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Acute toxicity and laxative effect of the aqueous extract of the leaves *Crossopteryx febrifuga (*Benth*)* in rats

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The leaves of *Crossepteryx febrifuga (*Euphorbiaceae) are commonly used in the Republic of Congo in traditional medicine as a laxative. The present study aims to justify the traditional use of *C. febrifuga* in the treatment of constipation. The acute toxicity of the aqueous extract was evaluated at the doses of 2000 and 5000 mg/kg in mice in accordance with the OECD (2001) guideline no. 423. The laxative activity of the aqueous extract at doses of 100, 200 and 400 mg/kg was evaluated in normal rats, in rats made constipated rats by loperamide hydrochloride as well as on the accumulation of liquid intestinal. The results obtained showed that the aqueous extract at a dose of 2000 mg/kg does not modify the general behavior of mice and does not cause mortality. However, sedation and mortality were observed at the dose of 5000 mg/kg of the aqueous extract. Thus, the aqueous extract would be weakly toxic, with an LD50 > 4000 mg/kg. Furthermore, it appears from this study that loperamide caused constipation in all normal rats during the three days of treatment, which resulted in a significant decrease ($p<0.01$ and $p<0.001$) in fecal parameters except the body weight of the animals which increased significantly ($p<0.01$) compared to the control group. In addition, Normacol* and aqueous extract (100, 200 and 400 mg/kg) significantly ($p<0.001$) increase faecal parameters compared to control group. In addition, the aqueous extract significantly increases ($p<0.001$) the accumulation of intestinal fluid and stimulates the excessive secretion of Na^+ ions. These results show that *C. febrifuga* has laxative properties which could be justified by the presence of anthraquinones, flavonoids, alkaloids and saponosides highlighted in this plant.

Key words: *Crossopteryx febrifuga*, aqueous extract, acute toxicity, laxative activity.

INTRODUCTION

Gastroduodenal disease remains a problem affecting many populations. They are very widespread and exist

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on all continents and especially in black Africa where the high dietary fiber content does not seem to fully protect populations (Camara et al., 1999); yet Africa has a great diversity of food plants. However, the eating habits of Africans are not very varied, which exposes them to very frequent digestive disorders, in particular constipation. Constipation is a gastrointestinal motility disorder characterized by infrequent bowel movements. An estimated 14% (3.2 million) of the world's population is constipated (Dimidi et al., 2014; Cirillo and Capasso 2015). The treatment of constipation involves the use of laxatives which act differently on intestinal transit; however, conventional laxatives pose a problem of accessibility to populations because of their cost and the distance from pharmacies (Johanson et al., 2007). Under these conditions, many populations are forced to resort to the leaves of medicinal plants presumed to be laxatives to seek treatment. Indeed, plants are the basis of traditional African medicine; they have existed for thousands of years and continue to bring new remedies to humanity. They have several pharmacological properties such as anti-inflammatory, antibacterial, gastrointestinal diseases and diabetes (Bennini and Merdaci, 2016). However, they can also have side effects when consumed in high concentrations, which will induce toxicity in different organs (Bennini and Merdaci, 2016). As a result, a number of toxicological, phytochemical and pharmacological studies of medicinal plants must be carried out, in order to provide a coherent scientific justification for the traditional use of it. In Congo, there are many herbal products in pharmacopoeias, at herbalists in the markets, or in the villages for the treatment of certain pathologies including constipation. Thus, *C. febrifuga* is well known in traditional medical practice in southern Congo where its leaves and roots are used to relieve or cure certain pathologies such as gonorrhea, epilepsy, heart and stomach aches, wounds, headaches; in eye drops it acts on the filaria which are in the connective tissue, and the oral affections of which the dental caries (Bouquet, 1969). The leaves of this plant are commonly used in herbal medicine as a laxative in the form of a suppository. Nowadays, no scientific study has been carried out on this laxative activity. Thus, the present study aims to understand the traditional use *of C. febrifuga* in the treatment of constipation.

MATERIALS AND METHODS

Plant material

The leaves of *C. febrifuga* species were used. Several plant leaves of the same species growing in the same place were collected in July 2019 in Kimbedi locality, Department of Pool (Congo). 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 herbarium of the National Institute for Research in Exact and Natural Sciences (NIRENS) in which a collected sample was compared to a reference samples (n° 8012). After identification, the plant material was dried at room temperature (27±50°C) for 14 days in the Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE), and then pulverized with a mortar. 5 g of *C. febrifuga* powder was mixed in 50 mL of distilled water. The whole was left under magnetic stirring for 24 h. The macerate (10%) obtained after filtration with the help of cotton wool was kept in a sterile bottle for study.

