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ANTI-CONSTIPATION POTENTIAL IN WISTAR RATS OF THE AQUEOUS LEAVES EXTRACT OF *Cassia occidentalis* (CAESALPINIACEAE), A PLANT USED IN TRADITIONAL MEDICINE FOR THE TREATMENT OF CONSTIPATION

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ABSTRACT

The aim of this study is to evaluate in rats the anti-constipant potential of *Cassia occidentalis* (Caesalpiniaceae), a plant whose leaves are used in traditional medicine in Côte d'Ivoire for the treatment of constipation. Loperamide (3 mg/kg bw in 0.9% sodium chloride), administered orally toWistar rats for 3 days, induces the emission of reduced, hard, dry faecal pellets, evidence that these animals had become constipated, as in healthy rats faeces are abundant, soft and moist. This induced constipation is accompanied by a reduction in food and water consumption, and in the number, weight and water content of the faecal pellets emitted by these rats. Aqueous extract of *Cassia occidentalis* (EACo), administered orally at doses of 100, 200 and 400 mg/kg bw to constipated rats, reduces these parameters in a dose-dependent manner, leading to a return to normal. This extract is therefore an anti-constipant. In addition, the use of activated charcoal as a marker of colonic movement shows that loperamide, when administered to rats, reduces the gastrointestinal transit ratio. EACo, at doses of 100, 200 and 400 mg/kg bw, increases this gastrointestinal transit ratio in a dose-dependent manner towards normalisation in rats made constipated by loperamide. These results indicate that EACo is also a laxative. The anti-constipant and laxative effects of AECo, at a dose of 400 mg/kg bw, and those of bisacodyl (0.25 mg/kg bw) are similar on loperamide-induced constipation in rats. This indicates that EACo acts like bisacodyl, a standard pharmaceutical drug used as a laxative in the treatment of constipation. These results provide scientific support for the anti-constipant and laxative potential of the aqueous extract of *Cassia occidentalis* leaves (Caesalpiniaceae) and justify the use of this plant in traditional medicine for the treatment of constipation.

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INTRODUCTION

Plants have long been an important source of medicines for a number of diseases. *Cassia occidentalis* (Caesalpiniaceae) is a well-known plant in traditional medical practice. It is widely used in traditional medicine for its analgesic, antifungal, anti-inflammatory and antipyretic properties (Aké-Assi, 2011), as well as for its antispasmodic properties (Fassassi, 2010). It is also used to treat hypertension, diabetes (Tra-Bi *et al.,* 2008), sexual impotence, oedema (Diakité, 2008) and asthma (Irié-N'guessan *et al.,* 2010). According to Pousset (2006) *Cassia occidentalis* is widely used in rural Senegal for its purgative properties. The aim of this study is to highlight the potential pharmacological effects of an aqueous extract of *Cassia occidentalis* (EACo) in the treatment of constipation.

MATERIALS AND METHODS

Materials

Plant material: The plant material consisted of fresh *Cassia occidentalis* (Caesalpiniaceae) leaves harvested in the Abobo district (Abidjan, Côte d'Ivoire). The botanical identification of this plant is carried out at the Centre National de Floristique (CNF) of the University Félix Houphouët-Boigny. A specimen of this species exists in this Centre under herbarium number 239 dated 28 June 1995.

Animal material: Rats of the species *Ratus norvegecus* (Muridae), weighing between 150 and 200 g, were used in this study. These rats are bred at the UFR Biosciences animal house at the Université Félix Houphouët-Boigny, in cages at room temperature, with light during

the day and darkness at night. They are fed as much as desired with pellets (supplied by Ivograins® of Abidjan, Côte d'Ivoire) and have free access to water. The conditions under which they are reared and used comply with the standards published by the Ivorian animal welfare laboratory and the experimental protocols are conducted in accordance with the European directive of 24 November 1986 (86/609/EEC) and the decree of 19 April 1988 (Anonymous) relating to the use of experimental animals in research.

Pharmacodynamic substances: The pharmacodynamic substances used are:

- Loperamide (JANSSEN-CILAG, France), to induce constipation in rats;
- Activated charcoal (AJC PHARM, France), to monitor gastrointestinal transit:
- Bisacodyl (Delpharm Reims, France), to treat occasional induced constipation.

