Effects of hydroethanolic extract of Cameroonian propolis (Promax-c) on castor oil-induced diarrhea in mice

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Abstract

The aim of our work was to evaluate the effect of hydroethanolic extract of Cameroonian propolis (Promax-c) on castor oil-induced diarrhea in mice. Diarrhea was induced in mice by oral administration 0.5 mL of castor oil in all mice. To determine the effective doses, each mouse received, 30 minutes after the administration of castor oil, one of the single oral doses of Promax-c: 0, 37.5, 75, 150, and 300 mg/kg bw. The mass and frequency of stool were measured and recorded per hour for five hours. The effect of Promax-c on the intestinal motility was evaluated by measuring the distance traveled by the charcoal meal in thirty minutes. The effects of Promax-c on intestinal secretion were evaluated by measuring the volume of the intestinal content and by dosing the electrolytes (Na+, K+ and Cl-) in the intestinal content by the colorimetric method. Promax-c produced significant (P <0.01) decreases, respectively, 29.16%, 62.50%, 62.50%, and 79.17% in the severity of diarrhea. Promax-c at 75 and 150 mg/kg bw exhibited significant (P <0.01) reduction on intestinal transit (12.30% and 24.86%, respectively) as compared to normal control and showed a significant (P <0.01) decrease of castor oil-induced enteropooling (48.64% and 56.75%, respectively) as compared to diarrheal control. This extract significantly (P <0.01) reduced the secretion of electrolytes (Na+, K+ and Cl-) and kept the stool osmotic gap at a normal value. The results we obtained allow us to affirm that the ethanolic extract of propolis (Promax-c) would have an antidiarrheal activity.

Keywords: Cameroonian propolis; Diarrhea; Castor Oil; Mice

1. Introduction

Diarrhea is an abnormal elimination of stool, above 100–200 g/day in adults or 5–10 g/kg/day in infants [1]. They can be of infectious origins (bacterial, viral, parasitic or fungal), inflammatory, tumoral or medicinal (antibiotics). Infectious diarrhea is caused by bacteria such as Salmonella, Shigella [2] and Entomopathogenic colibacilli [3]; by viruses such as paroviruses, rotaviruses, reoviruses [4], or by parasites such as amoebae and helminths [5]. These infections increase in case of lack of safe water for various household chores and for consumption. Diarrhea may be due to malnutrition and may occur as a result of a sudden change in overeating, or in the case of a diet containing enough poor quality protein [6]. Certain drugs such as tonicardiac, hypotensive, antiurecemic, antibiotics, anti-inflammatories, antimiotics may also be responsible for acute or chronic diarrhea. Diarrhea can be classified by the duration and etiology [7]. Diarrhea is acute when it has been going on for less than 2 weeks. In the majority of cases, acute diarrhea is sudden and preceded by normal transit. It is mainly caused by infectious agents (70% of cases), but may also be the consequence of the use of drugs, pathological conditions [7]. These acute diarrheas are responsible for at least four million deaths of children under five every year in developing countries [8]. Chronic diarrhea is characterized by an increase in fecal
output (> 300 g/day) that has been changing for more than a month [9,10]. Chronic diarrhea is thought to be associated with dietary deficiency due to malabsorption or increased consumption of the factors in question [11]. These acute or chronic diarrheas can be secretory, osmotic or motor. Secretory diarrhea is characterized by a high fecal volume following a considerable loss of fluids through the small intestine and colon. They have an osmotic hole of less than 50 mosmol/L. They can be caused by an excess of bile acids, laxatives (castor oil), certain pancreatic tumors, certain colitis and cryptosporidiosis [12]. Osmotic diarrhea is characterized by an osmotic hole greater than 125 mosmol/L [12]. Stress can change intestinal motility by directly affecting the enteric nervous system (ENS) which provides control of gastrointestinal functions such as motility, water and electrolyte secretion, blood flow and thus cause so-called motor diarrhea [13,14].