Animal material

Albino rats (100 to 150 g) and albino mices (20 to 30 g) of either sex aged 3 months were used. These animals were provided to us by the animal house of the Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE) of the Faculty of Science and Technical of the Marien Ngouabi University. They were fed with a standard food and with running water. They were acclimatized during one week before experimentation and were housed under standard conditions (12 h light and 12 h dark) and at the temperature of 27 ± 1°C. The rules of ethics published by the International Association for the Study of Pain have been considered (Zimmermann, 1980).

Acute toxicity of aqueous extract of *C. febrifuga*

The acute toxicity of the aqueous extract of the leaves of *C. febrifuga* was evaluated according to OECD no. 423 guideline adopted on December 17, 2001. Mice were fasted for 24 h before the treatment and then divided into three groups of three mice each and treated with different doses of distilled water (control group, 10 mL/kg) and aqueous extract of the leaves of *C. febrifuga* at the doses of 2000 and 5000 mg/kg. After rectal administration, macroscopic observations including mobility, ptosis, alertness, piloerection, aggressiveness, stool status, vomiting was evaluated at ½, 1, 2, 3 and 8^{th}. Mortality was evaluated in 48 h.

Laxative effect of aqueous extract of *C. febrifuga* in non-constipated rats

Method reported by Meité et al. (2010) was used. Five (5) groups of 5 rats each were formed and treated with the differents doses of aqueous extract of *C. febrifuga* **(**100, 200 and 400 mg/kg), Normacol* (standard drug, 2 mL/rat) and distilled water (control group, 10 mL/kg) were administered rectally to groups. After that, the animals of each groups were placed individually in metabolic cages and the following parameters were observed, onset, frequency, and the quantity of stool emission were evaluated for 8 h.

Laxative effect of aqueous extract of *C. febrifuga* in loperamide hydrochloride (Imoduim*) induced constipation

The method reported by Wintola et al. (2010) was used. Before the administration of the test products, the animals were selected. Constipation was induced in thirty (30) normal rats by oral administration of loperamide hydrochloride (3 mg/kg body weight in 0.9% sodium chloride) for three days. However, five rats were treated only with distilled water (control). During these three days, they were fed standard food and water. Thus, food consumption, water intake, number of faecal pellets, water content of pellets, weight of pellets and weight gain were determined every day at

eight hours for three days. The water content was calculated as the difference between the dry weight and the wet weight of the stools were determined. Three days later, animals showing signs of constipation (presence of hard or dry stools) were placed in metabolism cages and grouped into five (5) groups of five (5) rats each treated rectally for one week (7 days) with different doses of distilled water (control group, 0.5 mL/100 g), Normacol* (standard group, 2 mL/rat) and with aqueous extract of *C. febrifuga* leaves (100, 200 and 400 mg/kg). Food consumption, water intake, number of faecal pellets, water content of pellets, weight of pellets and weight gain were calculated every day at 8^{th}. for the duration of the experiment (7 days). The onset, the frequency and the quantity of stools excreted was determined each day for eight hours of observation of the animals after administration of the test products.

Evaluation of the effect of aqueous extract of *C. febrifuga* on intestinal fluid accumulation in mice

The method reported by Meité et al. (2010) was used. Mice were divided into groups of five mice each treated rectally with different doses of distilled water (control group, 0.5 mL/100 g), Sodium dihydrogen phosphate dihydrate (Normacol*, standard drug, 2 mL/rat), and aqueous extract of *C. febrifuga* leaves (100, 200 and 400 mg/kg). One hour after the administration of the products, the mice were treated with extract of *C. febrifuga* at the doses of 100, 200 and 400 mg/kg respectively received 0.5 mL/mouse of Normacol® 2 h after, the mice were sacrificed by cervical dislocation. The small intestine was removed and weighed (W1), then emptied of its contents and reweighed (W2) and its length (L) measured. The difference between the weights divided by the length of the total intestine gives the net quantity (Q) of accumulated stool:

$$Q = \frac{W1 + W2}{L}$$

Enteric contents of each group have been analyzed to define ions concentrations, sodium (Na^+), (potassium) K^+ by the aid of a spectrophotometer of mark Micro Touch Biochemistry Analyser.

Chemical profile

It was carried out using the classical tube reaction tests to identify the major chemical groups (Clarke, 1975).

