Activity of Oxalis barrelieri aqueous extract on rat secretory diarrhea and intestine transit

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ABSTRACT

Oxalis barrelieri is used in the folk medicine for diarrhea treatment. The effects of the aqueous extract of Oxalis barrelieri on castor oil-induced diarrhea and intestine transit were investigated in rats. To induce the diarrhea and enteropooling, animals orally received castor oil (1 mL/100 g bw). Each rat received 30 minutes after, one of the single oral doses of O. barrelieri aqueous extract: 0, 25, 50, 100, 200 and 250 mg/kg bw or loperamide (5 mg/kg bw). The frequency and the total diarrheal weight for diarrhea and the intestine content for enteropooling were measured. To value the extract effect on intestine transit, normal rats received or not acetylcholine 0.1 mg/kg bw i.p. and then different dose of plant extract 50 and 100 mg/kg bw. The extract produced significant (p<0.01) decrease: respectively 95%, 96.36%, 99% and 100% in the severity of diarrhea. The 50 and 100 mg/kg bw extract produced remarkable (p<0.01) decrease in castor oil-induced enteropooling (59% and 71.43%), intestine transit (42.12% and 46.50%), and reduced acetylcholine action (-65.90% and -53.73%) respectively. The results provide evidence that the aqueous extract of O. barrelieri could act on secretory diarrhea and intestinal motility, and thus could justify its traditional use.

INTRODUCTION

Diarrhea is defined as three or more watery stools in a 24 hour period (WHO, 2009). It is an alteration in normal bowel movement that leads to the increase in water content, volume or stool frequency and abdominal pain (Ezekwesili et al., 2004). According to the World Health Organization, there are approximately 2 billion annual cases of diarrhea worldwide. Diarrhea is the leading cause of death in children younger than 5 years and kills 1.5 million children each year (Kent and Banks, 2010). It is especially prevalent in the developing world, where mortality is related to dehydration, electrolyte disturbance, and the resulting acidosis. Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for diarrhea treatment but they have some side effects (Anup et al., 2007). Therefore, the search for safe and more effective agents has continued to be an important area of active research. Since ancient times, diarrhea has been treated orally with several medicinal plants or their extracts based on folklore medicine (Anup et al., 2007). O. barrelieri has shown anti-diabetic activity (Enock et al., 2006) and its decoction is traditionally used in Cameroon for diarrhea treatment. The present study was undertaken to evaluate the claimed anti-diarrheal potential of aqueous extract of O. barrelieri, in castor oil-induced diarrheic rats.

MATERIALS AND METHODS

Plant extract

The whole plants of O. barrelieri were collected from Yaoundé (Center Region of Cameroon) on September 2009. The plant was identified by the National Herbarium of Cameroon at Yaoundé, compared to a voucher specimen N° 49998 HNC. The whole plant was washed thoroughly with water, shade dried and ground. The powder (407 g) was mixed with distilled water (5 L) for 72 hours in a percolator. The filtrate was evaporated to dryness in rotavapor to yield 128 g of brown extract. Each rat received 30 minutes after, one of the single oral doses of O. barrelieri aqueous extract: 0, 25, 50, 100, 200 and 250 mg/kg bw or loperamide (5 mg/kg bw). To value the extract effect on intestine transit, normal rats received or not acetylcholine 0.1 mg/kg bw i.p. and then different dose of plant extract 50 and 100 mg/kg bw. The extract produced significant (p<0.01) decrease: respectively 95%, 96.36%, 99% and 100% in the severity of diarrhea. The 50 and 100 mg/kg bw extract produced remarkable (p<0.01) decrease in castor oil-induced enteropooling (59% and 71.43%), intestine transit (42.12% and 46.50%), and reduced acetylcholine action (-65.90% and -53.73%) respectively. The results provide evidence that the aqueous extract of O. barrelieri could act on secretory diarrhea and intestinal motility, and thus could justify its traditional use.

