Gastroprotective effect of ethylacetate fraction of the leaves of Hannoa klaineana on aspirin and histamine-induced gastric ulcer in rats

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Abstract

Background: Peptic ulcer disease remains endemic in our society affecting about four million people every year worldwide. Hannoa klaineana is used traditionally in the treatment of various gastrointestinal diseases including ulcer.

Aim: This study aims at evaluating the gastroprotective effect of ethylacetate fraction of the leaves of Hannoa klaineana (Simaroubaceae).

Methods: The gastroprotective effect of ethylacetate fraction of the Hannoa klaineana (50, 100 and 200mg/kg b.wt) was evaluated using aspirin and histamine induced ulcer models.

Results: In aspirin-induced ulcer model, the ethylacetate fraction of the Hannoa klaineana demonstrated significant (p<0.001) decreased in mean ulcer index with the maximum protective effect (99.84%) at 200 mg/kg against the gastric damages. While histamine-induced ulcer model, the solvent fraction significantly (p<0.001) decreased mean ulcer index with the protective effect up to 99.83% against the gastric lesions. In both models, a significant (p<0.001) increased in pH value coupled with significant (p<0.001) decreased in gastric volume, free and total acidity in rats pre-treated with varying doses of the ethylacetate fraction was found.

Conclusion: The mechanism of gastroprotective effects of ethylacetate fraction of the Hannoa klaineana could be attributed to its ability to stimulate prostaglandins secretion or possess prostaglandins like-substances or suppression of histamine-induced vasospastic effect and gastric secretion.

Keywords: Aspirin; Gastric acid; Hannoa klaineana; Histamine; Prostaglandin.

1. Introduction

Ulcer is a gastro-intestinal disease characterized by disruption of mucosal integrity of the stomach, duodenum or lower esophagos (Kumar et al., 2011). Every year, almost four million people are suffering by peptic ulcer disease (PUD) worldwide (Klein-Junior et al., 2010; Zelickson et al., 2011). Ulcers are classified base on their etiology into Type I and Type II ulcers. Type I ulcers occur as a result of impairment in mucosal protective factors and are characterized by less secretion of gastric acid. Type II ulcers are due to impaired negative feedback effects of acidification and are characterized by excess secretion of gastric acid (Andrade et al., 1988). Ulcers are also classified into gastric ulcer, duodenal ulcer, esophageal, and meckel’s diverticulum ulcer (Goroll and Mulley, 2009) depending on the site of occurrence in the gastro-intestinal tract.

Peptic ulcer disease (PUD) is caused by a lack of equilibrium between the luminal aggressive factors and the mucosal protective factors (Muralidharan and Srikanth, 2009). The predominant causes of peptic ulcer are H. Pylori infection and the therapeutic use of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin (Goroll and Mulley, 2009; Zhang et al., 2014; Feldman et al., 2016). Ulcers and their complications can also be caused by excessive alcohol consumption, smoking, diets (spicy food), emotional and psychosocial stress, genetic factors, ingestion of poisonous and other harmful substances and certain diseases (Andrade et al., 1988; Devi et al., 2011; Feldman et al., 2016).

Hannoa klaineana (Family: Simaroubaceae; genus: Hannoa) is traditionally used in many African countries in the treatment of many diseases such as cancer, malaria and fevers and various gastro-intestinal diseases including peptic ulcer (Basilevskai and Bouquet, 1969). In Nigeria, the plant is locally called “Takardar giwa” (in Hausa) and “Ofor” (in Igbo) and has been used in the North West, North East, and South East parts of the country for the treatment of various ailments including ulcer (François et al., 1998). Studies showed that the plant exhibited activities against P. fulciulcerrum in vitro and in vivo, and against a number of cancer cell lines (Monjour et al., 1987; Ang et al., 1995). The plant has been reported to possess antioxidant and antimicrobial activities as evidenced by its free-radical scavenging potential and significant LDH inhibitory effect (Odeghe et al., 2006).

