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ANTIDIARRHEAL EFFECT OF AQUEOUS AND HYDROETHANOLIC EXTRACTS OF ROOTS OF *COGNIAUXIA PODOLAENA BAILL* (CUCURBITACEAE)

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ABSTRACT

This work aimed to evaluate the antidiarrheic effect of the aqueous extracts (300 and 600 mg/kg) and hydroethanolic (300 and 600 mg/kg) of roots of *C. podolaena* (Cucurbitaceae). This effect was evaluated on frequency and quantity of the faces excreted by using castor oil method. Intestinal transit was evaluated in atropine and of acetylcholin presence by using the charcoal method. In addition, the effect of the two extracts (300 and 600 mg/kg) was evaluated on the intestinal fluid accumulation (enteropooling) and also on Na^+ and K^+ secretion. The results obtained show that these extracts significantly reduce ($p < 0,001$) the frequency and quantity of the faces excreted and also increase the onset of diarrheic faces compared to the control group.Moreover, these extracts at dose used significantly reduce ($P < 0,001$) the intestinal transit, the intestinal fluid accumulation and increase the ratio Na^+/K^+ compared to the control group. The antidiarrheic effect of the two extracts of *C.podolaena* could pass by the inhibition of the cholinergic receptors.

KEYWORDS: *Cogniauxia podolaena*, castor oil, acetylcholine, intestinal transit time, atropine.

INTRODUCTION

The diarrhoea is defined by the emission of too frequent faces, too abundant, of abnormal consistency (liquids or very soft) and of weight higher than 300 g/J. In clinical practice, one speaks about the diarrhoea according to WHO, when one makes three soft or liquid faces per day (WHO, 2001-2008). Several causes and circumstances can be at the origin of the diarrhoea, in particular bacterial, viral and parasitic infections. The infectious agents responsible of diarrhoea are generally propagated by the feco-oral way, especially by the food or water ingestion contaminated by the faces or a direct contact with infected faces (WHO, 2006). The diarrheal diseases cause approximately 1,8 million deaths each year in the world, of which 90 % are children under five years, most of whom live in developing countries (Cazaban et al., 2005). They are the third leading cause of death for infectious diseases of confused ages (WHO, 2011; Assogba and al., 2012) and the fifth leading cause of premature death in the worldwide scale (WHO., 2014). Faced with such an alarming prognosis, diarrhoea whose target group is consisted the children, of many international institutions such as WHO mobilize through various programs to reduce mortality and morbidity due to the diarrhoea. The Third world countries, particularly African whose plant heritage is rich in medicinal plants approaching the objectives set by WHO « Health for all by the year 2000 » by prioritizing treatments based on medicinal plants to enable of the low-income population of the world, to reach adapted therapeutic. At the present time, nearly 75 % of African look after themselves only with the medicinal plants which surround them (Kibungu Kembelo, 2004; Nkounkou-Loumpangou., 2005), though there are no yet specific statistical studies. In Congo, the flora is rich in plants with the antidiarrheic properties used in the traditional treatment. Among them, one can quote *C. podolaena* (Cucurbitaceae). The previous studies carried out with this plant showed many pharmacological activities such as, antidiabetic (Diatewa and al., 2004; Ahombo and al., 2012), antiplasmodiales and cytotoxic (Mbatchi and al., 2006; Banzouzi and al., 2008), analgesics (Makambila and al., 2011). However, no data on the possible antidiarrheic properties of the leaves is available. This is why, we aimed investigated the antidiarrheic effect of the aqueous extracts and hydroethanolic of roots of *C. podolaena*.