Methods

Preparation of Cassia occidentalis aqueous extract (EACo): Fresh leaves of *Cassia occidentalis* (Caesalpiniaceae) are dried in a room at room temperature and then ground using a grinder. One hundred and twenty grams (120 g) of dried leaves powder from this plant are boiled for 20 minutes in 2 litres of distilled water. The decoctate obtained is filtered twice on cotton wool and once on Whatman N° 2 filter paper. The filtrates collected are dried in an oven at 40°C for 72 hours. After drying, a water-soluble powder is obtained. This dry extract is the *Cassia occidentalis* aqueous extract (EACo) used in this study.

Induction of constipation in rats: Constipation is induced in rats by oral administration of 1 mL of loperamide (3 mg/kg bw in 0.9% sodium chloride) for 3 days. The emission of small, hard, dry faecal pellets indicates constipation in rats **(Bustos *et al.,* 1991)**.

Experimental study method for the effects of EACo on induced constipation in rats

- This study is carried out using the method described by Ashafa *et al*. (2011).
- A total of 36 Wistar rats are used for the experiment. These rats are divided into 6 batches, with 6 animals in each batch.
- Rats in batches 1 (healthy controls) and 2 (constipated controls) are fed distilled water.
- Rats in batches 3, 4 and 5 are constipated and received 100, 200 and 400 mg/kg bw/day, respectively, of *Cassia occidentalis* aqueous extract (EACo).
- Batch 6 consisted of constipated rats receiving bisacodyl (0.25 mg/kg bw/day).

The substances or water are administered by gavage through an oropharyngeal cannula and the treatment lasted 7 days. The pellets excreted by the rats are collected daily at 9 am. for the duration of the experiment (7 days). The number of faecal pellets, their weight and water content are determined. The moisture content is calculated as the difference between the wet and dry weights of the pellets. Water and food consumption, the number of faecal pellets and the weight evolution of the rats are measured daily during the experimental period. The gastrointestinal ratio is also measured using the method described by Nagakura *et al* (1996) and N'guessan (2019). On day 7, 1 mL of an activated charcoal solution (10% activated charcoal + 5% gum arabic), which is used as a marker to measure the distance travelled by the intestinal contents, is administered orally to the rats. One (1) hour after administration of the marker, the animals are euthanised by exsanguination under deep isoflurane anaesthesia and the small intestines are rapidly isolated. The distance travelled by the activated charcoal and the total length of the small intestine are measured. The TIG ratio is expressed as the percentage of the distance covered by the charcoal in relation to the total length of the small intestine. The transit of activated charcoal is given by the ratio of the distance travelled by the charcoal to the total length of the intestine (Pazhani *et al.,* 2001; Méité *et al.,* 2010).

$$P = \frac{1}{L} \times 100$$

P: Percentage of charcoal transit
l: Distance travelled by the charcoal
L: Total length of intestine

Data analysis: Statistical processing of the data is carried out using GraphPad Prism 8 software (Microsoft, San Diego, California, USA). Values are expressed as the mean plus the standard error of the mean (M ± SEM). Analysis of variance (Anova) of the Turkey-Kremer multiple comparison test is used to assess the significance of the differences observed, with a significance level of $p < 0.05$. In this case, if :

$p > 0.05$, the observed difference is not significant ;
$p < 0.05$, the observed difference is not very significant (*) ;
$p < 0.01$, the observed difference is significant (**);
$p < 0.001$, the observed difference is highly significant (***).

RESULTS

Effects of induction of constipation by administration of loperamide in rats: Oral administration of loperamide (3 mg/kg bw, in 0.9% sodium chloride, for 3 days) results in the emission of small, hard, dry faecal pellets in rats, indicating constipation, whereas in healthy rats the faeces are abundant, soft and moist. Loperamide, when administered to rats, also induces a reduction in food and water consumption, as well as in the number, weight and water content of faecal pellets emitted. In fact, in rats made constipated, food and water consumption decreases ($p < 0.05$) from 14.06 ± 1.64 g and 21.72 ± 0.63 mL respectively in healthy rats, to 9.85 ± 1.62 g and 19.94 ± 1.49 mL after administration of loperamide. Also, in these constipated rats, the number, weight and water content of faecal pellets decreases ($p < 0.01$) from 58.27 ± 5.09, 6.17 ± 0.76 g and 2.7 ± 0.22 mL, respectively, in healthy rats, to 24.26 ± 2.63, 2.51 ± 0.67 g and 1.43 ± 0.16 mL, respectively, when the rats are constipated (Table 1).