Conventional treatment of peptic ulcer disease (PUD) is associated with several challenges including high relapse rates and many side effects such as arrhythmias, gynaecomastia, hypergastrinaemia, nausea, dizziness, diarrhea, and constipation (Akah et al., 2007; Ahmad...
et al., 2013; BNF, 2018). Many studies confirmed the effectiveness of medicinal plants in the treatment of peptic ulcer diseases (PUDs) and their complications (Nwafor and Okeye, 2005; Akah et al., 2007; Jhanssi et al., 2010). Previous studies by the Authors showed that crude methanol extract and its solvent fractions of the leaves of Hannoa klaineana exhibited strong anti-ulcer activity and ethylacetate fraction was the most active fraction. This study aims at evaluating the gastro-protective effect of the ethylacetate fraction of the leaves of Hannoa klaineana (Simaroubaceae) and to elucidate its mechanism of action in different ulcer models.

2. Materials and methods

2.1. Experimental animals

A total of 60 Wistar rats weighing 180-200g were purchased from the Animal House Unit of Usmanu Danfodiyo University, Sokoto. The animals were housed in polycarbonate cages containing pine wood chip beddings; five rats per cage. They were acclimatized to the standard vivarium conditions (temperature 23±2°C, relative humidity 30-70%, and 14 hours light -10 hours dark cycle) for one week before the commencement of the experiment. They were fed with commercial rat’s pellets and water provided ad libitum.

2.2. Collection and identification of the plant sample

The fresh leaves of Hannoa klaineana were collected from Anka forest and Shinkafi town in Zamfara state with the help of traditional herbalists in Sokoto Old Market. The plant was identified and authenticatedated (UDUH/ANS/0335) at the Herbarium Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto.

2.3. Preparation of the plant extract

Fresh leaves of the Hannoa klaineana were dried in a clean ventilated room for one week. The dried leaves were weighted and grounded to fine powder using pestle and mortar. One thousand gram (1000g) of the dried powdered leaves was soaked in 2.5L of methanol for 72 hours with intermittent stirring. The extract was filtered through Whatman filter paper, and the filtrate was concentrated in rotary evaporator at 40°C under reduced pressure for 3 hours. The final residue was weighed and percentage yield was calculated and then stored at 4°C before use.

2.4. Fractionation

The crude methanol leaves extract of the Hannoa klaineana was fractionated by liquid-liquid fractionation in a separation funnel. The dried extract (20g) was suspended in distilled water, and then fractionated with ethylacetate. The partitioning was performed three times by mixing 300mL of ethylacetate with the aqueous phase, shaken vigorously and kept for 10 min to settle between the layers. The aqueous residue settled down and ethylacetate portion was collected and then labelled as ethylacetate fraction. The fraction was concentrated in rotary evaporator under reduced pressure. The solid fraction obtained was weighed and the percentage yield of the fraction was calculated.

2.5. Anti-ulcer activity

2.5.1. Aspirin-induced ulcer model

In aspirin-induced gastric ulcer model, the study was conducted according to the method described by Esplugues et al. (1982) with some modifications. Wistar rats (n=30) weighing 180-200g were randomly divided into six groups of 5 rats each. Group 1 received normal saline (10ml/kg bw./day) and served as normal control group. Group 2 was treated as negative control group in which no pre-treatments were given. Group 3 received reference standard drug, omeprazole (20mg/kg bw./day) (MFDS, 2014) and served as positive control group. Group 4, 5 and 6 were pre-treated with 50, 100 and 200 mg/kg bw doses of the fraction, respectively. The animals in the respective groups were pre-treated with the solvent fraction and the standard drug for 3 days. After the treatment, the animals were fasted for 24 hours but allowed free access to water. On the 4th day, thirty minutes after the final treatment, gastric ulcer was induced in rats by administration of aspirin (200 mg/kg, p.o.) (Datta et al. 2002). After 4 hours the animals were anaesthetized and then sacrificed by cervical dislocation.