Oral rehydration (OR) is the first course of action for the treatment of diarrhea [4], to quickly correct the hydroelectrolytic deficits. This replenishment reduces the abnormal increase in intestinal permeability observed in cases of acute diarrhea [15] and can also promote the regeneration of enterocytes and the recovery of disaccharides in the membrane of the brush border [16]. Drug treatment of diarrhea involves the use of intestinal motility inhibitors such as loperamide, atropine, antisecretory agents such as loperamide, racecadotril [17]. Several medicinal plants have shown antidiarrheal activity. Among these plants may have been Mallotus oppositifolium [18], Euphorbia hirta [19], Euphorbia scordifolia [20], Oxalis barrellieri [21], Albizia lebbek [22], Aegle marmelos [23], Rumex maritimus [24], Calotropis gigantean [25]. It has been reported that the ethanolic extract of propolis has anti-diarrheal effects. Propolis is a natural substance harvested by bees and consists of various types of secretions (lipophilic and mucilaginous substances, gum, oil and perhaps wax) or exudates (resin, latex) of plants [26]. Propolis has been used for a long time in traditional medicine and is a popular remedy currently used to treat various diseases [27]. Cameroonian propolis is more widely available and even out of the country in the form of Promax-c, a product that allows for body maintenance and various treatments [28]. The main objective of this work is to study the pharmacological properties of ethanolic extract of propolis (Promax-c) in vivo on castor oil-induced diarrhea in Mus musculus Swiss mice.

2. Material and methods

2.1. Biological material

2.1.1. Preparation of Promax-c doses

The Promax-c samples are hydroethanolic extracts (70% V/V) of Cameroonian propolis, prepared, stored in bottles of 30, 60, 125, 250 or 1000 mL and marketed by the AFH/BFM (Abeille, Fleur, Homme/Bee, Flower, Man) Association of Ngaoundere Cameroon for the treatment of various diseases such as the flu, cough, dental infections, sore throat, angina, sores, headaches, hemorrhoids, digestive and visual disturbances, gastric ulcers and hyperacidity, kidney, skin, urogenital tract and respiratory tract infections, menstrual problems, appendicitis, diabetes, hypertension and heart problems [29]. To determine the doses of Promax-c to be administered, a 125 mL bottle was offered to us by Professor Fernand-Nertor Tchuenguem Fohouo. Two (2) teaspoons (10 mL) were taken dried in an oven at 40°C and the extract obtained was weighed. The mass of the extract allowed us to determine the concentration of Promax-c by the following formula, as well as the maximum dose to use.

\[ \text{[promax-c]} = \frac{\text{(Dry mass)}}{\text{Volume}} \]

This maximum dose was subsequently diluted by 2, 4 and 8 with distilled water for the other doses. The solutions are administered to mice in a volume of 10 mL/kg bw. The different doses of Promax-c administered to the mice were 37.5, 75.0, 150.0, and 300.0 mg/kg bw.

2.1.2. Experimental animals

The experimental animals were white Mus musculus Swiss mice of two sexes, 8 to 12 weeks old and weighing between 18 and 24 g. These mice were acclimatized in the Laboratory of Medicinal Plants, Health and Galenic Formulation of the Department of Biological Sciences of the University of Ngaoundéré for two weeks. Experiments on animals have been carried out according to the guidelines on animal care of European Union (EEC Council No 86/609) [30]. Their diet consisted of a mixture of corn flour (60%), palm oil (3%), fish meal (12%), soy flour (15%) and a little salt which is in the form of a granule [31].

2.2. Promax-c activities on castor oil-induced diarrhea in mice
Induction of diarrhea was done according to the method described by Fokam et al. [21]. Six groups of five mice each were arranged in cages differently. The mice were left to fast for eighteen (18) hours with free access to water. Before handling, the mice were weighed and each animal received oral castor oil (0.5 mL) using an esophageal tube.

Thirty (30) minutes after the castor oil administration, group I (diarrheal control: DC) received distilled water orally, group II (Lop5) was treated with loperamide 5 mg/kg bw (ELDOPER, Micro Labs. 92, sipcot, Hosur-635126, India), group III (Pro37.5), group IV (Pro75), group V (Pro150) and group VI (Pro300) received respectively Promax-C at 37.5 mg/kg, 75 mg/kg, 150 mg/kg and 300 mg/kg bw by the oral route. The animals, thus treated were placed individually in metabolic cages having previously weighed papers and mounted under the grid. The frequency (F) and quantity of stools were noted and recorded every hour for 5 hours. The percentage inhibition (I) of diarrhea was determined by the following formula:

\[
F=\frac{\text{Total number of stool}}{\text{Time (5 h)}} \quad (2)
\]

\[
I(\%)=\frac{\text{SMDC}-\text{SMDT}}{\text{SMDC}}\times 100 \quad (3)
\]

Where, F: frequency; I: inhibition; SMDC: stool mass of diarrheal control; SMDT: stool mass of diarrheal test.