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Animals
For the studies animals of either sex: mice (25 – 30 g) for toxicity and wistar albino rats (115 – 175 g) for secretory diarrhea and intestine transit were selected. All animals were allowed to acclimatization for a week to our laboratory environment (temperature and dark/light cycle) prior to the study. Animal housing and experiments in vivo were done according to the guidelines of the European Union on Animal Care (CEE Council 86/609) (Smith et al., 2007) that was adopted by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon.

During the experiment, the animals were randomly distributed in groups and housed in polypropylene cages (4 animals per cage), and were fed with standard rat diet: carbohydrates 50-55%, fats 15-20% and proteins 25-30% (Kamgang et al., 2008).

Acute toxicity
Seven groups of ten (10) normal albino mice each (five males and five females) were fasted for 16 h with free access to water. After which, they were orally administered one of the increasing doses of O. barrelieri aqueous extract: 0, 5, 10, 15, 20, 25 or 30 g/kg body weight (bw). The mice were then observed for at least 48 h and up to 7 days, for death, lethargy, jerkiness, sensitiveness to noise and touch, stool quality and frequency. The total feces weight was recorded within a period of four hours. The diarrheal feces weight of the control group was considered as 100 %. The results were expressed as percentage of inhibition of diarrhea (Meite et al., 2009).

Preliminary phytochemical screening test
The phytochemical properties of the extract was determined using the following chemicals and reagents (Sofowora, 1998; Trease and Evans, 1997): Mayer and freshly prepared Dragendorff’s reagents for alkaloids, Liebermann-Buchard test for terpenoids and sterols, FeCl₃ and K₃Fe(CN)₆ for phenols and tannins, Shinoda test for flavonoids, Molish test for polysaccharides, frothing test for saponins, UV lamps for coumarins, FeCl₃ and HCl for phlobotannins and NH₄OH for antheraquinones.

Assessment of O. barrelieri extract effects
Enteropooling
Five groups of five rats each were fasted for 18 h with free access to water. The normal control (NC) group received distilled water (1 mL/100 g bw). The other groups received castor oil (1 mL/100 g bw). One hour before the castor oil administration, the control group received distilled water (1 mL/100 g bw), the other groups respectively received loperamide 5 mg/kg bw (Lop5), 50 mg/kg bw (WOb50) and 100 mg/kg bw (WOb100) O. barrelieri aqueous extract. Two hours later, the rats were sacrificed; the small intestine was removed (after tying the ends with threads) and weighed. The intestine was emptied of its content by milking into a graduated tube, the volume measured and the intestine weighed (Anup et al., 2007; Ezeja et al., 2012).

Secretary diarrhea
Seven groups of six rats each were fasted for 18 h with free access to water. Castor oil (bought in the local market) was given orally (1 mL/100 g bw) to all animals to induce the diarrhea (Doherty, 1981).

Thirty minutes later, the first group (Diarrheic Control) received distilled water (1 mL/100 g bw) while each of the five following groups were given one of the aqueous extract doses 25, 50, 100, 200 and 250 mg/kg bw by oral route. The seventh group received the reference drug, loperamide (ELDOPER, Micro Labs. 92, sipcot, Hosur-635126, India) 5 mg/kg bw. Animals of all groups were placed separately in individual cages lined with filter paper. The filter paper was changed every hour and the severity of diarrhea was assessed hourly for four hours. The total feces weight was recorded within a period of four hours. The diarrheal feces weight of the control group was considered as 100 %. The results were expressed as percentage of inhibition of diarrhea (Meite et al., 2009).

Intestine transit
Normal transit
Four groups of five normal rats each were fasted for 18 h with free access to water. The first group (normal control: NC) was administered distilled water (1 mL/100 g bw). The three other groups respectively received O. barrelieri aqueous extract 50 mg/kg (WOB50), 100 mg/kg (WOB100) and the standard drug (Atropine sulphate, Gland Pharma. Pally. Dundigal. Post, Hyderabad, India) 0.2 mg/kg bw i.p (AT0.2). Each animal was orally given 2 mL of charcoal meal (10% activated charcoal by 5% gum acacia) 30 min later, as died marker, and was sacrificed 30 min after administration of the charcoal.