2.5.2. Histamine-induced ulcer model

Histamine-induced gastric ulcer model was performed according to the method described by Shan et al. (2006) and Malairajan et al. (2007). Wistar rats (n=30) weighing 180-200g were randomly divided into six groups of 5 rats each. Group 1 received normal saline (10ml/kg bw./day) and served as normal control group. Group 2 was treated as negative control group in which no pre-treatments were given. Group 3 received reference standard drug, famotidine (20mg/kg bw. p.o.) (Bharti et al., 2010) and served as positive control group. Group 4, 5 and 6 were pre-treated with 50, 100 and 200 mg/kg bw doses of the fraction, respectively. The animals in the respective groups were pre-treated with the solvent fraction and the standard drug for 3 days. After the treatment, the animals were fasted for 24 hours but allowed free access to water. On the 4th day, thirty minutes after the final treatment, gastric ulcer was induced in rats by administration of histamine acid phosphate (100 mg/kg i.p.) (Maity et al., 1995). Animals were sacrificed after 4 hours of the ulcer induction by cervical dislocation.

2.6. Isolation of stomachs and collection of gastric juice

The carcass stomachs were removed after midline incision in the abdomen of the rats, and then fixed in 10% neutral buffered formalin prior to examination of ulcer. The entire gastric content was drained into centrifuge tubes through a small nick along the greater curvature and the gastric volume was read directly from the graduation on the centrifuge tubes.
2.7. Gross and microscopic examination of ulcers

The carcass stomachs were dissected opened along the greater curvature and rinsed with distilled water to remove gastric contents and blood clots. The stomachs were mounted on a glass slide and observed for detection of gastric lesions using 10× magnification lens and dissecting microscope and then the number and the length of gastric lesions were evaluated (Sánchez et al., 2010).

2.8. Scoring of ulcers

Ulcers were scored based on their severity according to a method adopted by Kulkarni (2002) as follows:

0 = Normal appearance of stomach
0.5 = Red appearance of stomach
1 = Spot ulcer
1.5 = Hemorrhagic streaks
2 = Ulcer ≥ 3 mm² but ≤ 5 mm²
3 = Ulcer >5 mm²

2.9 Calculation of ulcer index and percentage gastro-protection

Ulcer index was calculated using the formula below:

\[ UI = UN + US + UP \times 10^{-1} \] (Jyoti et al., 2012)

Where: UI = Ulcer Index; UN = Average of number of ulcer per animal; US = Average of severity score; UP = Percentage of animals with ulcer

The percentage gastro-protection was calculated using the formula below:

\[ \% \text{ gastro protection} = \frac{UIC - UIT}{UIC} \times 100 \]  (Jyoti et al., 2012)

Where; UIC = Ulcer Index of Control; UIT = Ulcer Index of Test

2.10. Evaluation of pH

Five ml (5mL) of distilled water was added into the centrifuge tubes containing the gastric juice and centrifuged at 10000rpm for 5 minutes. The clear supernatant was separated and the pH of gastric acid was measured using pH meter (Nwinyl and Kwanashie, 2013).

2.11. Determination of free and total acidity

One ml (1mL) of the gastric juice was pipetted out in 100mL conical flask and two-three drops of topfer’s reagent (Dimethyl-azo-benzene with phenolphthalein) was added into the conical flask. The solution was titrated with 0.01N NaOH until all traces of pink colour disappears and the colour of the solution turns to yellowish orange, then the volume of alkali added was recorded. This volume corresponds to free acidity. Titration was continued until pink color of solution reappears. Again the total volume of alkali added was recorded. This volume corresponds to total acidity. Acidity (mEq/L/100gm) was calculated using the formula below:

\[ \text{Free/Total acidity (mEq/L/100gm)} = \frac{\text{Volume of NaOH used} \times 100}{1000} \]  (Shay et al., 1954)