### 2.3. Effect of Promax-c on intestinal transit

Twenty-five mice divided into five lots of five mice each were fasted for 18 hours with free access to water. The first group (normal control: NC) received distilled water (10 mL/kg bw). The other groups received Promax-c at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75), 150 mg/kg (Pro150) or the reference drug, atropine sulfate (Gland Pharma. Pally. Dundigal. Post, Hyderabad, India) at 0.3 mg/kg (AT0.3) intraperitoneally. Thirty (30) minutes after the administration of these treatments, each animal received orally 1 mL of activated charcoal meal (5% activated charcoal of arabic gum) and thirty (30) minutes later, the animals were killed by cervical dislocation and the abdomen was opened [32]. The intestine was removed and the distance from which charcoal meal progressed from the pylorus and the cecum was measured using the measuring tape, and expressed as a percentage of the total distance from the small intestine. The inhibition was calculated by the following formula:

\[
\text{Inhibition (\%)}=\frac{(\text{DCDC}\%)-\text{DCDT}\%)}{(\text{DCDC}(\%)\times 100} \quad (4)
\]

Where, DCDC: distance traveled by charcoal in the diarrheal control (DC), DCDT: distance traveled by charcoal in the diarrheal treated

### 2.4. Effect of Promax-c on intestinal secretion

Six (6) groups of five (5) mice each were each were fasted for 18 h with free access to water. All groups received oral castor oil (10 mL/kg bw) except the normal control group. 30 minutes after administration of castor oil, the diarrheal control group (DC) and the normal control group (NC) received distilled water (10 mL/kg bw) and the other groups received orally loperamide 5 mg/kg (Lop5) or Promax-C at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75) or 150 mg/kg (Pro150) orally. 1 hour later, all the animals were sacrificed, the abdomen opened, the small intestine was removed and weighed. The content of the small intestine was emptied into graduate tubes and the volume of the content was measured and then stored in the freezer for dosing electrolytes (Na⁺, K⁺ and Cl⁻). Inhibition was determined by the following formula:

\[
\text{Inhibition (\%)}=\frac{(\text{VDC}-\text{VDT})}{\text{VDC}}\times 100 \quad (5)
\]

Where VDC: volume of the diarrheal control, VDT: Volume of diarrheal treated

### 2.5. Determination of sodium, potassium and chloride ion concentration

#### 2.5.1. Determination of sodium ions

The sodium ions were determined by the colorimetric method using the kit (LIQUIZYME SODIUM, BEACON DIAGNOSTICS PVT.LTD., 424, NEW GIDC, KABILPORE, NAVSARI-396 424. INDIA), according to the manufacturer's protocol. 500 µL of sodium reagent were put in all the tubes. Then, 10 µL of distilled water were added to the white tube, 10 µL of standard solution (150 mEq/L) were added to the standard tube and 10 µL of the sample were added to the test tubes. Each mixture was incubated at 37°C in a water bath for five (5) minutes and then the absorbance was read at 630 nm with a spectrophotometer (UNICO © ULTRA VIOLET). The concentration of sodium ions in each tube was determined by the following formula:
[\text{Na}^+] \text{(mEq/L)} = \frac{(\text{Abs T})}{(\text{Abs S})} \times [S] \quad (6)

Where, [\text{Na}^+]$: concentration of sodium ions in the stool; \text{Abs T}: Absorbance of the test tubes; Abs S: absorbance of standard tube; [S]: Concentration of the standard (150 mEq/L).