The distance covered by the charcoal meal in the intestine was expressed as a percentage of the total distance traveled from the pylorus to the coecum (Anup et al., 2007; Ezeja et al., 2012; Meite et al., 2009).

Acetylcholine (ACh)-induced intestine transit
Five groups of five normal rats each were fasted for 18 h with free access to water. The normal control (NC) group was administered the distilled water (1 mL/100 g bw). The four other groups respectively received distilled water 1 mL/100 g bw (ACh), O. barrelieri extract 50 mg/kg bw (WOB50), 100 mg/kg bw (WOB100) and 0.2 mg/kg bw i.p. atropine (AT0.2). Thirty minutes later each animal of the ACh, WOB50, WOB100 and AT0.2 groups received acetylcholine (Acetylcholine, A6625 SIGMA, MFCD00011698) 0.1 mg/kg bw i.p. After acetylcholine administration, all the animals orally received 2 mL charcoal meal and were sacrificed 30 min later. The distance covered by the charcoal meal in the intestine was expressed as a percentage of the total distance traveled from the pylorus to the coecum.
Statistical analysis

Data were expressed as mean ± standard error of mean (X± S.E.M). Data were analyzed by one-way ANOVA followed by Dunnett’s t-test and Tukey test using computerized Graph Pad InStat 3.05 version (Graph Pad software, U.S.A.).

RESULTS

Acute toxicity

Single doses of O. barrelieri aqueous extract did not elicited any overt signs of toxicity up to 10 g/kg bw. The LD_{100} was 25 g/kg bw; theoretical and graphical estimated LD_{50} were 15 g/kg bw.

Phytochemical properties

Phenols, terpenoids, anthocyanidines, anthraquinones, coumarins, saponins, lipids and volatile oils were identified in the aqueous extract of O. barrelieri, Alkaloids were present in traces.

Effect of the extract on castor oil-induced enteropooling

The diarrheic control (DC) rat intestinal content fluid was 3.22 ± 0.47 mL against 0.76 ± 0.05 mL for the normal control (NC). The O. barrelieri 50 and 100 mg/kg bw extract, as the loperamide (5 mg/kg bw), significantly (P<0.01) inhibited castor oil-induced intestinal accumulation (Fig. 1): -59.01%, -71.43% and -68.32% (P<0.01) respectively.

Effect of the extract on secretory diarrhea

The total diarrheic feces weight (DF) in diarrheic control (DC) was 3.30 ± 0.39 g. A single oral administration of each dose of O. barrelieri extract (50, 100, 200 and 250 mg/kg bw) to diarrheic rats produced significant decrease in the severity of diarrhea, reducing the defecation rate in rats (Table 1). The frequency of stool emission was respectively: 2.7, 0.2, 2.3, O.2, 0.5, 0.3 and 0.0 /hrs for DC, Lop5, WOb25, WOb50, WOb100, WOb200 and WOb250.

The inhibition rate of wet feces mass was significant (P<0.01): 93.94%, 19.70%, 94.55%, 96.36%, 99.09% and 100% respectively for Lop5, WOb25, WOb50, WOb100, WOb200 and WOb250.

**Table 1:** Feces weight of castor oil-induced diarrhea in rats treated with O. barrelieri water extract 25 (WOb25), 50 (WOb50), 100 (WOb100), 200 (WOb200) and 250 (WOb250) mg/kg bw.