Table 1. Effects of loperamide on food and water consumption and on the number, weight and water content of rat faecal pellets

Parameters	Healthy rats (Control)	Rats constipated by loperamide administration
Food intake (g)	14.06 ± 1.64	9.85 ± 1.62*
Water consumption (mL)	21.72 ± 0.63	19.94 ± 0.49*
Number of faecal granules	58.27 ± 5.09	24.26 ± 2.63**
Weight of faecal granules (g)	6.17 ± 0.76	2.51 ± 0.67**
Water content of faecal pellets (mL)	2.76 ± 0.22	1.43 ± 0.16**

n = 6; * $p < 0.05$; ** $p < 0.01$ compared with healthy rats

Effects of *Cassia occidentalis* aqueous extract (EACo) on loperamide-induced constipation in rats

Effects of EACo and bisacodyl on food and water intake, number, weight and water content of faecal pellets and body weight of constipated rats

Table 2 shows that food consumption decreases ($p < 0.01$) when rats are given loperamide, from 15.16 ± 0.90 g in healthy animals to 10.66

± 0.52 g when they are made constipated. This food intake, compared with that of constipated control rats (10.66 ± 0.52 g), increases in a dose-dependent manner in all constipated rats given 100, 200 and 400 mg/kg bw of *Cassia occidentalis* aqueous leaves extract (EACo) for 7 days, to 11.65 ± 0.35 g, 12.14 ± 0.38 g and 12.46 ± 1.41 g, respectively. Similarly, in constipated rats treated with bisacodyl (0.25 mg/kgbw), food consumption increases to 12.96 ± 0.56 g. However, in these treated constipated rats, food consumption remains lower ($p < 0.05$) than in healthy rats. Water consumption is also lower ($p < 0.05$) in untreated constipated rats (19.68 ± 1.77 mL) than in healthy control rats (21.53 ± 0.86 mL).

to 6.25 ± 0.80 g. Thus, in constipated rats treated with EACo at 400 mg/kg bw or with bisacodyl, the weight of faecal granules becomes approximately equal ($p > 0.05$) to that of healthy rats. When the rats are made constipated, the water content of the faecal pellets falls very significantly ($p < 0.01$) to 1.39 ± 0.08 mL, whereas in healthy rats the water content is 2.82 ± 0.27 mL. Treatment of constipated rats for 7 days results in an increase in the water content of faecal pellets in rats receiving EACo at doses of 200 and 400 mg/kg bw and bisacodyl (0.25 mg/kg bw), without however returning this water content to normal (2.82 ± 0.27 mL).

Table 2. Effects of *Cassia occidentalis* aqueous leaves extract (EACo) and bisacodyl on food and water consumption and on the number, weight and water content of faecal pellets in loperamide-constipated rats

Parameters	Healthy rats (Control)	Constipated rats	Constipated rats treated with EACo (mg/kg bw)			Constipated rats treated. with Bisacodyl(0.25 mg/kg bw)
			100	200	400	
Feed consumption (g)	15.16 ± 0.90	10.66 ± 0.52**	11.65 ± 0.35*	12.14 ± 0.38*	12.46 ± 1.41*	12.96 ± 0.56*
Water consumption (mL)	21.53 ± 0.86	19.68 ± 1.77*	21.9 ± 1.48*	23.03 ± 0.47*	25.56 ± 0.83**	19.33 ± 0.91*
Number of faecal granules	63.27 ± 2.44	25.8 ± 1.70**	32.9 ± 3.05**	36.7 ± 4.97**	54.35 ± 1.09*	50.33 ± 4.92*
Weight of faecal granules (g)	6.81 ± 0.78	2.23 ± 0.17**	2.62 ± 0.61**	2.82 ± 0.55**	5.97 ± 0.48	6.25 ± 0.80
Water content of faecal granules (mL)	2.82 ± 0.27	1.39 ± 0.08**	1.57 ± 0.11**	1.70 ± 0.14**	2.74 ± 0.14*	2.79 ± 0.15*
Body weight gain (g)	10.46 ± 2.02	11.76 ± 1.46*	10.76 ± 2.45	10.71 ± 1.61	10.5 ± 2.02	10.49 ± 1.25

n = 6; * $p < 0.05$; ** $p < 0.01$ compared with healthy rats

Table 3. Effects of *Cassia occidentalis* aqueous leaves (EACo) and bisacodyl on gastrointestinal motility in lopéramide induced constipated rats