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). An analysis of variance using Excel version 2016 software followed by Student-Fischer t test “t” was performed. The significance level was set at $p<0.05$

RESULTS

Acute toxicity of the aqueous extract of *C. febrifuga* leaves

The results of macroscopic observation show that the aqueous extract of the leaves of *C. febrifuga* administered at a single dose of 2000 mg/kg rectally did not cause any change in general behavior compared to the control group. However, the aqueous extract of the leaves of *C. febrifuga* administered rectally at a single dose of 5000 mg/kg modified the general behavior characterized by immobility of the mice following the rectal administration of the products. Furthermore, mortality was observed after the dose of 5000 mg/kg with a calculated LD50≥3500 mg/kg.

Effect of aqueous extract of *C. febrifuga* leaves on the onset, frequency and quantity of excreted sells in normal rats

Tables 1 to 3 show the effect of aqueous extract of *C. febrifuga* leaves on onset appearance of sells, frequency and quantity excreted sells in normal rats respectively. They show that animals mice treated with Normacol* (2 mL/rat) as well as those treated with the aqueous extract at increasing doses of 100, 200 and 400 mg/kg significantly ($p<0.001$) reduced the onset of sells emission compared to the control group (Table 1). On the other hand, Normacol* (2 mL/rat) as well as aqueous extract (100, 200 and 400 mg/kg) significantly ($p<0.05$, $p<0.01$, and $p<0.001$) increased the frequency and quantity of sells emission (Tables 2 and 3) during 6 h of experimentation compared to control group. However, no significant change in the frequency of excreted sells was observed at 8 h (Table 2).

Effect of aqueous extract of *C. febrifuga* in constipated rats

The loperamide induced constipation in all normal animals during the three days of treatment (Table 4). Thus, there is a significant decrease ($p <0.01$ and $p<0.001$) in the faecal parameters (food consumption, water intake, number of faecal pellets, water content of the pellets, weight of stool excreted), except body weight of the animals which increased significantly ($p <0.01$) compared to the control group (Non-constipated rats). In addition, Normacol* (standard drug) and the aqueous extract of the leaves of *C. febrifuga* at the doses used (100, 200 and 400 mg/kg) significantly increase ($p<0.001$) food consumption, water intake, number of faecal pellets, water content of the pellets, weight of stool excreted) and body weight of constipated rats during the seven days of treatment (Table 5) compared to the control group.

Effect of aqueous extract of *C. febrifuga* leaves on the accumulation of intestinal fluid: Enteropooling

The results of the effect of aqueous extracts of *C. febrifuga* leaves by rectal administration on the

Table 1. Effect of aqueous extract of *Crossopteryx febrifuga* on stool onset in normal rats.

Treatment	Dose	Onset (Min)
E. distilled	0.5 mL/100 g	360±00
Normacol	2 mL/rat	4.40±0.40***
Extract of *C. febrifuga*	(100 mg/kg)	3.60±0.24**
	(200 mg/kg)	3.80±0.20***
	(400 mg/kg)	4.00±0.31***

Each value represents the mean ± ESM of onset. **p<0.01; ***p <0.001 significant different (Student t-test) versus control group.
Source: Authors

Table 2. Effect of aqueous extract of *C. febrifuga* on the frequency of stool emission in normal rats.

Treatment	Doses	Frequency of excreted sells			
		2 h	4 h	6 h	8 h
Distilled water	(0.5 mL/100 g)	00±00	00±00	00±00	00±00
Normacol*	(2 mL/rat)	3.01±0.10***	2.16±0.20***	2.6±0.24***	$0.45±00^{ns}$
	(100 mg/kg)	3.09±0.43***	2.22±0.58**	2.26±0.20***	$0.45±0.22^{ns}$
C. febrifuga	(200 mg/kg)	3.8±1.150*	2.52±0.21***	2.26±0.19***	$0.65±0.38^{ns}$
	(400 mg/kg)	4.2±0.37***	2.58±0.22***	2.36±0.20***	$0.8±0.37^{ns}$

Each value represents the mean ± ESM. p<0.05; **p<0.01; ***p <0.001 significant different (Student t-test) versus control group.
Source: Authors

Table 3. Effect of aqueous extract of *C. febrifuga* leaves on the quantity of excreted stool in normal rats.