3. Results

3.1. Aspirin-induced gastric ulcer model

Fig.1 shows the gross appearance of the stomachs of rats in aspirin-induced ulcer model. Administration of aspirin caused severe lesions with extensive haemorrhages in the gastric mucosa of the negative control rats (Fig.1B). Pre-treatment of the rats with the ethylacetate fraction reduced the damage in a dose dependent manner. The gastric mucosa of the rats pre-treated with 50 mg/kg dose of the solvent fraction showed spot ulcer (Fig.1D). The rats pre-treated with 100 and 200 mg/kg doses of the solvent fraction and the omeprazole (20 mg/kg) showed normal appearance of the mucosa (Fig.4E, F and C, respectively).
The effect of ethylacetate fraction of the *Hanna klaineana* on the aspirin-induced gastric mucosal damage is shown in Fig.2. The result showed a significant ($p<0.05$, $p<0.01$, $p<0.001$) decreased in mean ulcer index in the pre-treated rats with 50, 150 and 200 mg/kg doses of the solvent fraction and the omeprazole (20 mg/kg), the standard drug as compared to the negative control rats (Fig.2).

Fig.3 shows the percentage gastroprotection of ethylacetate fraction of the *Hanna klaineana* against the aspirin-induced gastric lesions. The solvent fraction exhibited protective effect in a dose dependent manner. The maximum protective effect (99.84%) against the gastric damages was found at 200mg/kg dose of the solvent fraction and is more than that demonstrated by the reference standard drug, omeprazole (64.36%) (Fig.3).

The effect of ethylacetate fraction of the *Hanna klaineana* on pH, gastric volume, free acidity and total acidity in aspirin-induced ulcer is shown in Table 1. The result showed a significant ($p<0.001$) increased in pH of gastric juice coupled with significant ($p<0.001$) decreased in volume of gastric juice, free and total acidity in the pre-treated rats with all the three doses of the solvent fraction and the standard drug as compared to the negative control group (Table 1).

**Table 1: Effects of Ethylacetate Fraction of the *Hanna Klaineana* on pH, Gastric Volume, Free and Total Acidity**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Gastric pH</th>
<th>Gastric Volume (mL)</th>
<th>Free Acidity (mEq/L)</th>
<th>Total Acidity (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROL</td>
<td>5.80 ± 0.35</td>
<td>2.50 ± 0.13</td>
<td>35.60 ± 0.50</td>
<td>102.60 ± 0.50</td>
</tr>
<tr>
<td>NEGATIVE CONTROL</td>
<td>3.62 ± 0.37</td>
<td>4.52 ± 0.21</td>
<td>78.80 ± 1.15</td>
<td>153.20 ± 2.67</td>
</tr>
</tbody>
</table>
### 3.2. Histamine-induced gastric ulcer model

Fig.4 shows the gross appearance of the stomachs of rats in histamine-induced ulcer model. The gastric mucosa of the negative control rats showed prominent ulcers after administration of histamine (Fig.4B). The rats pre-treated with 50 mg/kg dose of the solvent fraction showed red appearance of the gastric mucosa (Fig.4D). The gastric mucosa of the rats pre-treated with 100 and 200 mg/kg doses of the solvent fraction and the standard drug, famotidine showed normal appearance of the gastric mucosa (Fig.4E, F and C, respectively).

![Gross Appearance of Stomachs](image)

The effect of ethylacetate fraction of the *Hannoa klaineana* on histamine-induced gastric ulcer is shown in Fig.5. Pre-treatment of the rats with 50, 150 and 200 mg/kg doses of the solvent fraction and the famotidine (20 mg/kg) showed a significant (*p*<0.001) decrease in mean ulcer index in comparison to the negative control group (Fig.5).