		Total length of intestine (cm)	Distance covered by coal par (cm)	Percentage of coaltransit (%)
Healthycontols		98.53 ± 0.89	77 ± 3.14	78.19 ± 3.29
Constipated rats (Constipatedwitnesses)		102.35 ± 1.78	38.42 ± 1.95	37.7 ± 2.52***
Constipated rats treated for 7 days with EACo or Bisacodyl	EACo 100 mg/kg bw.	105.6 ± 1.71	57.94 ± 1.75	54.92 ± 1.95**
	EACo 200 mg/kg bw.	103.6 ± 2.33	71 ± 0.61	68.65 ± 1.54*
	EACo 400 mg/kg bw.	100.4 ± 2.56	79.71 ± 1.74	79.4 ± 1.20
	Bisacodyl 0.25 mg/kg bw.	106.5 ± 3.98	77.16 ± 2.08	73.09 ± 4.38

n = 6; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with healthy rats

In constipated rats treated with 7 days of EACo, their water consumption increases ($p < 0.05$ or $p < 0.01$) compared with that of healthy control rats, whereas when constipated rats are treated for 7 days with bisacodyl, their water consumption was identical ($p > 0.05$) to that of untreated constipated rats (constipated controls). Water consumption in constipated rats given doses of 100, 200 and 400 mg/kg bw of EACo increases to 21.9 ± 1.48, 23.03 ± 0.47 mL and 25.56 ± 0.82 mL respectively, compared with 19.68 ± 1.77 mL in untreated constipated rats and 21.53 ± 0.86 mL in healthy control rats. That of constipated rats given bisacodyl (0.25 mg/kg bw) decreased, compared with that of healthy control rats, to 19.33 ± 0.91 mL. In healthy rats, the number of faecal granules is 63.27 ± 2.44. This number falls ($p < 0.01$) when the rats became constipated (25.8 ± 1.70 g). In constipated rats treated for 7 days with EACo, the number of faecal granules increased in a dose-dependent manner and is 32.9 ± 3.05, 36.7 ± 4.97 and 54.35 ± 1.09 for EACo doses of 100, 200 and 400 mg/kg bw respectively. An increase in the number of faecal granules is also recorded when constipated rats are treated for 7 days with bisacodyl, with 50.33 ± 4.92 faecal granules counted. The effect of EACo at a dose of 400 mg/kg bw on faecal granule emission is slightly greater than that of the standard drug, bisacodyl (0.25 mg/kg bw), but the number of faecal granules emitted following treatment of constipated rats with these substances remains lower ($p < 0.05$) than that of healthy rats. Also, the weight of faecal granules in constipated rats falls ($p < 0.01$) to 2.23 ± 0.17 g, compared with 6.81 ± 0.78 g in healthy rats. The weight of these faecal granules in constipated rats treated with EACo for 7 days at doses of 100, 200 and 400 mg/kg bw increases in a dose-dependent manner to 2.62 ± 0.61 g, 2.82 ± 0.55 g and 5.97 ± 0.48 g, respectively. In constipated rats treated for 7 days with bisacodyl (0.25 mg/kg bw), faecal granule weight also increases

In these cases, the water content becomes 1.70 ± 0.14 mL, 2.74 ± 0.14 mL and 2.79 ± 0.15 mL respectively. Body weight gain in healthy rats is 10.46 ± 2.02 g. This weight gain increases ($p < 0.05$) when the rats are constipated, rising to 11.76 ± 1.46 g. When constipated rats are treated for 7 days with EACo at doses of 100, 200 and 400 mg/kg bw or with bisacodyl (0.25 mg/kg bw), weight gain increases, bringing them to values approximately equal ($p > 0.05$) to those *of healthy* rats, with respective values of 10.76 ± 2.45 g; 10.71 ± 1.61 g; 10.5 ± 2.02 g and 10.49 ± 1.25 g.