<table>
<thead>
<tr>
<th>Group</th>
<th>TF (g)</th>
<th>DF (g)</th>
<th>Inhibition rate (%)</th>
<th>Frequency (number/hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>3.56 ± 0.39</td>
<td>3.30 ± 0.39</td>
<td>-</td>
<td>2.70 ± 0.33</td>
</tr>
<tr>
<td>Lop5</td>
<td>0.28 ± 0.08**</td>
<td>0.20 ± 0.06**</td>
<td>-</td>
<td>93.94 ± 0.17**</td>
</tr>
<tr>
<td>WOb25</td>
<td>2.77 ± 0.58**</td>
<td>2.65 ± 0.66**</td>
<td>-</td>
<td>94.55 ± 0.17**</td>
</tr>
<tr>
<td>WOb50</td>
<td>0.18 ± 0.08**</td>
<td>0.18 ± 0.08**</td>
<td>-</td>
<td>96.36 ± 0.17**</td>
</tr>
<tr>
<td>WOb100</td>
<td>0.53 ± 0.46**</td>
<td>0.12 ± 0.07**</td>
<td>-</td>
<td>99.09 ± 0.17**</td>
</tr>
<tr>
<td>WOb200</td>
<td>0.23 ± 0.17**</td>
<td>0.03 ± 0.03**</td>
<td>-</td>
<td>100 ± 0.00**</td>
</tr>
<tr>
<td>WOb250</td>
<td>0.07 ± 0.07**</td>
<td>0.00 ± 0.00**</td>
<td>-</td>
<td>0.00 ± 0.00**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=5). Significant difference: **P<0.01 compared with Diarrheic Control (DC); *P<0.01 compared with diarrhetic rat treated with loperamide 5 mg/kg (Lop5); TF: Total feces; DF: Diarrheal feces.

Effect of the extract on intestine transit

The total length covered rate (TLCR) in normal control rats was 59.67 ± 2.63% (Table 2). The atropine, 50 and 100 mg/kg bw O. barrelieri extract significantly (p<0.01) inhibited the normal propulsion: respectively -48.80%, -42.12% and -46.50%.

**Table 2:** Intestine transit in normal rats treated with O. barrelieri water extract: 50 (WOb50), 100 (WOb100) and Atropine 0.2 mg/kg (AT0.2) mg/kg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ITL (cm)</th>
<th>CCL (cm)</th>
<th>TLCR (%)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>112.40±3.140</td>
<td>67.20±3.997</td>
<td>59.67±2.631</td>
<td>-</td>
</tr>
<tr>
<td>AT0.2</td>
<td>120.60±2.46</td>
<td>37.07±2.48</td>
<td>30.55±2.98**</td>
<td>-48.80</td>
</tr>
<tr>
<td>WOb50</td>
<td>104.20±3.44</td>
<td>35.87±1.29</td>
<td>42.12</td>
<td>42.12</td>
</tr>
<tr>
<td>WOb100</td>
<td>112.20±2.08</td>
<td>35.87±2.56</td>
<td>37.07±1.29</td>
<td>42.12</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=5). Significant difference: **P<0.01 compared with control. ITL: Intestine total length; CCL: Charcoal covered length; TLCR: Rate of total length covered.

ACh has increased the intestine propulsion (+26%) in rats compared with the normal control (NC) rat (85.42% versus 59.67%). The atropine, 50 and 100 mg/kg bw O. barrelieri extract significantly (p<0.01) inhibited acetylcholine (ACh)-induced intestine transit by 79.91%, 65.90% and 53.73% respectively (Table 3).

**Table 3:** Effect of O. barrelieri water extract: 50 mg/kg bw (WOb50), 100 mg/kg bw (WOb100) and Atropine 0.2 mg/kg (AT0.2) mg/kg on acetylcholine (ACh)-induced intestine transit in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ITL (cm)</th>
<th>CCL (cm)</th>
<th>TLCR (%)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>112.40±3.140</td>
<td>67.20±3.997</td>
<td>59.67±2.631</td>
<td>-</td>
</tr>
<tr>
<td>ACh</td>
<td>94.40±6.79</td>
<td>61.00±5.44</td>
<td>85.42±2.41</td>
<td>-</td>
</tr>
<tr>
<td>AT0.2+ACh</td>
<td>106.20±3.23</td>
<td>18.27±2.11</td>
<td>17.16±1.80**</td>
<td>-9.91</td>
</tr>
<tr>
<td>WOb50+ACh</td>
<td>97.00±5.14</td>
<td>28.80±4.36</td>
<td>29.13±3.19**</td>
<td>-65.90</td>
</tr>
<tr>
<td>WOb100+ACh</td>
<td>95.20±5.77</td>
<td>37.73±3.08</td>
<td>39.52±1.69**</td>
<td>-53.73</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=5). Significant difference: **P<0.01 compared with Normal control (NC); *P< 0.01 compared with acetylcholine control (ACh). ITL: Intestine total length; CCL: Charcoal covered length; TLCR: Rate of total length covered.

