine powder.

The methanol extract was prepared by macerating 300 g of the fine powder in 1.8 Liters of methanol. The mixture was shaken for 72 hours and then filtered. The filtrate was evaporated; the concentrate was weighed and stored in the refrigerator until required. The extract was reconstituted in 2% DMSO prior to administration.

For the aqueous extract, 200g of the plant was macerated in 1.5 liters of water and was well shaken and left for 6h. The mixture was filtered and the resulting filtrate stored in the refrigerator until needed. Slight modifications of the methods of Trease & Evans (1989) were used to determine the phytochemical constituents of the crude extract and the values recorded in percentage per gram.

**Experimental design.** Groups I and II served as baseline and negative controls respectively and were untreated; Groups III - V received 75mg/kg, 150mg/kg and 300 mg/kg of methanol leaf extract of Moromdica balsamina (MEMB) respectively while VI – VII received 75 mg/kg, 150mg/kg and 300 mg/kg aqueous leaf extract of Moromdica balsamina (AEMB) for 7 days and then 40 minutes prior to the induction of the ulcers. Gastric ulcer was induced in the rats by administering 1ml/kg of 95 % ethanol orally. After 24 hours of food deprivation, all animals except those in Group I (baseline control) received a single oral dose of ethanol. Group II (negative control) did not receive any treatment before the induction of ulcer.

The animals were euthanized with chloroform 4 hours after induction of the ulcers; their stomachs were removed, rinsed in saline, and dissected along the greater curvature. A magnifying lens was used to observe and score the ulcers. The ulcerative lesion index was calculated as follows: ulcerative lesion < 1 mm = 1, ulcerative lesion > 1 mm = 2, ulcerative lesion > 2 mm = 3. The sum of the scores was divided by 10 to derive the ulcer index (UI) for each rat. The ulcer preventive index of the extracts was calculated with the formula; Ulcer Preventive index (UPI) (%) = ulcer index of negative control – ulcer index of treated/ulcer index of control x 100 (Kumar *et al.*, 2013, Ode *et al.*, 2011).

**Histological processing and Microscopy.** The excised stomachs were processed into formalin-fixed, paraffin-embedded, haematoxylin and eosin stained- slides using standard protocols. The sections were analyzed and representative micrographs of each group of animals taken.

**Statistical Analysis.** Numerical data obtained was analyzed with SPSS version 20 and the findings were expressed as mean SEM. The Dunnett’s post hoc test was used to compare the treated groups to the negative control group, with significance level defined as P values less than 0.05.
RESULTS

Phytochemical Analysis. Phytochemical analysis of the crude extracts of M. balsamina (Table I) showed the presence of alkaloids, saponins, tannins, glycosides, flavonoids, terpenoids and phenols. Flavonoids were the most abundant phytochemical present while saponins were the least. No steroids were detected in the extracts.

Ulcer index and Ulcer Preventive Index. All the methanol extract treated groups had lower UI compared to the negative control group. The low and high dose aqueous extract treated groups also had lower UI compared to the negative control (Table II). However, no animals in the treatment groups had a significantly lower UI compared to the negative control (P > 0.05). The UPI of the extracts ranged from approximately 2% to 50%; with the high dose AEMB having the highest UPI.

Gross and histological findings. Assessment of the gross appearance of the stomach mucosa of the test groups of animals revealed varying degrees of lesions mostly seen as haemorrhagic streaks and spots (Fig. 1). The groups that received high doses of AEMB and MEMB had visibly less ulcer lesions in their stomach mucosae.

Histologically, induced ulcers were observed in the gastric mucosa in various forms in the test groups ranging from regions of epithelial erosion, submucosa inflammation and gastric epithelial necrosis (Fig. 1). Histological findings correlated positively with ulcer scores with the animals that received high doses of both extracts having less severe ulcer lesions in their gastric epithelia.

Table I. Phytochemical constituents of crude extracts of M. balsamina.

<table>
<thead>
<tr>
<th>Phytochemical (%/g)</th>
<th>Alkaloid</th>
<th>Saponin</th>
<th>Tannin</th>
<th>Glycosides</th>
<th>Flavonoid</th>
<th>Terpenoid</th>
<th>Phenol</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mormodica balsamina</td>
<td>1.24</td>
<td>0.97</td>
<td>3.99</td>
<td>1.12</td>
<td>12.53</td>
<td>2.15</td>
<td>3.68</td>
<td>-</td>
</tr>
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</table>

Table II. Mean ulcer indices and ulcer preventive indices in different treatment groups following ethanol induced gastric ulcer lesion.