Effects of EACo and bisacodyl on gastrointestinal motility in constipated rats: In rats made constipated by administration of loperamide, gastrointestinal motility is reduced ($p < 0.001$). Thus, in constipated rats not treated with EACo (constipated controls), gastrointestinal transit is 37.52 ± 2.76%, compared with 78.15 ± 4.12% in healthy rats (healthy controls). Treatment of constipated rats for 7 days with EACo at doses of 100, 200 and 400 mg/kg bw results in a dose-dependent increase in this gastrointestinal mobility and, for the 400 mg/kg bw dose, gastrointestinal transit becomes identical ($p > 0.05$) to that of healthy rats (Figure 1). In fact, when constipated rats are given the aqueous extract of *Cassia occidentalis* leaves, gastrointestinal transit increases in animals given the 100 mg/kg bw dose of EACo ($p < 0.05$) and in those given the 200 and 400 mg/kg bw doses of EACo ($p < 0.01$), giving gastrointestinal transit ratios of 54.92 ± 1.95%, 68.65 ± 1.54% and 79.40 ± 1.20% respectively (Table 3). Thus, in constipated animals given EACo at 100 and 200 mg/kg bw over 7 days, the decrease in gastrointestinal mobility induced by loperamide administration to rats is reduced by an average of 42.34% and 76.32% respectively, and this decrease in gastrointestinal mobility is reversed (100% reduction) when the dose of EACo

reaches 400 mg/kg bw. In addition, the reduction in gastrointestinal transit in constipated rats is also reversed when the rats are given bisacodyl (0.25 mg/kg bw) for 7 days. The gastrointestinal transit ratio in constipated rats given bisacodyl at 0.25 mg/kg bw is 73.09 ± 4.38%, compared with 78.19 ± 3.29% ($p > 0.05$) in healthy rats.

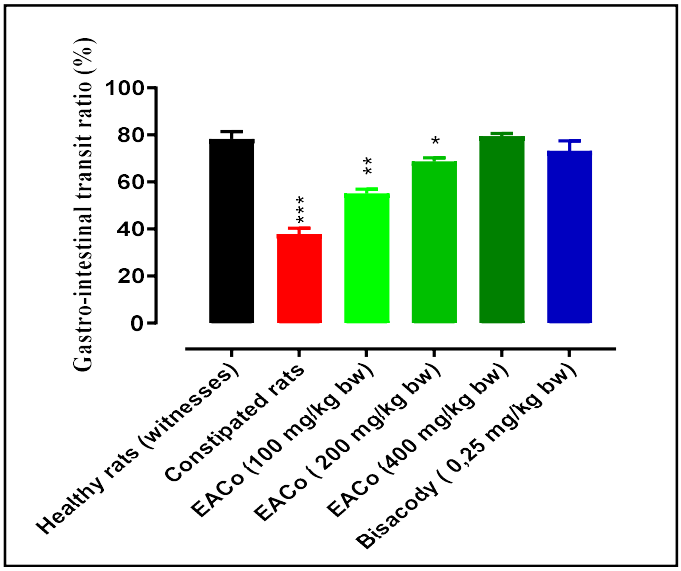

Figure 1. Effects of *Cassia occidentalis* aqueous leaves extract (CALE) and bisacodyl on the gastrointestinal transit ratio in rats made constipated by loperamide

Healthy controls: healthy rats given distilled water

Constipated controls: constipated rats given distilled water

- EACo (100 mg/kg bw): constipated rats given EACo at 100 mg/kg bw/day
- EACo (200 mg/kg bw): constipated rats given EACo at 200 mg/kg bw/day
- EACo (400 mg/kg bw): constipated rats receiving EACo at 400 mg/kg bw/day.
- Bisacodyl (25 mg/kg bw): constipated rats given bisacodyl at 0.25 mg/kg bw/day.