### 2.5.2. Determination of potassium ions

The potassium ions were determined by the colorimetric method using the kit (LIQUIZYME POTASSIUM, BEACON DIAGNOSTICS Pvt.Ltd., 424, NEW GIDC, KABILPORE, NAVSARI-396 424. INDIA), according to the manufacturer's protocol. 500 µL of potassium reagent were put in all the tubes. 20 µL of distilled water were then added to the white tube, 20 µL of standard solution (5 mEq/L) were added to the standard tube and 20 µL of samples were added to the test tubes. Each mixture was incubated at 37°C in a water bath for five (5) minutes. Then the absorbance of the content of each tube was read at 630 nm with a spectrophotometer (UNICO © ULTRA VIOLET). The concentration of potassium ions in each test tube was determined by the following formula:

\[
[\text{K}^+] \text{(mEq/L)} = \frac{(\text{Abs T})}{(\text{Abs S})} \times [S] \quad (7)
\]

Where, [\text{K}^+]$: Concentration of potassium ions in the stool; \text{Abs T}: Absorbance of the test tubes; Abs S: Absorbance of the standard tubes; [S]: Concentration of the standard (5 mEq/L).

### 2.5.3. Determination of chloride ions

The chloride ions were measured in the intestinal contents by the colorimetric method using the kit (SGM Italia-Via Pindaro 28C-Roma) according to the manufacturer's protocol. 1000 µL of chloride reagent were placed in all the tubes. 10 µL of distilled water were then added to the white tube, 10 µL of standard solution (100 mEq/L) were added to the standard tube and 10 µL of the sample were added to the test tubes. The mixture was incubated at 37°C for five (5) minutes in a water bath and the optical densities (OD) were read with a spectrophotometer (UNICO © ULTRA VIOLET) at 480 nm. The concentration of chloride ions in the different test tubes was determined by the following formula:

\[
[\text{Cl}^-] \text{(mEq/L)} = \frac{(\text{Abs T})}{(\text{Abs S})} \times [S] \quad (8)
\]

Where, [\text{Cl}^-]$: concentration of chloride ions in the stool; \text{Abs T}: Absorbance of the test tubes; Abs S: Absorbance of the standard tube; [S]: Concentration of the standard (100 mEq/L).

### 2.6. Determination of Stool Osmotic Gap (SOG)

The Stool Osmotic Gap was calculated by the following formula [12]:

\[
\text{SOG} = 290 - 2 \times ([\text{Na}^+] + [\text{K}^+]) \quad (9)
\]

Where, [\text{Na}^+]$: concentration of sodium ions in the stool; [\text{K}^+]$: Concentration of potassium ions in the stool.

### 2.7. Statistical analysis

The data were expressed as mean ± standard error of mean (X ± S.E.M). The data were analyzed using Graph Pad Instat 3.05 software (Graph Pad software, U.S.A.) at one path followed by the ANOVA test and the Dunnett t-test for comparison of means. The difference was significant when the probability was less than 0.05% (P <0.05).

### 3. Results

#### 3.1. Antidiarrheal effects of Promax-c on the mass and frequency of diarrheal stools in mice

Five hours after the administration of castor oil, the diarrheal control emitted 0.25 ± 0.02 g of diarrheal stools with a frequency of 3.26 ± 0.50 stools/hour. These diarrheal stools, decreased significantly (P <0.01) in mass and frequency in mice treated with Promax-c at doses 75, 150 and 300 mg/kg bw. The inhibition was 62.50%, 62.50% and 79.17% respectively in animals treated with Promax-cat 75, 150 and 300 mg/kg bw. Loperamide significantly reduced (P <0.01) the mass of diarrheal stools and the frequency (Table 1).
Table 1 Effects of Promax-c on the mass and frequency of diarrheal stools

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>MDS (g)</th>
<th>FDS (n/h)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>DW 10 mL/kg</td>
<td>0.24±0.02</td>
<td>3.26±0.50</td>
<td>-</td>
</tr>
<tr>
<td>Lop5</td>
<td>5 mg/kg</td>
<td>0.05±0.05**</td>
<td>0.52±0.52**</td>
<td>79.17</td>
</tr>
<tr>
<td>Pro37.5</td>
<td>37.5 mg/kg</td>
<td>0.17±0.03</td>
<td>2.17±0.30</td>
<td>29.16</td>
</tr>
<tr>
<td>Pro75</td>
<td>75 mg/kg</td>
<td>0.09±0.07**</td>
<td>1.15±0.92**</td>
<td>62.50</td>
</tr>
<tr>
<td>Pro150</td>
<td>150 mg/kg</td>
<td>0.09±0.09**</td>
<td>0.92±0.92**</td>
<td>62.50</td>
</tr>
<tr>
<td>Pro300</td>
<td>300 mg/kg</td>
<td>0.05±0.04**</td>
<td>0.64±0.40**</td>
<td>79.17</td>
</tr>
</tbody>
</table>

Mean ± ESM (n = 6). Significant difference: **P <0.01 compared to the diarrheal control (DC). FDS: Frequencies of diarrheal stools. MDS: mass of diarrheal stools. Animals treated with distilled water (DW), loperamide 5 mg/kg (Lop5), Promax-c at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75), 150 mg/kg (Pro150) or 300 mg/kg (Pro300).