MATERIALS AND METHODS

Vegetable Material

The dry and pulverized roots *C. podolaena* (Cucurbitaceae) were used. These roots were collected in Boulou dia Moundelé, District Kiélé Tenard, Brazzaville in Mars 2017. Botanical identification of the plant material was done by Mousamboté, botanist systematist of (Higher Normal School of Agronomy and Forestry (HNSAF) and confirmed at the botanical laboratory of Research Institute in Exact and Natural Sciences (RIENS) in Brazzaville where the samples were compared with the reference samples of the herbarium (n°7083). After that, plant material were dried and pulverized with a mortar. The aqueous extract of root of *C. podolaena* were prepared by decoction. 20 g of powder of plant are mixed with 200 mL of distilled water. The mixture was boiled for 15 min. After cooling and filtration, the filtrate obtained was concentrated on a double boiler (60°C). The hydroethanolic extract of root of *C. podolaena* was prepared by maceration. 20 g of powder of *C. podolaena* were mixed with 200 mL ethanol 70% and put under magnetic agitation during 24 hours. After filtration, the filtrate was concentrated on a double boiler (60°C). The dry extracts obtained (aqueous and hydroethanolic extract) were preserved to evaluate their antidiarrheic effects.

Animal Material

Male and female albino rats weighting between 150 - 250 g and male and female albino mice weighting between 25- 30 g obtained from the Faculty of Science and Technical of Marien NGOUABI-University were used. All animals were acclimated during one week before the experiments. They were fed and maintained under standard lighting conditions (12 h light and 12 h dark) at a temperature of 27 ± 1° They were fasted for 24 h before experiments, water was given ad libitum. The rules of ethics published by the International Association for the Study of Pain have been considered (Zimmermann., 1983).

Effect of the aqueous and hydroethanolic extracts of root of *C.podolaena* on the frequency and the quantity of the faces excreted in normal rats

The method described by Souleymane et al., (2010) was used. The animals were divided into groups of 5 rats each.The various doses of the aqueous extracts (300 and 600 mg/kg), hydroethanolic (300 and 600 mg/kg), distilled water (control group, 0, 5 mL/100g), castor oil (reference molecule, 3mL/rat) were administered orally to groups. After oral administration of the products tests, the animals were placed in metabolism cage to evaluate the frequency of faeces emitted as well as the onset of appearance of the diarrheal faeces (soft or liquids) during 6 hours. In continuation, the excreted faeces were collected on dry paper and were weighed to evaluate the quantity of faeces excreted during the 6 hours.

Castor oil induced diarrhoea in Rat

Diarrhoea was induced by intragastric administration of the castor oil in rats (Elion Itou et al., 2018). The animals were divided into groups of 5 rats each. The different doses of the aqueous extracts (300 and 600 mg/kg), hydroethanolic (300 and 600 mg/kg), saline water (control group, 0,5 mL/100g), loperamide (reference molecule, 10 mg/kg) were administered orally to groups, one hour prior castor oil administration (0.6 mL/100g/rat). After castor oil administration, the animals were placed in metabolism cages to evaluate the frequency, the quantity of the faeces emitted as well as the onset of appearance of the diarrheal faeces (soft or liquids) during 6 hours. In continuation, the excreted faeces were collected on dry paper and were weighed to evaluate the quantity of faeces excreted during the 6 hours.

Intestinal transit

The intestinal transit was evaluated according to the method of charcoal (Elion Itou et al., 2018). The animals were divided into groups of 5 rats each .The various doses of the aqueous extract (300 and 600 mg/kg), aqueous extracts (600 mg/kg + acetylcholine 1,5 mg/kg), hydroethanolic extract (300 and 600 mg/kg), hydroethanolic extract (600 mg/kg + acetylcholin 1,5 mg/kg), saline water (control group, 0,5 mL/100g), saline water (0,5 mL/100g + acetylcholine 1,5 mg/kg), atropin (3mg/kg s.c + acetylcholine 1,5mg/kg) and loperamide (reference molecule, 10 mg/kg) were administered orally to groups, one hour prior of 10% charcoal (Norit *, 10 ml/kg) in mice. Acetylcholine and atropine were administered subcutaneously 10 min before extracts (Robert et al., 1976). 30 minutes after administration of the charcoal, the animals were sacrificed by cervical dislocation, the abdomen opened, the small intestine removed and placed on blotting paper. The small intestine is inspected; the distance travelled by the charcoal was measured using a scale and expressed as a percentage of the intestinal transit.

