Ulcer-protective and Antidiarrhoeal Effects of the Aqueous Stem Bark Extract of Bridelia ferruginea in Rodents

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Abstract: Bridelia ferruginea which have some ethnomedicinal applications was investigated for ulcer-protective and anti-diarrhoeal effects. The ulcer-protective and anti-diarrhoeal activities of the aqueous stem bark extract were examined in rats and mice. Acute toxicity studies were also carried out. The extract exhibited ulcer-protective properties against ethanol and indomethacin induced ulceration activity in rats with maximal anti-ulcer activity observed at 400 mg kg⁻¹. The extract dose dependently decreased intestinal propulsion of charcoal meal in mice. The aqueous extract of Bridelia ferruginea also exerted significant anti-enteropooling in mice. A significant antidiarrhoeal activity was also recorded in mice. The frequency of defaecation as well as the wetness of faecal droppings was reduced. In addition, the extract produced 100% inhibition of castor oil-induced diarrhoea in mice. The oral LD₅₀ obtained was greater than 5000 mg kg⁻¹ in mice. The results show that Bridelia ferruginea stem bark probably contains some active ingredients that potentially could be developed as useful drug for the treatment of ulcer and diarrhoea in Nigerian herbal traditional medicine.

Key words: Bridelia ferruginea, ulcer-protective, anti-enteropooling, herbal medicine, rodents

INTRODUCTION

Plants form the major part of treatments used by traditional healers in many societies, thus many plants have acquired reputation for being useful against diseases. Ethnomedicines play a central role in the search for and development of new drugs (Kirby, 1997; Heinrich, 2000). Many herbal remedies have been used traditionally for treatment of diseases in Nigeria. The World Health Organization (WHO) encourages the inclusion of herbal medicine in health care because of the great potentials they possess. Also, the long historical use of medicinal plants has demonstrated the safety and efficacy of traditional medicine. A number of medicinal plants are traditionally endowed with gastrointestinal properties. One of such medicinal plants is Bridelia ferruginea.

Bridelia ferruginea (Family: Euphorbiaceae) is an indigenous medicinal plant in Nigeria. It is commonly found in the savannah. It is usually a gnarled shrub which sometimes reaches the size of a tree in suitable condition. Its common names in Nigeria include Kirri, Kizni (Hausa), Maren (Fulani), Iralodan (Yoruba), Ola, Ede (Igbo). Its habitat is the savannah, especially in the moister regions from Guinea to Zaire and Angola. The bark is dark grey, rough and often marked scaly (Rashid et al., 2000). Bridelia ferruginea has diverse uses. The leaves have been used to treat diabetes. The plant is also used as a purgative and a vermifuge (Cimanga et al., 1999). The bark extract is being used for milk coagulation and also in lime juice for the formulation of traditional gargele “ogun efu” (Orafidiya et al., 1996). Magistretti et al. (1988) reported that the bark extract of the plant possess antimicrobial activities against some micro-organisms known to cause enteric and secondary upper respiratory tract infections, while Olajide et al. (1999) reported that the plant has anti-inflammatory activity. Its potential for water treatment has also been reported by Kolawole and Olayemi (2003). The present study was designed to investigate the ulcer-protective and anti-diarrhoeal potentials of Bridelia ferruginea stem bark extract in experimental animal models.

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MATERIALS AND METHODS

Plant material: The stem bark of Bridelia ferruginea was collected from Odenigbo, Nkalagu-Obukpa, Nasuksa, Enugu state, Nigeria. The leaves, fruits and stem bark were identified and authenticated by Mrs. Ibrahim Jemilat a Taxonomist of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. Voucher specimen (No: NIPRD (H) 6414) was deposited in the herbarium of NIPRD. The international plant name index is Euphorbiaceae Bridelia ferruginea Niger Fl. (W.J. Hooker). 511. 1849 (Nov-Dec. 1845) (IK).

Extraction method: The bark was cut into pieces and air-dried at room temperature for 7 days and ground to fine powder using mortar and pestle and soaked in boiled distilled water over night. The filtrate was evaporated to dryness on a water bath to obtain hot water extract of Bridelia ferruginea and the yield calculated to be 50.29 w/w.

Phytochemical screening: The phytochemical composition of the aqueous extract was determined using standard method (Trease and Evans, 1983).