![Effect of Ethylacetate Fraction](image)

The percentage gastroprotective effect of ethylacetate fraction of the *Hannoa klaineana* against the gastric lesion in histamine-induced ulcer model is shown in Fig.6. The solvent fraction exhibited highest protective effect (99.83%) against the gastric lesions at 200 mg/kg and was more than that demonstrated by the famotidine (65.91%), the reference standard drug (Fig.6).
Table 2 shows the effect of ethylacetate fraction of the *Hannoa klaineana* on pH, gastric volume, free and total acidity in histamine-induced gastric ulcer. The result showed a significant ($p<0.001$) increased in the pH of gastric juice along with significant ($p<0.001$) decreased in volume of gastric juice, free and total acidity in the rats pre-treated with 50, 100 and 200 mg/kg doses of the solvent fraction and the standard drug as compared to the negative control group (Table 2).

### Table 2: Effects of Ethylacetate Fraction of the *Hannoa klaineana* on pH, Gastric Volume, Free and Total Acidity

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Gastric pH (mEq/L)</th>
<th>Gastric Volume (mEq/L)</th>
<th>Free Acidity (mEq/L)</th>
<th>Total Acidity (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>5.80 ± 0.35</td>
<td>2.50 ± 0.13</td>
<td>35.60 ± 0.50</td>
<td>102.60 ± 0.50</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1.84 ± 0.15</td>
<td>6.38 ± 0.35</td>
<td>109.60 ± 3.99</td>
<td>252.0 ± 2.75</td>
</tr>
<tr>
<td>Positive Control</td>
<td>4.00 ± 0.25***</td>
<td>3.64 ± 0.14***</td>
<td>46.60 ± 2.04***</td>
<td>101.00 ± 2.64***</td>
</tr>
<tr>
<td>EtyAc (50mg/kg)</td>
<td>3.98 ± 0.21***</td>
<td>4.02 ± 0.13***</td>
<td>48.80 ± 2.55***</td>
<td>107.80 ± 2.81***</td>
</tr>
<tr>
<td>EtyAc (100mg/kg)</td>
<td>5.28 ± 0.22***</td>
<td>2.68 ± 0.11***</td>
<td>18.60 ± 1.20***</td>
<td>45.40 ± 2.01***</td>
</tr>
<tr>
<td>EtyAc (200mg/kg)</td>
<td>7.04 ± 0.20***</td>
<td>1.18 ± 0.08***</td>
<td>8.60 ± 1.43***</td>
<td>17.00 ± 1.64***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=5 rats/group).

***$p<0.001$ statistically significant when compared with negative control (One-way ANOVA) followed by Tukey’s multiple comparison test. Normal control (10ml/kg, Normal saline), Negative control (100mg/kg, Histamine), Positive control (20mg/kg, Famotidine), ethylacetate (EtyAc).

### 4. Discussion

#### 4.1. Effect of ethylacetate fraction of the *Hannoa klaineana* on aspirin-induced gastric ulcer in rats

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin induce mucosal injury by several mechanisms. The biochemical actions common to all conventional NSAIDs are inhibition of prostaglandin synthesis, decreased mucus and bicarbonate secretion, and topical irritation of the gastric epithelium (Whittle, 1977; Whittle, 1992; Wallace, 2008). Non-steroidal anti-inflammatory drugs (NSAIDs) disrupt mucus phospholipids of the gastric mucosa leading to uncoupling of mitochondrial oxidative phosphorylation thereby resulting to mucosal damage (Glarborg et al., 1976; Spenny and Bhown, 1977; Bhala et al., 2013).

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit activity of cyclooxygenase enzyme (COX-1 and COX-2) the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins (Del et al., 2003). Prostaglandins play a vital protective role via enhancing the mucosa’s resistance to injury under certain conditions, perhaps by increasing mucosal blood flow (Robert, 1979; Gaskill et al., 1982), stimulating the secretion of mucus and bicarbonate (Hogan et al., 1994), strengthening of the gastric mucosal barrier (Takeuchi and Nobuhara, 1981), increasing release of endogenous mediators of gastric cytoprotection (Szabo et al., 1981) and regulating mucosal cell turnover and repair (Hawkey and Ramton, 1985).