Intestinal accumulation of the fluid: Enteropooling

The method described by Robert et al., (1976) was used. This study involves assessing the net quantity of fluid accumulated in the small intestine. The various doses of the aqueous extracts (300 and 600 mg/kg), hydroethanolic (300 and 600 mg/kg), saline water (control group, 0,5 mL/100g) and loperamide (reference molecule, 10 mg/kg) were administered orally to groups, one (1) hour prior administration of castor oil (0,6 mL / 20 g / rat). 2 hours after castor oil administration, the mice sacrificed by cervical dislocation. The small intestine was removed and weighed (W1).Subsequently it was emptied of its contents then and reweighed (W2) and its length (L) measured. The difference between the weights (W1-W2) divided by the total length of the intestine gives net quantity (Q) of the fluid accumulate. The intestinal contents of each group were collected in a tube and sent to the laboratory to determine the concentrations of Na^+(sodium), K^+ (potassium) ions

using a flame photometer (Micro Touch Biochemistry Analyzer).

Statistical analyze

All values were expressed as mean ± ESM. Analysis of variance followed by Student-Fischer t test "t" was performed. The significance level was set at $p<0.05$

RESULTS

Effect on the frequency of emission and the quantity of faeces in the normal rats

The results of the effect of the extracts aqueous and hydroethanolic of the roots of *C. podolaena* on the fecal excretion are presented on Table 1.These results show that the animals treated with the reference laxative (castor oil) significantly ($p< 0.001$) increase the frequency of faeces emitted with a percentage of production of 70.40 % compared to the control group. In addition, the two extracts (aqueous and hydroethanolic) caused a significant reduction ($p<0.01$; $p<0.001$) of the frequency of emission of the faeces compared to the control group. However, no significant difference ($p>0.05$) was observed on the quantity of the faeces excreted with the two extracts compared to the control group.

Table 1: Effect of aqueous and hydroethanolic extracts on the frequency and the quantity of faeces excreted in the normal rats.

Traitement	Doses/volume	Frequency /6 hours	Quantity /6 hours
Salin water	0.5 mL/100g	1.74±0.11	0.78±0.19
Castor oil	3 mL	5.88±0.33***	2.66±0.21***
Aqueous extract	300 mg/kg	0.86±0,08*	0.51±0.17 ns
	600 mg/kg	0.92±0.08***	0.7±0.14 ns
Hydroethanolic extract	300 mg/kg	0.41±0.14***	0.45±0.06 ns
	600 mg/kg	1.44±0.08**	0.63±0.19 ns

Each value represents the mean ± ESM; *p<0.05, **p<0.01, ***p<0.001 (Student t-test); versus control group. ns= p>0,05.

Antidiarrheal effect

The oral administration of the castor oil (3 mL / rat) caused the diarrhoea during the six hours of observation (Table 2). These two extracts at the doses used significantly delays (P<0,001) the onset of fecal excretion compared to the control group (figure 1).The loperamide (reference molecule) as well as the aqueous and hydroethanolic extracts of the roots of *C. podolaena* at the doses used significantly reduce (P<0,001) the frequency and the quantity of the fecal excretion six hours of observation with a maximum of effect during the first two hours of observation where the animals are completely constipy. However, this effect decreases with time. The effect observed continues until the fourth hour of observation in the rats treated with the loperamide and the hydroethanolic extract (600 mg/kg, Table 2).