Acute toxicity (LD₅₀) study: The LD₅₀ of stem bark extract was tested to determine the safety of the agent using (Lorke, 1983) method. Dose levels used ranged from 10-5000 mg kg⁻¹ p.o. The acute toxicity was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all. The animals were kept under the same conditions and observed for toxicity signs and mortality for 24 h.

Animals: Adult Wistar rats (200-250 g) and Swiss albino mice (22-25 g) of either sex maintained at the Animal Facility Centre, NIPRD and Abuja, Nigeria were used for the experiments. The animals were housed in cages with saw animals were housed in cages with saw dust as bedding and given food and water ad libitum, except in cases where fasting was needed. The animals were maintained under standard conditions of humidity, temperature and 12-h light/12-h darkness cycle. They were used in accordance with NIH Guide for the care and use of laboratory Animals. NIH publication (No. 83-23) revised (NRC, 1985).

Ethanol-induced gastric ulceration in rats: The experimental rats were fasted for 48 h but had water ad libitum. They were randomized into 5 groups of 6 each. Group 1 received 20 mL kg⁻¹ normal saline. Group 2 received standard drug (Ranitidine 20 mg kg⁻¹). Group 3, 4 and 5 received 100, 200 and 400 mg kg⁻¹ of the extract, respectively. All the drugs were administered orally. One hour later, ulceration was induced by intragastric instillation of 0.5 mL of 90% ethanol and one hour after ethanol administration, rats were anaesthetized using ether and the stomachs were removed and opened along the greater curvature to examine ulcerative lesions. The number, length and severity of the ulcers were noted and scored on an arbitrary 0-6 point scale (Magistretti et al., 1988):

0 = No lesion
1 = 1-3 small lesions
2 = 1-3 large lesions
3 = 1-3 thick lesions
4 = More than 3 small lesions
5 = More than 3 large lesions
6 = More than 3 thick lesions

Indomethacin-induced ulceration in rats: The rats were fasted for 48 h having access to water ad libitum. The animals were randomized into 5 groups of 6 each. Group 1 received 20 mL kg⁻¹ normal saline. Group 2 received standard drug (Ranitidin 20 mg kg⁻¹). Groups 3, 4 and 5 received 100, 200 and 400 mg kg⁻¹ of the aqueous extract, respectively. All the drugs were administered by oral route. One hour later, ulceration was induced by oral administration of 25 mg kg⁻¹ of indomethacin. Five hours after, rats were anaesthetized using ether and the stomach were removed and opened along the greater curvature to examine any ulcerative lesions. Ulcers were also scored according to severity (Nwafor et al., 1996):

0 = No ulcer
1 = Haemorrhagic and slightly dispersed ulcers less than 2 mm length
2 = 1 ulcer, haemorrhagic and up to 5 mm length
3 = More than 1 ulcer, each up to 5 mm length
4 = 1 ulcer above 5 mm in length
5 = More than 1 ulcer above 5 mm in length

Castor oil-induced diarrhoea in mice: The method of Capasso et al. (2008) was adopted with slight modifications. The animals were deprived of food for 24 h but had free access to water. Thirty mice were randomized into five groups of six mice each. Group 1, which served as negative control was given 20 mL kg⁻¹ normal saline. Group 2 which served as the positive control received 3 mg kg⁻¹ of loperamide. Group 3, 4 and 5 received graded doses of Bridelia ferruginea stem bark extract (100-400 mg kg⁻¹), all administered by oral route. One hour
after pre-treatment with these agents, mice in all the groups were given 0.3 ml of castor oil. The mice in each group were then placed singly in cages with absorbent paper on their floors. The number of defecations per animal was recorded for 4 h.

**Intestinal transit test:** The effect of the stem bark extract on gastrointestinal motility was evaluated using the method described by Rao *et al.* (1997) with slight modification. Thirty mice were randomly divided into 5 groups of 6 mice each and fasted for 24 h prior to the experiments but were allowed free access to water. Group 1 served as negative control and received 20 ml kg\(^{-1}\) normal saline. Group 2 served as positive control and received 3 mg kg\(^{-1}\) of atropine (Standard drug). Group 3-5 received graded doses of *Bridelia ferruginea* stem bark extract (100-400), all administered orally. Thirty minutes after drug administration, each mouse was given 0.5 ml of charcoal meal orally (5% deactivated charcoal in 10% aqueous tragacanth). The animals were sacrificed thirty minutes later and the abdomen opened. The small intestine was dissected out from the pylorus to the caecum and the total distance travelled by the charcoal plug a long the small intestine was estimated for both the control and treated groups. For each group, the results were expressed as percentage of th e distance travelled from the pylorus to the caecum (Gamariel and Akah, 1996).