3.2. Effects of Promax-c on intestinal transit

The progression of activated charcoal in the gastrointestinal tract was 76%, 54%, 76%, 67% and 57% respectively in the normal control (NC), the animals treated with atropine (AT0.3) or Promax-c at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75) and 150 mg/kg (Pro150). The percentage of inhibition of intestinal transit was 28.92%, 0.13%, 12.30% and 24.86% respectively in the control treated with atropine (0.3 mg/kg) and the animals treated with Promax-c at 37.5, 75 and 150 mg/kg (Table 2).

Table 2 Effects of Promax-c on intestinal transit in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>TLSI (cm)</th>
<th>DTC (cm)</th>
<th>PDCC (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>DW 10 mL/kg</td>
<td>51.80±1.50</td>
<td>39.60±3.00</td>
<td>76.40±5.00</td>
<td>-</td>
</tr>
<tr>
<td>AT0.3</td>
<td>5 mg/kg</td>
<td>48.20±0.60</td>
<td>26.20±1.10**</td>
<td>54.30±1.70**</td>
<td>28.92</td>
</tr>
<tr>
<td>Pro37.5</td>
<td>37.5 mg/kg</td>
<td>48.20±1.00</td>
<td>36.80±1.00</td>
<td>76.30±2.00</td>
<td>0.13</td>
</tr>
<tr>
<td>Pro75</td>
<td>75 mg/kg</td>
<td>48.00±1.00</td>
<td>32.60±2.00**</td>
<td>67.00±3.00**</td>
<td>12.30</td>
</tr>
<tr>
<td>Pro150</td>
<td>150 mg/kg</td>
<td>52.80±1.10</td>
<td>30.20±2.00**</td>
<td>57.40±4.00**</td>
<td>24.86</td>
</tr>
</tbody>
</table>

Mean ± ESM (n = 5). Significant difference: **P <0.01 compared to the normal control (NC). TLSI: total length of the small intestine; DTC: distance traveled by charcoal; PDCC: percentage of the distance covered by charcoal meal. Mice treated with distilled water (DW), with atropine 0.3 mg/kg (AT0.3), with Promax-c at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75) or 150 mg/kg (Pro150).

3.3. Effects of Promax-c on induced intestinal secretion in mice

The volume of intestinal contents was 0.37 mL in the diarrheal control (DC). This volume was reduced in the normal control (NC), the mice treated with loperamide 5 mg/kg (Lop5), with Promax-c at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75) and 150 mg/kg (Pro150) and was 0.08 mL, 0.14 mL, 0.30 mL, 0.19 mL and 0.16 mL, respectively. The inhibition of intestinal secretion was, 62.16%, 18.91%, 48.64%, 56.75% respectively in animals treated with loperamide 5 mg/kg (Lop5), with Promax-c at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75) and 150 mg/kg (Pro150). This inhibition was significant (P <0.01) in the normal control and in mice treated with loperamide 5 mg/kg (Lop5), with Promax-c at 75 mg/kg and 150 mg/kg (Fig. 1).
Figure 1 Effect of ethanolic extract of propolis on the intestinal secretion. Data are expressed as mean ± S.E.M (n=5). NC: normal control; DC: diarrheal control; Lop5: Loperamide 5 mg/kg; Pro37.5, Pro75 and Pro150: hydroethanolic extract of propolis respectively at 37.5, 75 and 150 mg/kg; (%): Inhibition. Significant difference: *P <0.05 and **P <0.01 compared with DC; aP <0.05 and bP <0.01 compared with NC.