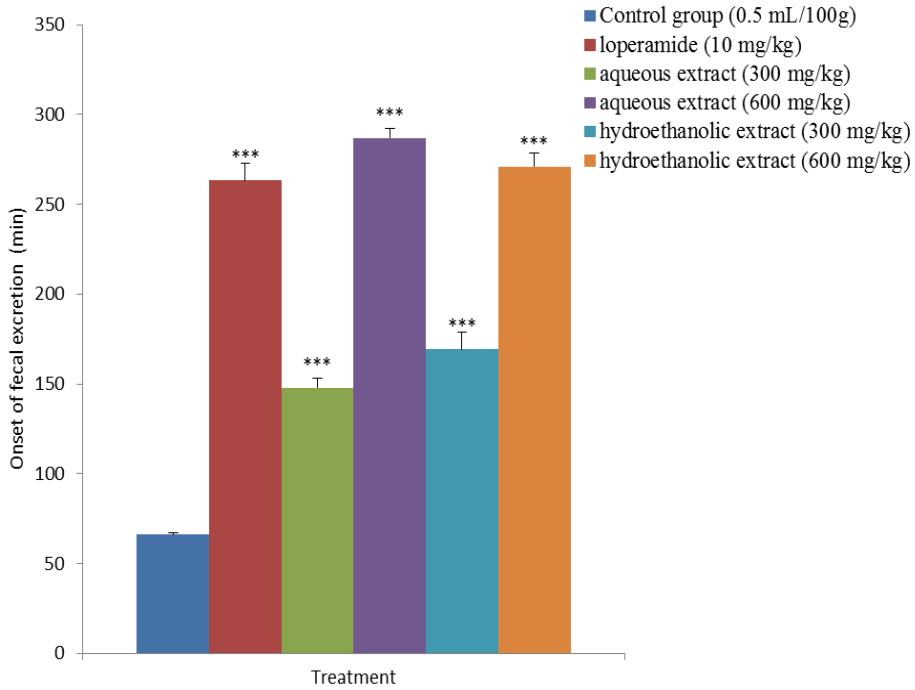

Figure 1: Effect of aqueous et hydroethanolic extracts on onset of fecal excretion.

Each value represents the mean ± ESM, ***p<0.001 (Student t-test).; versus control group.

Effects of the extracts aqueous and hydroethanolic of the roots of *C. podolaena* on the intestinal transit

The effect of the extracts aqueous and hydroethanolic on the intestinal transit time is presented by figure 2. It shows that the loperamide as well as the two extracts with the amounts used significantly reduce ($p<0.01$, $p<0.001$) the intestinal transit time compared to the reference group distilled water. However, this effect observed with the two extracts appears definitely lower than that of the loperamide. The values of the transit are 93.42; 23.98; (79.26 and 78.90) and (80.14 and 82.26) respectively for salin water, loperamide, aqueous extract (300 and 600 mg/kg) and the hydroethanolic extract (300 and 600 mg/kg). The acetylcholine in the aqueous extract presence as well as hydroethanolic extract significantly reduced the percentage of the intestinal transit time ($p<0,05$ $p<0.01$,$p<0,001$) compared to the group controls (figure 3).

Tableau 2: Effect of aqueous (AE) and hydroethanolic (HE) extracts on the frequency and the quantity of faeces induced by castor oil (C.o).

Treatment	Frequency of fecal excretion			Quantity of faeces excretion			Fecal reduction (%)		
	2H	4H	6H	2H	4H	6H	2H	4H	6H
C.g (0.5 ml/100g) + C.o (3mL/rat)	14,33±0,98	18.66±0,54	20.83±0.61	6.76±0.17	8.73±0.28	10.13±0.19	/	/	/
Lop (10 mg/kg) + C.o (3mL/rat)	0±00***	0±00***	1.94±0.26-***	0±00***	0±00***	0.78±0.10***	100	100	92.30
AE (300 mg/kg) + C.o (3mL/rat)	0±00***	3.17±0.39***	5.68±0.76***	0±00***	1.17±0.21***	2.61±0.83***	100	86.59	74.23
AE (600 mg/kg) + C.o (3mL/rat)	0±00***	0±00***	2.67±0.31***	0±00***	0±00***	2.75±0.17***	100	100	72.85
HE (300 mg/kg) + C.o (3mL/rat)	0±00***	2.78±0.26***	5.22±0.58***	0±00***	2.47±0.14***	3.90±0.16***	100	71.70	61.50
HE (600 mg/kg) + C.o (3mL/rat)	0±00***	0±00***	5±0.57***	0±00***	0±00***	3.78±0.62***	100	100	62.68