**Castor oil-induced enteropooling in mice:** In this method, Swiss albino mice of either sex (22-25 g) were divided into 5 groups of 6 mice each. The animals were fasted 24 h prior to the experiment but allow free access to water. Group 1 was treated 20 ml kg\(^{-1}\) of normal saline which served as negative control. Group 2 was treated with standard drug (Loperamide 3 mg kg\(^{-1}\)). Group 3-5 received graded doses of *Bridelia ferruginea* stem bark extract (100-400 mg kg\(^{-1}\)), all administered orally. Thirty minutes later, each mouse was given 0.3 ml of castor oil orally. The animals were sacrificed thirty minutes later and the whole length of the intestine from the pylorus to the caecum was ligated and dissected out. The contents were expelled into a measuring cylinder and the volume measured.

**Statistical analysis:** Results were expressed as Mean±SEM. The difference between mean was determined using One Way Analysis of Variance (ANOVA). p<0.05 was considered significant.

**RESULT**

**Phytochemical:** Results of phytochemical screening of the hot water extract of *B. ferruginea* stem bark revealed the presence of alkaloids, tannins, saponins, steroids, flavonoids, terpenes, phenols and resins.

**Acute toxicity test:** The LD\(_{50}\) of the extract was estimated to be greater than 5000 mg kg\(^{-1}\) p.o. in mice. The behavioral signs of toxicity exhibited by animals are respiratory distress and abdominal constriction.

**Ethanol-induced gastric ulcer:** The extract was found to possess remarkable ulcer-protective properties at 200 and 400 mg kg\(^{-1}\).

Four hundred milligrams per kilogram produced the maximal effect (100% inhibition of ulceration) whereas the standard drug (ranitidine) gave 91.38% protection (Table 1).

**Indomethacin induced gastric ulcer:** The pretreatment test with aqueous extract of *Bridelia ferruginea* stem bark revealed a protective action against indomethacin-induced gastric ulcer. The extract showed a significant (p<0.05) reduction in gastric hyperemia in both number and severity of the lesion. Four hundred milligram per kilogram produced maximal effect of 91.75% inhibition ulcer protection whereas the standard drug (Ranitidine) produced 87.5% protection (Table 2).

**Effect on castor oil-induced anteropooling:** The aqueous extract of *Bridelia ferruginea* stem bark was found to possess an anti-enteropooling activity. The extract significantly (p<0.05) inhibited castor oil-induced intraluminal accumulation of fluid in a dose-dependent manner, an effect that favourably compares with inhibition caused by 3 mg kg\(^{-1}\) of loperamide (Table 3).

**Effect on castor oil-induced diarrhoea:** The aqueous stem bark extract of *Bridelia ferruginea* exhibited a remarkable dose-dependent anti-diarrhoeal activity in the study. The

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**Table 1:** Effects of aqueous stem bark extract of *B. ferruginea* on ethanol-induced gastric ulceration in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Extract dose (mg kg(^{-1}))</th>
<th>Ulcer index</th>
<th>Maximal protection of ulceration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline</td>
<td>3.8±0.21</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Ranitidine 20</td>
<td>0.3±0.21</td>
<td>91.38*</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>2.67±0.21</td>
<td>91.75*</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>2.60±0.26</td>
<td>47.78*</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>0.60</td>
<td>100.00*</td>
</tr>
</tbody>
</table>

*Significantly different from control at p<0.05

**Table 2:** Effects of aqueous stem bark extract of *B. ferruginea* on indomethacin-induced gastric ulceration in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Extract dose (mg kg(^{-1}))</th>
<th>Ulcer index</th>
<th>Maximal protection of ulceration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline</td>
<td>4.60±0.00</td>
<td>87.50*</td>
</tr>
<tr>
<td>2</td>
<td>Ranitidine 20</td>
<td>0.50±0.34</td>
<td>33.25*</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>2.67±0.21</td>
<td>54.25*</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>1.83±0.31</td>
<td>91.75*</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>0.33±0.21</td>
<td>91.75*</td>
</tr>
</tbody>
</table>