<table>
<thead>
<tr>
<th>Groups (treatment)</th>
<th>Ulcer Index</th>
<th>Ulcer preventive index (UPI %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II (Negative control)</td>
<td>2.85 ± 0.45</td>
<td>-</td>
</tr>
<tr>
<td>III (75mg/kg MEMB)</td>
<td>2.68 ± 0.17</td>
<td>5.96</td>
</tr>
<tr>
<td>IV (150mg/kg MEMB)</td>
<td>1.8 ± 0.90</td>
<td>36.84</td>
</tr>
<tr>
<td>V (300mg/kg MEMB)</td>
<td>1.65 ± 0.43</td>
<td>42.11</td>
</tr>
<tr>
<td>VI (75mg/kg AEMB)</td>
<td>1.78 ± 0.59</td>
<td>37.72</td>
</tr>
<tr>
<td>VII (150mg/kg AEMB)</td>
<td>2.80 ± 0.29</td>
<td>1.75</td>
</tr>
<tr>
<td>VIII (300mg/kg AEMB)</td>
<td>1.43 ± 0.48</td>
<td>50.00</td>
</tr>
</tbody>
</table>

UI values are presented as mean ±SEM.

Fig 1. Grid showing exposed stomach mucosa and histology sections of representative animals in selected groups showing the major findings. The haemorrhagic ulcer streaks are shown with the yellow arrows. Animals in the negative (ulcer control) had eroded gastric epithelial surfaces (red arrows). While those in the high dose extract treatment groups generally showed less severe stomach lesions like submucosal infiltration (black arrow) and patches of necrosed gastric epithelia cells (blue arrows) seen in the high dose groups of the aqueous and methanol extracts respectively. Histology sections were stained with haemotxylin and eosin; display magnification is X 100.
DISCUSSION

This study was designed to assess the antiulcer potentials of crude extracts of M. balsamina and determine its ability to protect the gastric histo-morphology against ethanol-induced gastric ulcer.

In this study, administration of a single dose of 1 ml/kg body weight of ethanol produced haemorrhagic ulcer lesions in the gastric mucosa of the rats. Alcohol has been documented to significantly increase susceptibility to gastric ulcers (Lee et al., 2017). Ethanol causes gastric ulcerations by inducing lipid peroxidation on the gastric mucosal surface it comes in contact with. Highly reactive free radicals produced in the process are thought to cause gastric epithelial cell death via induced intracellular oxidative stress (Amaral et al., 2013).

High doses (300 mg/kg body weight) of the extracts offered noticeable protection to the gastric mucosa of treated animals and prevented more severe lesions compared to the untreated animals. Mormodica balsamina leaves are reported to contain numerous phytochemicals including; flavonoids, glycosides, tannins, and alkaloids that have anti-plasmodial, anti-viral, hypoglycemic, and anti-oxidant effects (Otimenyin et al., 2008) which may have contributed to the observed gastro protection, even though it was not significant.

The crude leaf extracts of M. balsamina tested positive for tannins, alkaloids, saponins, glycosides, flavonoids, terpenoids and phenols in the preliminary screening. These phytochemicals present in Mormodica balsamina are known to possess cytoprotective abilities. For example, Flavonoids are well-known antioxidants that protect the stomach mucosa by clearing reactive oxygen species, stabilizing mucous membranes, and increasing gastric mucosal prostaglandin levels (Ahmed & Rahman, 2016; Onwukwe et al., 2018). These active bioactive compounds, according to previous studies, have the ability to boost mucus, bicarbonate, and prostaglandin secretion, as well as counteract the negative effects of reactive oxidants in the gastrointestinal lumen (Sahoo et al., 2016; Danai et al., 2021).

Tannins, because of their capacity to precipitate microproteins and promote vasoconstriction, have been proposed to provide gastric protection by forming an additional protective layer on the mucous membranes of the stomach, making them less vulnerable to irritating injury (El-Komy & Mouafi, 2016). Saponins have been shown to have antiulcer capabilities, presumably as a result of their surfactant-like qualities and the presence of antioxidants as documented by a previous study (Paguigan et al., 2014). Terpenoids have also been reported to have high antioxidant and antiulcer properties (Mohod & Bodhankar, 2013). In various experimental models of gastric ulcer, terpenoid has also shown anti-secretory and cytoprotective effects (Danai et al., 2021). Thus, the antiulcer activity exhibited by MEMB may due to the presence of tannins, flavonoids, and terpenoids (Sahoo et al., 2016).

In conclusion, the findings from the present studies showed that higher doses of methanol leaf extract of Mormodica balsamina protected the stomach of the experimental rat models against ethanol induced gastric ulcer.

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Graves. En extractos de M. balsamina puede poseer actividades antiuclerosas razonables en ratas contra la úlcera gástrica inducida por etanol.

PALABRAS CLAVE: Úlceras estomacales; Plantas medicinales; Mormódica balsamina; Mucosa gástrica.

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