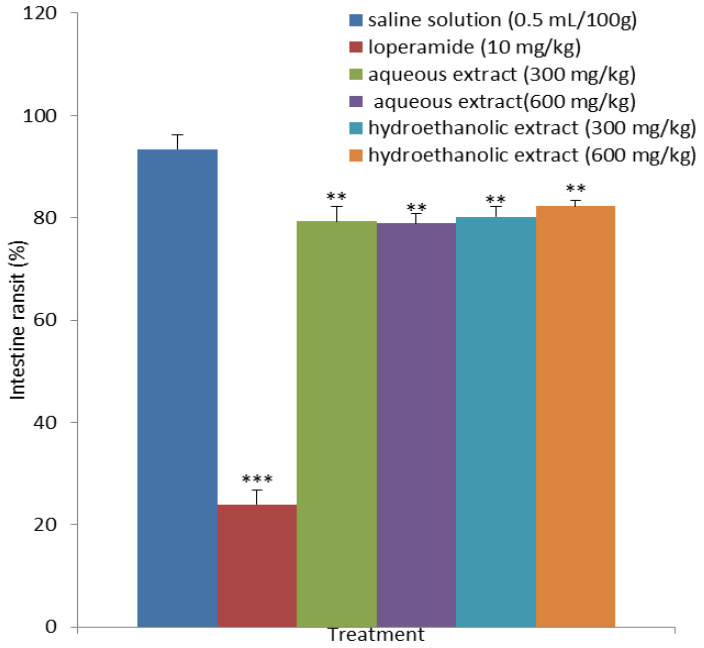

Figure 2: Effect of aqueous and hydroethanolic extracts on normal transit.

Each value represents the mean ± ESM. ***P<0.001 **p<0.01 significant different (Student t-test) versus control group

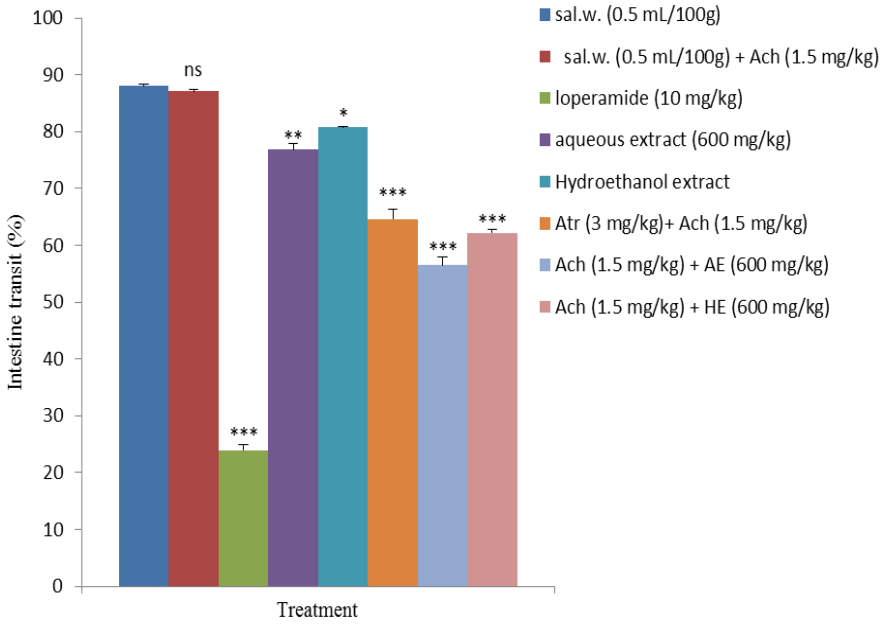

Figure 3: Effect of aqueous (AE) and hydroethanolic (HE) extract on intestine transit in acetylchiline (Ach) presence.

Each value represents the mean ± ESM. *p<0.05; **p<0.01 and ***P<0.001 significant different (Student t-test) versus control group. ns= no significant different Each value represents the mean ± ESM. ***P<0.001 significant different (Student t-test) versus control group (C.g); Lop= loperamide.

