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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. Prior to the oral administration, the extract was dissolved in distilled water so that each animal received less than 1 mL/100 g body weight solution.

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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 dose of the extract that would kill 50% of the animal population (LD_{50}) was estimated graphically and with the formula (Murali *et al.*, 2002):

$$LD_{50} = X_s - d(\sum p\text{-}1/2)$$

Xs= 100 % lethal dose, d= intreval between two successive doses, ∑p= sum of all death ratio per group.

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 Dragendoff's reagents for alkaloïds, Liebermann-Buchard test for terpenoids and sterols, $FeCl_3$ and $K_3Fe[(CN)_6]$ for phenols and tannins, Shinoda test for flavonoids, Molish test for polysaccharides, frothing test for saponins, UV lamps for coumarins, $FeCl_3$ and HCl for phlobotannins and NH_4OH for anthraquinones.

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).

Secretory 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 ($\overline{X}$ ± 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; theorical 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.

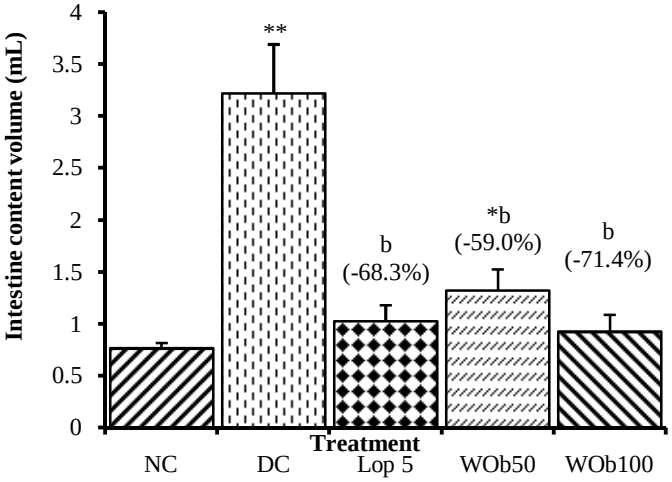

Fig. 1: Enteropooling in castor oil-induced diarrheic rats treated with water extract of *O. barrelieri* 50 (WOb50), 100 (WOb100) and Loperamide 5 (Lop5) mg/kg bw. Values are mean ± S.E.M, (n=5). Significant difference: *P<0.05; **p<0.01 compared with Normal control; ^{b}P<0.05 compared with Diarrheic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

Effect of the extract on secretory diarrhea

The total diarrheic feces weight (DF) in diarrheal 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.

Group	TF (g)	DF (g)	Inhibition rate (%)	Frequency (number/hrs)
DC	3.56 ± 0.39	3.30 ± 0.39	-	2.70 ± 0.33
Lop5	0.28 ± 0.08**	0.20 ± 0.06**	-93.94	0.17 ± 0.05**
WOb25	2.77 ± 0.58^{b}	2.65 ± 0.66^{b}	-19.70	2.30 ± 0.55^{b}
WOb50	0.18 ± 0.08**	0.18 ± 0.08**	-94.55	0.17 ± 0.05**
WOb100	0.53 ± 0.46**	0.12 ± 0.07**	-96.36	0.50 ± 0.34**
WOb200	0.23 ± 0.17**	0.03 ± 0.03**	-99.09	0.33 ± 0.21**
WOb250	0.07 ± 0.07**	0.00 ± 0.00**	-100	0.00 ± 0.00**

Values are expressed as mean ± S.E.M (n=6). Significant difference: **P<0.01 compared with Diarrheic Control (DC); ^{b}P<0.01 compared with diarrheic 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 bw (AT0.2) mg/kg.

Treatment	ITL (cm)	CCL (cm)	TLCR (%)	Inhibition rate (%)
NC	112.40±3.140	67.20±3.997	59.67±2.631	-
AT0.2	120.60±2.46	37.07±4.28	30.55±2.98**	-48.80
WOb50	104.20±3.44	35.87±1.29	34.54±1.47**	-42.12
WOb100	112.20±2.08	35.87±2.56	31.93±2.06**	-46.50

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 bw (AT0.2) on acetylcholine (ACh)-induced intestine transit in rats.

Treatment	ITL (cm)	CCL (cm)	TLCR (%)	Inhibition rate (%)
NC	112.40±3.140	67.20±3.997	59.67±2.631	
ACh	94.40±6.79	61.00±5.44	85.42±0.41	
AT0.2+ACh	106.20±3.23	18.27±2.11	17.16±1.80**^{b}	-79.91
WOb50+ACh	97.00±5.14	28.80±4.36	29.13±3.19**^{b}	-65.90
WOb100+ACh	95.20±5.77	37.73±3.08	39.52±1.69**^{b}	-53.73

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

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 LD_{50} (15 g/kg bw) value was much higher than 5 g/kg. This indicates that the aqueous extract of *O. barrelieri*could be consider safe for all practical purpose 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-mediate 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 antidiarrheic 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 compare 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 probably by other mechanisms that lead to the inhibition of intracellular calcium mobilization such as inhibition IP_3 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 anti-diarrheal drug by traditional healers.

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