Aspirin is known to be potent inhibitor of prostaglandin (PG) biosynthesis (Whittle, 2003) characterized by gastric damage and hemorrhage (Toruner, 2007). The drug is frequently used as a model in studies on *in vivo* cytoprotective effect of a chemical substance (Rajka-poor et al., 2002). In this study, pre-treatment of the rats with ethylacetate fraction of the *Hannoa klaineana* significantly decreased mean ulcer index with the maximum protective effect (99.84%) at 200 mg/kg against the gastric damages. Hydrolytic (HCl) and proteolytic (pepsin) activities in stomach are important determinants of development of gastric ulcer (Lüllmann et al., 2000). Secretion of gastric (hydrochloric) acid and pepsin is responsible for the potential of causing mucosal damage (Das et al., 1997). The result in this study showed a significant ($p<0.001$) increased in pH of gastric juice coupled with significant ($p<0.001$) decrease in gastric volume, free and total acidity in rats pre-treated with varying doses of the ethylacetate fraction. Changes in pH value are associated with pathogenesis of ulcer and gastric damage in experimental animals (Lüllmann et al., 2000). High pH value is associated with decrease in hydrogen ion (H+) concentration in gastric juice. Decrease in gastric acidity is an important improvement in the treatment of peptic ulcer disease (Proctor and Deans, 2014).

#### 4.2. Effect of ethylacetate fraction of *Hannoa klaineana* on histamine-induced gastric ulcer in rats

The induction of gastric ulcers by histamine administration has long been recognized (Amagase and Okabe, 2003). The basis of the histamine induced gastric ulcer model is mediating formation of gastric ulcers by the release of histamine (Adinortey et al., 2013). Histamine released from mast cells binds with receptors present on the surface of parietal cells and causes activation of adenylate cyclase which converts ATP into c-AMP subsequently enhanced secretion of gastric acid (Sander et al., 2006). The model is use to evaluate anti-secretory effects of a chemical agent against ulcers and agents that function as H2-receptor antagonists (Hay et al., 1942). In this study, pre-treatment of the rats with ethylacetate fraction significantly ($p<0.001$) decreased mean ulcer index with the protective effect up to 99.83% against the histamine-induced gastric lesions.
Histamine (2-[4-ethylazolyl] ethylamine) is a biogenic molecule synthesized by L-histidine decarboxylase (HDC) from the amino acid histidine (Moody and Davis, 1970). Histamine released from gastric mucosa can affect mucosal blood vessels and change their permeability (O'Brien and Silen, 1973). Fiocca et al. (1974) and Moody and Davis, 1970 have shown that administration of histamine increased the ionic permeability of the gastric mucosal barrier. In the present study, pre-treatment of the rats with 50, 100 and 200 mg/kg doses of the ethylacetate fraction showed a significant increased (p<0.001) in pH of gastric juice along with significant (p<0.001) decreased in gastric volume, free and total acidity. High pH value is associated with decreased in hydrogen ions concentration in the gastric juice (Lullmann et al., 2000).

5. Conclusion and recommendation

The mechanism of gastroprotective effect of ethylacetate fraction of the Hannoa klaineana in aspirin-induced gastric ulcer model could be attributed to its ability to stimulate prostaglandins secretion or possess prostaglandins like-substances. Also, the protective effect of the solvent fraction against the histamine-induced gastric ulcer could be attributed to the suppression of histamine-induced aspirin-induced gastric effect and gastrosecretion. Further study could be done to characterize the specific components responsible for the gastroprotective effect of ethylacetate fraction of the Hannoa klaineana.

References