DISCUSSION

Loperamide, administered orally to Wistar rats, induces the emission of fewer, hard, dry faecal pellet output, whereas in healthy rats the faeces are abundant, soft and moist. It also leads to a reduction in food and water consumption, and in the number, weight and water content of the faecal pellets emitted. These results are similar to those of Nafiu *et al* (2015) which showed that loperamide reduces the water content of faecal pellets and the amount of water consumed. These effects observed after the treatment of rats with loperamide indicate the induction of constipation in these animals. Indeed, according to Ashafa *et al* (2011), the reduction in the number, weight and water content of faecal pellets indicates episodes of constipation in the animals. Shimotoyodome *et al* (2000) also indicate that the reduction in these indices, characteristic of constipation, may be a direct consequence of the significant reductions in food and water intake resulting from exposure to loperamide. Loperamide induces constipation in rats by inhibiting intestinal water secretion and colonic peristalsis. This inhibition prolongs faecal evacuation time and delays intestinal transit. Loperamide-induced constipation is therefore considered to be a model of spastic constipation (Takasaki *et al.,* 1994). Administration of *Cassia occidentalis* aqueous leaves extract (EACo) at doses of 100, 200 and 400 mg/kg bw to loperamide-induced constipated rats resulted in a dose-dependent normalisation of the faeces of these animals with, for the EACo dose of 400 mg/kg bw, the emission of abundant, soft, moist faecal pellets similar to those of healthy rats. This extract also produces a dose-dependent increase in food and water consumption, and in the number, weight and water content of the faecal pellets emitted, in the direction of normalisation of these parameters. These indications attest to the anti-constipant properties of EACo. A similar observation is reported by Nafiu*et al* (2015) who used the aqueous extract of *Lecaniodiscus cupanioides* (Sapindaceae) for the treatment of constipation induced by loperamide in rats. Our results also indicate that EACo at a dose of 400 mg/kg bw and bisacodyl at 0.25 mg/kg bw exert similar effects in correcting loperamide-induced constipation. Bisacodyl is a standard laxative drug used in the treatment of constipation. It belongs to the group of stimulant laxatives. After oral administration, bisacodyl induces the transport of electrolytes across the intestinal mucosa and increases colonic motricity. It inhibits water and electrolyte reabsorption, stimulates colonic motility by direct action on the myenteric plexuses and accelerates transit through the colon Manabe *et al.,* 2009). As a result, its effects are mainly directed at the colon, where it stimulates peristalsis and, like other laxatives, reduces water reabsorption, leading to stool softening. These results show that the aqueous extract of Cassia occidentalis leaves is an anti-constipant substance which could act like bisacodyl on gastrointestinal transit. This supports the use of herbal remedies in the treatment of constipation in many countries (Van *et al.,* 1997). Activated charcoal is used as a marker to measure colonic movement. Measurement of the gastrointestinal transit ratio of activated charcoal is useful in the diagnosis of constipation (Wintola *et al.,* 2010). Loperamide administered to rats causes a decrease in the gastrointestinal transit ratio of activated charcoal. This decrease in the gastrointestinal transit ratio of charcoal indicates constipation (Sagar*et al.,* 2005; Méité *et al.,* 2010). In this study, a dose-dependent increase in the gastrointestinal transit ratio of activated charcoal and a return to normal are observed in constipated rats treated with *Cassia occidentalis* aqueous leaves extract at doses of 100, 200 and 400 mg/kg bw. EACo thus increases intestinal motility, which in turn improves colonic peristalsis in the constipated rats. This provides evidence that this extract exerts a laxative effect favourable to the normalisation of gastrointestinal transit. The possible mechanism of EACo in this process may be to increase fluid release, thereby increasing intestinal secretion. The laxative effect of the extract could also be linked to changes in intestinal motility, leading to an increase in intestinal transit and colonic movement (Capasso *et al.,* 1997). The effects of EACo on gastrointestinal transit are similar to those of the aqueous extract of Konjac flowers (*Amorphophallus muelleri*) (Widjanarko *et al.,* 2013). These authors showed that this extract is effective in the treatment of loperamide-induced constipation in rats. The effect of EACo at 400 mg/kg bw on gastrointestinal motility was similar to that of bisacodyl (0.25 mg/kg bw). This is an indication that this extract is effective in improving intestinal deobstruction by facilitating intestinal movement.

CONCLUSION

The present study reveals that oral administration of an aqueous extract of *Cassia occidentalis* leaves (EACo) induces anti-constipant activity and promotes intestinal motility in rats rendered constipated by oral administration of loperamide. These effects of EACo at a dose of 400 mg/kg bw are similar to those of bisacodyl (0.25 mg/kg bw), a reference laxative used in the treatment of constipation. EACo is therefore an anti-constipant and a laxative. These results provide scientific support for a potential pharmacological effect of the aqueous extract of *Cassia occidentalis* leaves as an anti-constipant and laxative substance and justify the use of this plant in traditional medicine for the treatment of constipation.

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