3.4. Effects of Promax-c on the concentration of electrolytes (Na⁺, K⁺ and Cl⁻) in the intestinal contents

The concentration of sodium ions in the intestinal contents was 129.60±8.24 mEq/L in diarrheal control (DC) mice. This concentration of sodium ions was significantly (P<0.01) lower in normal control and treated animals. These values were 102.84±4.22, 103.16±3.82, 114.82±4.46, 112.91±6.47 and 105.33±4.85 mEq/L, respectively in the normal control (NC), mice treated with loperamide 5 mg/kg or with Promax-c at 37.5 mg/kg bw, 75 mg/kg bw and 150 mg/kg bw (Table 3).

The concentration of potassium ions in the contents of the small intestine was 6.75±1.01 mEq/L in diarrheal control (DC) mice. This concentration of potassium ions was significantly (P<0.05) increased in normal control (10.51±2.74 mEq/L) and in animals treated with loperamide 5 mg/kg bw (11.05±3.41 mEq/L) or with Promax-c 150 mg/kg bw (9.02±2.91 mEq/L). However, there is a relative increase in potassium ions in mice treated with Promax-c at 37.5 mg/kg bw (7.89±0.68 mEq/L) and 75 mg/kg bw (7.54±0.43 mEq/L).

In the intestinal content, the chloride ion level was 126.39±4.18 mEq/L in diarrheal control (DC) mice. This concentration of chloride ions was significantly (P<0.01) lower in normal control and treated animals. These values were 97.59±7.08, 91.27±8.17, 113.85±5.11 and 107.60±4.22 mEq/L, respectively in NC, Lop5, Pro37.5, Pro75 and Pro150 (Table 3).

Table 3 Effects of Promax-c on concentration of electrolytes (Na⁺, K⁺ and Cl⁻) in the intestinal contents

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>[Na⁺] (mEq/L)</th>
<th>[K⁺] (mEq/L)</th>
<th>[Cl⁻] (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>DW 10 mL/kg</td>
<td>102.84±4.22**</td>
<td>10.51±2.74*</td>
<td>97.59±7.08**</td>
</tr>
<tr>
<td>DC</td>
<td>DW 10 mL/kg</td>
<td>129.60±8.24</td>
<td>6.75±1.01</td>
<td>126.39±4.18</td>
</tr>
<tr>
<td>Lop5</td>
<td>5 mg/kg</td>
<td>103.16±3.82**</td>
<td>11.05±3.41*</td>
<td>91.27±8.17**</td>
</tr>
<tr>
<td>Pro37.5</td>
<td>37.5 mg/kg</td>
<td>114.82±4.46**</td>
<td>7.89±0.68</td>
<td>103.43±2.67**</td>
</tr>
<tr>
<td>Pro75</td>
<td>75 mg/kg</td>
<td>112.91±6.47**</td>
<td>7.54±0.43</td>
<td>111.38±5.11**</td>
</tr>
<tr>
<td>Pro150</td>
<td>150 mg/kg</td>
<td>105.33±4.85**</td>
<td>9.02±2.91*</td>
<td>107.60±4.22**</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM (n = 5). Significant difference: *P <0.05 and **P <0.01 compared to the diarrheal control (DC). NC: normal control; group of animals treated with loperamide 5 mg/kg (Lop5), or with Promax-c at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75) or 150 mg/kg (Pro150).
3.5. Effects of Promax-c on stool osmotic gap of intestinal contents

The stool osmotic gap was 63.30±1.32, 17.30±0.38, 44.58±1.53, 49.10±1.95 and 61.30±0.90 mEq/L, respectively in the normal control (NC), diarrheal control (DC), mice treated with loperamide 5 mg/kg (Lop5), or with Promax-C at 37.5 mg/kg (Pro37.5), 75 mg/kg (Pro75) and 150 mg/kg (Pro150) (Fig. 2).

Figure 2 Effect of Promax-c on stool osmotic gap. Data are expressed as mean ± S.E.M (n=5). NC: normal control; DC: diarrheal control; Lop5: Loperamide 5 mg/kg; Pro37.5, Pro75, and Pro150: hydroethanolic extract of propolis respectively at 37.5, 75 and 150 mg/kg. Significant difference: **P <0.01 compared with DC; ᵃP < 0.05 and ᵇP <0.01 compared with NC.

4. Discussion

Pathologies of the gastrointestinal system are a major health problem in developing and developed countries[6]. These digestive disorders are generally manifested by diarrhea, which, according to the WHO, is the emission of at least three bowel movements per day. We proceeded by the evaluation of the effectiveness of the ethanolic extract of propolis on castor oil-induced diarrhea in mice.