Treatment	Doses	Quantity of excreted sells			
		2 h	4 h	6 h	8 h
	(0.5 mL/100 g)	00±00	00±00	00±00	00±00
Normacol distilled water	(2 mL/rat)	1.64±0.05 ***	1.46±0.12***	2.12±0.18***	2.26±0.27***
Ext. *C. febrifuga*	(100 mg/kg)	1.64±0.06 ***	1.52±0.11***	2.18±0.11***	2.34±0.08***
	(200 mg/kg)	1.76±0.06***	1.72±0.42**	2.22±0.59**	2.56±0.45***
	(400 mg/kg)	1.78±0.05 ***	1.84±0.44**	2.36±0.30***	2.88±0.09***

Each value represents the mean ± ESM. p<0.05; **p<0.01; ***p <0.001 significant different (Student t-test) versus control group.
Source: Authors

Table 4. Effect of loperamide on faecal parameters in normal rats.

Faecal parameter	Non-constipated rats	Constipated rats
Food consumption	165.42±2.68	18.02±2.00***
water intake	161.16±3.66	16.39±1.37***
Number of faecal pellets	185.53±2.18	5.65±0.24***
Water content of pellets	7.04±0.39	0.82±0.53**
Weight of stool excreted	13.94±0.56	0.69±0.07***
Body weight	221.63±2.57	226.35±0.98**

Each value represents the mean ± ESM. p<0.05; **p<0.01; ***p <0.001 significant different (Student t-test) versus control group.
Source: Authors

Table 5. Effect of aqueous extract of *C. febrifuga* leaves on the constipate rats.

Faecal parameter	Control group (Constipated rats + distilled water)	Constipated rats + Normacol*	Constipated rats + aqueous extract		
			100 mg/kg	200 mg/kg	400 mg/kg
Food consumption	135.81±11.28	159.93±13.37***	202.15±16.82***	221.53±16.37***	238.63±7.27***
water intake	142.03±3.38	281.66±4.78***	288±3.22***	288±3.22***	288.66±4.50***
Number of faecal pellets	00±00	348±5.44***	364±13.17**	393.33±33.86	401.66±41.97***
Water content of pellets	00±00	12.33±0.77***	17.66±1.42**	20±1.18***	22.66±2.06***
Weight of stool excreted	00±00	13.70±1.34***	19.33±1.45***	23.66±1.83***	33.96±2.37***
Body weight	258.87±0.81	252.60±0.94	262±0.89***	262.31±8.77***	262.31±7.47***

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

Table 5. Effect of aqueous extract of *C. febrifuga* leaves on intestinal fluid.

Treatment	Dose	Fluid accumulation
Control group	1 mL/100 g	0.01±0.00
Normacol*	0.5 mg/kg	0.04±0.01**
Aqueous extract of *C. febrifuga*	100 mg/kg	0.04±0.00***
	200 mg/kg	0.05±0.00***
	400 mg/kg	0.06±0.01***

Source: Authors

Table 6. Effect of aqueous extract of *C. febrifuga* in electrolytes secretion.

Treatment	Doses	Na+	K+
Control group	1 mL/100 g	199.38	0.61
Normacol*	0.5 mg/kg	286.37	16.81
Aqueous extract of *C. febrifuga*	100 mg/kg	251.62	25.13
	200 mg/kg	307.20	39.48
	400 mg/kg	357.79	41.67

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

accumulation of intestinal fluid are recorded in Table 5. From this study it is found that Normaco*l as well as aqueous extract of *C. febrifuga* at increasing doses of 100, 200 and 400 mg/kg significantly (p<0.01 and p<0.001) increases the accumulation of intestinal fluid as compared to the control group. The amount of intestinal fluid is 0.01±0.001 for control group, 0.04±0.01 for Normacol*; 0.04±0.00; 0.05±0.00 and 0.06±0.01 for aqueous extract at 100, 200 and 400 mg/kg, respectively. In addition, Normacol* and aqueous extract at increasing doses of 100, 200 and 400 mg/kg also increased the secretion of Na+ ions more than K+ ions (Table 6).

Phytochemical analysis of the aqueous extract of *C. febrifuga* leaves

Phytochemical analysis of the macerated leaves of *C. febrifuga* by tube reactions revealed the presence of anthraquinones, alkaloids, flavonoids, saponosides, tannins, reducing sugars and mucilages (Table 7).