*Significantly different from control at p<0.05
Table 3: Effects of aqueous Stem bark extract of *B. ferruginea* on castor oil-induced diarrhoea in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Extract dose (mg kg⁻¹)</th>
<th>Mean±SEM (h) frequency of diarrhoea in 4 h</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal saline</td>
<td>10.67±2.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Loperamide 3</td>
<td>1.50±0.88</td>
<td></td>
<td>85.94*</td>
</tr>
<tr>
<td>3 100</td>
<td>6.67±0.49</td>
<td></td>
<td>37.49*</td>
</tr>
<tr>
<td>4 200</td>
<td>3.67±0.34</td>
<td></td>
<td>65.60*</td>
</tr>
<tr>
<td>5 400</td>
<td>0.00</td>
<td></td>
<td>100.00*</td>
</tr>
</tbody>
</table>

*Significantly different from control at p<0.05

Table 4: Effect of aqueous Stem bark extract of *B. ferruginea* on castor oil-induced enteroaggregating in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Extract dose (mg kg⁻¹)</th>
<th>Mean±SEM volume of intestinal contents</th>
<th>Inhibition of secretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal saline</td>
<td>0.07±0.01</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2 Loperamide 3</td>
<td>0.04±0.01</td>
<td></td>
<td>83.71*</td>
</tr>
<tr>
<td>3 100</td>
<td>0.04±0.01</td>
<td></td>
<td>42.86*</td>
</tr>
<tr>
<td>4 200</td>
<td>0.03±0.01</td>
<td></td>
<td>57.14*</td>
</tr>
<tr>
<td>5 400</td>
<td>0.01±0.00</td>
<td></td>
<td>85.71*</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05

Table 5: Effect of aqueous extract of *B. ferruginea* on intestinal transit time in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Extract dose (mg kg⁻¹)</th>
<th>Mean distance travelled by marker (cm)</th>
<th>Intestinal transit (h)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal saline</td>
<td>37.33±0.99</td>
<td>55.5±1.65</td>
<td>95.10</td>
<td></td>
</tr>
<tr>
<td>2 Atropine 3</td>
<td>38.0±0.73</td>
<td>71.7±0.83</td>
<td>18.87</td>
<td>76.23*</td>
</tr>
<tr>
<td>3 100</td>
<td>39.0±0.19</td>
<td>20.5±0.69</td>
<td>51.00</td>
<td>42.10*</td>
</tr>
<tr>
<td>4 200</td>
<td>39.5±0.80</td>
<td>17.8±0.60</td>
<td>45.33</td>
<td>49.77*</td>
</tr>
<tr>
<td>5 400</td>
<td>36.6±0.88</td>
<td>9.3±0.49</td>
<td>25.44</td>
<td>69.66*</td>
</tr>
</tbody>
</table>

*Significantly different from control at p<0.05

In the evaluation of anti-ulcer drugs for the treatment of gastric ulcers, induced in laboratory animals in various experimental models.

Ethanol, indomethacin and hypothymic restraint stress are among the most commonly utilized experimental models for the evaluation of anti-ulcer activity in rats (Lewis and Hanson, 1991; Akah et al., 1997). Ethanol treatment induces a direct damage of gastric mucosal cells, probably by the development of free radicals and hyper oxidation of lipid (Puruinen et al., 1980; Pihan et al., 1987; Ito et al., 1993). Moreover, in the stomach, ethanol causes solubilization of mucus constituents and depresses tissue levels of proteins, leading to flow stasis in gastric blood (Szabo et al., 1986). Endogenous glutathione and prostaglandin (PG) levels are also lowered by ethanol while the release of histamine, influx of calcium ions and generation of free radicals and production of leukotrienes are all increased (Gavin and Szabo, 1992; Akudod et al., 2010). It is possible that an increase in gastric mucus or a possible leukotriene antagonism may contribute to the gastroprotective effect of *Bridelia ferruginea*. Also, the fact that significant antpyretic and analgesic activities have been demonstrated (Akudod et al., 2011) lend credence to the notion that the inhibition of prostaglandins might also be a possible mechanism for the ulcer protective effects of *Bridelia ferruginea* aqueous extract.