Fig. 1: Enteropooling in castor oil-induced diarrheic rats treated with water extract of O. barrelieri 50 (WOb50), 100 (WOb100) and Loperamide 5 (Lop5) mg/kg bw. Values are mean ± S.E.M. (n=5). Significant difference: *P<0.05; **p<0.01 compared with Normal control; bP<0.05 compared with Diarrheic Control (DC). ( ) inhibition rate.
DISCUSSION

Diarrhea results from unsteady between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in intestine motility and an excess loss of fluid in the feces (Anup et al., 2007). In traditional medicine, *O. barrelieri* is used in the management of diarrhea. The present study sought to assess the anti-diarrheal activity of the *O. barrelieri* aqueous extract.

In the preliminary acute toxicity test, aqueous extract of *O. barrelieri* at doses below 10 g/kg bw did not provoke any change in the behavior of normal animals and moreover the LD₅₀ (15 g/kg bw) value was much higher than 5 g/kg. This indicates that the aqueous extract of *O. barrelieri* could be considered safe for all practical purposes in the laboratory and for all medical uses, according to the WHO criteria (Diezi, 1992).

Castor oil increased intestine content volume that lead to diarrhea. However, the *O. barrelieri* extract led to a marked reduction in intestinal contents volume on castor oil-induced enteropooling and significantly inhibited castor oil-induced diarrhea in rats. Castor oil causes diarrhea by its active metabolite, ricinoleic acid which stimulates peristaltic activity in the small intestine, and modify the electrolyte (Na⁺, K⁺) permeability by inhibiting the intestinal Na⁺/K⁺ ATPase activity (Kent and Banks, 2010; Meite et al., 2009). The inhibition of intestinal Na⁺/K⁺ ATPase activity reduces normal fluid absorption, by activating the adenylate cyclase or mucosal cAMP-mediated active secretion. Ricinoleic acid stimulates the prostaglandin formation and activates the platelet factor (Anup et al., 2007). The *O. barrelieri* inhibited castor oil-induced enteropooling and its anti-diarrheal activity was comparable to loperamide effect with regard to the severity of diarrhea. The anti-diarrheic action of loperamide is mediated by antisecretory mechanism. The loperamide reduces intestinal motility through direct effect on the circular and longitudinal muscles of the intestinal wall (Ooms et al., 1984). This could evident that *O. barrelieri* extract effect might be mediated by an anti-secretory mechanism as loperamide by activating the intestinal Na⁺/K⁺ ATPase activity. *O. barrelieri* contains terpenoids, phenols, saponins and volatile oils which possess antioxidant properties (Koudou et al., 2009). The presence of these constituents could be presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (Mora et al., 1980).

Aqueous extract of *O. barrelieri* also significantly reduced intestinal transit compared to atropine sulfate and significantly prevented acetylcholine action. Atropine decreases intestinal transit through its anti-cholinergic effect which blocks the muscarinic receptor (Teixeira-Neto et al., 2012). The extract inhibiting action was less important in normal transit than in acetylcholine-induced intestine transit. *O. barrelieri* effect on intestine transit could then result from partial capability of the extract activity on muscarinic receptor function, and/or by other mechanisms that lead to the inhibition of intracellular calcium mobilization such as inhibition IP₃ and prostaglandin synthesis, or calcium channel blocking. *O. barrelieri* chemical compounds such as tannins, saponins, reducing sugar, sterols and terpenes could also act on opioid receptors located on gut smooth muscle and hence inhibited gastrointestinal motility (Longanga et al., 2000; Venkatesan et al., 2005).

CONCLUSION

The results of this investigation revealed that *O. barrelieri* extract could reduce secretory diarrhea and intestine transit. This provides the rationale for the use of the extract of *Oxalis barrelieri* as an anti-diarrheal drug by traditional healers.

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REFERENCES


Mora A, Paya M, Rios JL, Alcaraz MJ. Structure activity relationships of polymethoxy flavones and other flavonoids as inhibitors

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