Effect of the extract on castor oil-induced enteropooling

The results of the effect of the extracts aqueous and hydroethanolic on castor oil-induced intestinal accumulation fluid are presented on the figure 4. It shows that the loperamide as well as the extracts aqueous and hydroethanolic significantly ($p<0.05$; $p<0.01$) reduce the intestinal content fluid compared to the group control. The diarrheic mice intestinal content fluid is 0.864± 0.10 control group against, 0.409±0.06 for the lopéramide, 0.385±0.12 and 0.348±0.05 for the aqueous (300 and 600 mg/kg) ; 0.576±0.079 and 0.632 ±0.06 for the extract hydroethanolic (300 and 600 mg/kg). In addition, control group, loperamide, aqueous and hydroethanolic extracts at the doses used (300 and 600mg/kg) eliminate more Na^+ than K^+ (Table 3).

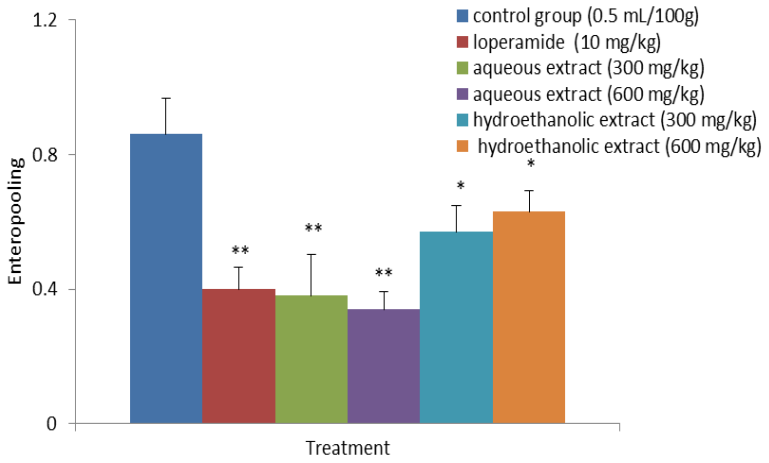

Figure 4: Effect of aqueous (AE) and hydroethanolic (HE) extract on castor oil-induced intestinal accumulation fluid. Each value represents the mean ± ESM. *p<0.05 and **p<0.01 significant different (Student t-test) versus control group.

Table 3: Effect of aqueous and hydroethanolic extracts at the doses used (300 and 600mg/kg) on the Na^+ and K^+ excretion.

Traitement	Doses	Na^+ (mmol/L)	K^+ (mmol/L)	Ratio Na^+/K^+
Control group	0.5 mL/100 g	192.69	20.58	9.36
loperamide	10 mg/kg	89.00	21.53	4.13
Aqueous extract	300 mg/kg	793.20	10.59	74.90
	600 mg/kg	354.06	3.52	100.58
Hydroethanolic extract	300 mg/kg	564.8	17.72	31.87
	600 mg/kg	560.2	16.71	33.52

DISCUSSION

The present study was initiated for investigate the antidiarrheic effect of the aqueous and hydroethanolic extracts of roots of *C. podolaena*. Before that, we had evaluating the effect of these two extracts on the frequency and the quantity of the fecal excretion in the normal rats in order to know if the two extracts would behave either like a laxative or an antidiarrheic. This show that the two extracts at the doses used do not increase the frequency and the quantity of the fecal excretion in the normal rats compared to the control group. On the other hand, the castor oil used as standard laxative significantly increased the frequency and the quantity of fecal excretion in the normal rats. This result would thus suggest an antidiarrheic effect.This is why the effect of these extracts was evaluated on the diarrhoea induced by the castor oil. Several mechanisms explain the diarrheal effect of the castor oil, the inhibition of the intestinal activity of N^{a+}/K^+ ATPase, reducing consequently the normal absorption of the fluid and electrolytes (Ebuehi et al., 2006); the activation of the adenylate cyclase (AMpc) (Okeke et al., 2007) and the stimulation of the prostaglandin formation and the factor of activation of plates (Capasso et al., 1994).Usually, the castor oil is metabolized in acid ricinoleic (C_{18}) in the small intestine what causes an irritation and an inflammation in the intestinal mucous membrane, involving the release of inflammatory mediators (for example, prostaglandins and histamine).The released prostaglandins initiate a vasodilatation, a contraction of the smooth muscles and a mucosa secretion in the small intestine. In the experimental animals as well as in the human beings, the prostaglandins E are regarded as good diarrhegenic. It was suggested that the inhibitors of the biosynthesis of prostaglandins delay the diarrhoea induced by the castor oil. The results obtained show that the loperamide (reference molecule) as well as the aqueous and