Indomethacin is known to induce relative increase in leukotriene C4 at the cost of reduced PG E2 levels which may induce mucus vasoconstriction and enhance non-steroidal anti-inflammatory drugs-induced injury (Hawkey, 1989). The ulcerogenic action of indomethacin especially in an empty stomach has already been established (Rasool et al., 2008). Indomethacin-induced ulceration mostly affects the mucosal part of the stomach (Nwafor et al., 1996). Indomethacin is known to inhibit the cyclooxygenase enzyme (COX) responsible for the production of prostaglandins involved in maintenance of gastric mucosal integrity (Rasool and Varalashmi, 2006). Inhibition of COX-1 enzyme may result in the formation of ulcers in many human and hence the selective inhibition of COX-2 enzyme by compounds has a major advantage over non-selective non-steroidal anti-inflammatory drugs (Smith et al., 2000). The mechanism for the mucosal protective action of the extract may be due partly to the stimulation of PG synthesis since endogenous PGs play a crucial role in gastroprotection. The significant delay in gastrointestinal transit caused by the extract was demonstrated in the charcoal meal treated mice. The findings indicated a decrease in peristaltic activity and ultimately decrease gastrointestinal motility. Moreover, a delay in gastric emptying of stomach will

**DISCUSSION**

Peptic ulcer and gastritis have been associated with multipathogenic factors and could be due to disturbances in natural balances between the aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism (Shetty et al., 2008; Abdalla et al., 2010). Compounds from natural products like plants products are some of the most attractive sources of new drugs and have shown promising results
prevent speedy evaluation of the stomach contents which in turn increases the absorption of orally administered anti-ulcer agents and promotes ulcer healing. These activities are considered to be beneficial to ulcer patients (Bertaccini et al., 1981; Akah et al., 1998). The active constituents of the extract revealed by the phytochemical screening especially tannins may also have contributory role to play in its anti-ulcer activity. Tannins are known to ‘tarn’ the outer most layer of the gastric mucosa, rendering it less permeable and more resistant to chemical and mechanical injury or irritant (Asuzu and Omu, 1990). Thus, the result of this study has shown that Bridelia ferruginea stem bark extract probably antagonizes the aggressive factors like acid, pepsin and H. pylori, which play an important role in the pathogenesis of gastric ulcer (Kumar and Clarke, 2002) while augmenting the defensive mucosal factors that protect the gastric mucosa from injury (Germano et al., 1998). This assertion is further buttressed by the fact that the extract has been shown to have a remarkable antimicrobial activity against five strains of bacteria (Adebayo and Ishola, 2009; Jose and Kayode, 2009). The aqueous extract of Bridelia ferruginea demonstrated a dose-dependent reduction in castor oil-induced diarrhoea in mice. The extract at 400 mg kg⁻¹ produced 100% inhibition of diarrhoea more than the standard anti-diarrhoea drug, loperamide which at 3 mg kg⁻¹ produced 85.94% inhibition of diarrhoea.

Phytochemical analysis of the plant extract revealed the presence of alkaloid, sterols, tannins, saponins, flavonoids, terpenes. These secondary metabolites previously reported for their anti-diarrhoeal activity (Otshudi et al., 2000), may be responsible for the anti-diarrhoea activity of the aqueous extract. A possible mechanism may be by precipitation of proteins in enterocyte and production of protein tanninates that lead to reduced secretion and peristaltic movement (Yu et al., 2000). Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate and consequently any effect on colonic mortality (Theodora et al., 1991).

The anti-muscarinic drug, atropine and different doses of the extract decreased the propulsive movement in the charcoal meal, atropine being more potent than the aqueous extracts of Bridelia ferruginea stem bark. This is possible due to its anticholinergic effect (Brown and Taylor, 1996). The significant inhibition of castor oil-induced enteropooling in mice suggests that the extract produced relief in diarrhoea by spasmylytic activity in vivo and anti-enteropooling effects.

The results obtained revealed that Bridelia ferruginea is a potential therapeutic option in the effective management of gastrointestinal disorders, thus justifying its wide spread use by the local population for these purposes.

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