Castor oil induced an increase in the mass and frequency of diarrheal stools in mice. Castor oil contains an active ingredient, ricinoleic acid, which is a hydroxylated unsaturated fatty acid produced by the action of lipase in the intestine. Ricinoleate in the small intestine is poorly absorbed and alters the permeability of the intestinal mucosa to electrolytes (Na⁺ and K⁺), peristalsis and transport of electrolytes (Na⁺ and Cl⁻), which leads to hypersecretion and diarrhea [33]. Ricinoleic acid also stimulates epithelial cells to produce nitric oxide and Adenylate cyclase which lead to the production of prostaglandin-induced diarrhea [21]. These effects result from the inhibition of the activity of the Na⁺/K⁺ ATPase pump as a result of the increased mass and frequency of diarrheal stools. Increasing dose Promax-c inhibited beaver oil-induced diarrhea in mice. These results would be justified by the presence in propolis of compounds such as flavonoids (galangin, pinocembrine, chrysin, quercetin) and phenolic acids (benzoic, ferulic, cinnamic, p-coumaric, caffeic) [34] which act by reducing intestinal peristalsis or by activating Na⁺/K⁺ ATPase activity [21,35].

Promax-c and loperamide reduced the intestinal secretion induced by castor oil as well as the secretion of sodium and potassium ions. Loperamide binds not only to the opioid receptors μ, but also to the enterocyte receptors. This results in a decrease in the production of cAMP (cyclic adenosine monophosphate) in the epithelial cells of the intestine, which reduces the hypersecretion of electrolytes in the event of diarrhea [36]. In addition, loperamide binds to calmodulin. This protein activates calcium, which causes the opening of certain chloride channels and stimulates secretion. In other words, inactivation of calmodulin reduces hypersecretion [37]. Ricinoleic acid changes the permeability and transport of electrolytes leading to hypersecretion by intestinal inhibition of Na⁺/K⁺ ATPase activity [21,24]. The anti-secretory activity of Promax-c would result either from the activation of Na⁺/K⁺ ATPases pumps inhibited by ricinoleic acid [35], or from the inhibition of production of nitric oxide and cyclic adeny late by epithelial cells [38], either by blocking the opening of the chloride channels or by inhibiting the production of prostaglandins E2 [1].
Promax-c and atropine slowed down bowel movements. Atropine a muscarinic antagonist [39]. It prevents the muscle contraction induced by the intracellular accumulation of Ca²⁺ ions, and this by blocking the muscarinic receptor (M3) responsible for the establishment of the inositol 1,4,5-triphosphate (IP₃) at the origin of increase in intracellular Ca²⁺ [21]. Like atropine, Promax-c would act either by blocking the membrane calcium channels, by blocking the muscarinic receptors or by activating the µ receptors of opiates [39]. Activation of the opiate receptors µ by certain drugs such as loperamide inhibits the release of acetylcholine and prostaglandins, thereby reducing the contraction of the intestinal smooth muscles. This results in spasmodic activity, which increases, the intestinal transit time [40].

The stool osmotic gap in all animals treated with castor oil is negative, unlike the normal control where it is 118 mosmol/L. When the stool osmotic gap is less than 50 mosmol/L, the diarrhea is said to be secretory and when it is greater than 125 mosmol/L, it is said to be osmotic [12]. Castor oil-induced diarrhea in mice is indeed secretory diarrhea since all stool osmotic gap were less than 50 mosmol/L.

5. Conclusion
The hydroethanolic extract of Cameroonian propolis (Promax-c) inhibited diarrhea in mice by reducing the frequency of diarrheal stool emission, by inhibiting intestinal motility and intestinal secretions. These effects prove the anti-diarrheal activity of the hydroethanolic extract of Cameroonian propolis and would justify the use in the treatment of diarrheal diseases.

Compliance with ethical standards

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Disclosure of conflict of interest
The authors declare that they have no known competing interests.

Statement of ethical approval
Animal housing and in vivo experiments were done according to the guidelines of the European Union on Animal Care (CEE Council 86/609) that were adopted in Cameroon by the Institutional Committee of the Ministry of Scientific Research and Innovation.

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