hydroethanolic extracts of the roots of *C.podolaena* at the doses used reduce significantly the frequency and the quantity of the excreted fecal and significantly delay ($p<0,001$) the onset of the fecal excreted compared to the control group.The fact that these extracts at doses used are opposed to the diarrhoea induced by the castor oil suggests that they could interfere with the mechanisms of induction of the diarrhoea by the castor oil. Put aside the abnormal exchanges of the electrolytes which explain an excessive excretion of water, the diarrhoea can be also due by an increase in the intestinal peristalsis which is influenced by acetylcholine and noradrenalin (Elion Itou et al., 2018) that why the effect of the two extracts was evaluated in the acetylcholine presence and of the atropine its antagonist. The results obtained in the presence of acetylcholine as those of acetylcholine in the atropine presence showed a reduction of the intestinal transit compared to the control group. The atropine is a cholinergic antagonist acts while being fixed at the muscarinic-receptors (M1 and M3), blocks the M1-receptors on the gastric parietal cells and contributes to the reduction of gastric secretions (Heinz et al., 2010). Moreover, it blocks the M3-receptors on the visceral smooth muscles of the stomach and the intestine, which involves a relieving of these muscles and decreases the tonality and the amplitude of these bodies (Sharma., 2011). The inhibiting effect of the intestinal transit could be explained by the fact why the two extracts of the roots of *C.podolaena* would be fixed on the same receptors as the atropine. Apart from this mechanism, the diarrhoea, can be also due by an osmotic increase in load in the intestine.The increase in the intestinal motility and the disturbances of the secretion of the electrolytes remain usually a common denominator in the majority of the cases of diarrhoea (Harrison et al.,, 2005) this is why the effect of the aqueous and hydroethanolic extracts of the roots of *C.podolaena* on the intestinal accumulation of the fluid was evaluated. The results show that the two extracts at the doses used as well as the loperamide significantly inhibit ($p<0,001$) the accumulation of intestinal fluid and increases the ratio Na^+/K^+ compared to the control group. Indeed, it's well-known that the loperamide is a agonist of µ-receptors; its fixing on its receptors slows down the intestinal transit (Elion Itou et al., 2017). The molecule also has an antisecretory effect by increase of the hydro-electrolytic flow of the intestinal light towards the plasmatic pole of the enterocyte (reabsorption), and opposite reduction of flow (secretion). In this study, it was shown that the two extracts at the doses used excrete more the Na^+ than ions K^+ compared to the control group. In addition, to the physiopathological mechanisms of the diarrhoea, the hypermotility characterizes the diarrhoea where the secretary component is not the causal factor of the electrolytes and water in the intestinal mucous membrane. For that the implied mechanism would be associated for a double purpose on the one hand on the intestinal peristalsis by reducing accumulation of the intestinal fluid and on the other hand, on the transport of water and electrolyte (reduction in the absorption of Na^+ and K^+) through the intestinal mucous membrane what caused a significant excretion of Na^+ than K^+ in the faeces (Kouitcheu et al., 2006; Ngo et al., 2010 ; Khalilur et al., 2015).Thus, it is possible that the two extracts reduce the diarrhoea by increasing the reabsorption of the electrolytes and of water or by inhibiting the accumulation of the intestinal contents fluid like would do it certain traditional drugs like the loperamide and the diphenoxylate.

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Conflict of interest: The authors declare that they have no conflict of interest.

CONCLUSION

This study was initiated to confirm or cancel the use of the roots of *C.podolaena* in the treatment of the diarrhoea. The results showed an effect antidiarrheic of the extract aqueous and extract hydroethanolic of its roots. The effect antidiarrheic of the extracts of the roots of *C.podolaena* could pass by an anticholinergic effect.

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