DISCUSSION

The present study was initiated to justify the laxative

Table 7. Phytochemical profile of aqueous extract of *C. febrifuga.*

Chemicals compounds	Result
Antraquinons	+++
Alkoids	+++
Saponosids	+++
Tannins	+++
Oses	++
Mucilage	++

Meaning of symbols: Low presence "+"; Moderate presence "+ +"; High presence "+++"
Source: Authors

properties of *C. febrifuga* used in traditional Congolese medicine before evaluating the laxative effect of the aqueous extract of the leaves of *C. febrifuga*. It is an aim in this study of estimating beforehand acute toxicity in mice in order to determine the LD50 and the therapeutic doses of the extract used. To do this, the OECD guideline 423, which provides reliable guidance on the assessment of toxicity in animals was used (OECD 423, 2001). It appears from this study that the dose of 2000 mg/kg of the aqueous extract of the leaves of *C. febrifuga* did not modify the general behavior of the animals; no mortality was observed. Moreover, at a dose of 5000 mg/kg, the aqueous extract of the leaves of *C. febrifuga* modified the general behavior of the animals, which resulted in a reduction in mobility and drowsiness compared to the control. The decreased mobility and drowsiness observed suggest a sedative effect. In addition to this sedative effect, the aqueous extract at a dose of 5000 mg/kg caused the partial death of the mice with a calculated LD50 equal to 4000 mg/kg. A previous study also showed the toxic effect of the methanolic extract based on the bark of *C. febrifuga* by intra-peritoneal administration. The behavioural signs of toxicity are decreased respiratory rate, inactivity, increased abdominal contractions. The intra-peritoneal LD50 of the extract in mice was estimated to be 774 mg/Kg (Salawu et al., 2008). That is why the doses of 400, 200 and 100 mg/kg corresponding respectively to the tenth (1/10th), twentieth (1/20th) and to the fortieth (1/40th) of the dose of 4000 mg/kg were chosen to evaluate the laxative effect of the aqueous extract of *C. febrifuga* leaves. The laxative effect of the aqueous extract was evaluated in normal rats and in rats constipated by loperamide. It appears from this study that Normacol* (2 mL/rat) and the extract (100, 200, and 400 mg/kg) significantly reduce the time of stool emission; significantly increase ($p<0.05$, $p<0.01$ and $p<0.001$) the frequency and quantity of stool emission compared to the control group. The onset, frequency and quantity of stools excreted are three important parameters for assessing the laxative effect of a drug. The fact that the aqueous extract used (100, 200, and 400 mg/kg) reduced the time for the appearance of stools on the one hand and on the other hand increased the frequency and quantity of stools excreted as the Normacol* (2 mL/rat, reference molecule) suggests a laxative effect of this extract. In fact, Normacol* (sodium dihydrogen phosphate dihydrate) is a rectal laxative whose stool onset is between 10 to 60 min and triggers the defecation reflex by stimulating intestinal activity (Dumont et al., 2010; Faures 2012). Therefore, the effect of the aqueous extract used (100, 200, and 400 mg/kg) was evaluated on loperamide-induced constipation in normal rats. Indeed, the use of loperamide as an inducer of constipation is well known. This inhibition prolongs the purgative time.

This compound possibly exerts its action by disturbing the balance between the absorption of water from the intestinal lumen by active sodium transport (Wintola et al., 2010) and the secretion of water into the intestinal lumen by a mechanism dependent on prostaglandins (Niwa, 2002; Wintola et al., 2010). In addition, the increase in food consumption, water intake, number of faecal pellets, water content of the pellets and weight of the pellets of the animals after the seven days of treatment with the aqueous extract (100, 200 and 400 mg/kg) and Normacol* (2 mL/rat, reference molecule) suggests a laxative effect of the aqueous extract which would act like normacol* or go through an interference with the mechanisms of induction of constipation by loperamide. Some authors have also shown similar effects in the treatment of morphine-induced constipation in rats treated with dietary fiber (Shimotoyodome et al., 2000; Niwa et al., 2002). In addition, the disruption of electrolyte excretion mechanisms in the intestine and the direct effect of the products on the faeces may explain the increase in the frequency and therefore the quantity of stools produced. Therefore, the effect of the aqueous extract on the accumulation of intestinal fluid was evaluated.

The results obtained show that the aqueous extract at the doses used as well as Normacol* significantly increase ($p<0.001$) the accumulation of intestinal fluid and would stimulate the secretion of Na^+, K^+ ions compared to the control group. These results suggest an effect of the aqueous extract on intestinal physiology as would most natural laxatives which exert their effects on the colon epithelium by stimulating Cl- secretion and/or inhibiting Na^+ absorption, leading to accumulation of intestinal fluid and increased intestinal motility (Chatsri et al., 2005). Phytochemically, the laxative effect attributed to this plant could be due to the presence of secondary metabolites contained in the aqueous extract of the leaves of *C. febrifuga* known to have laxative properties such as anthraquinones (Pasricha, 2006), flavonoids (Nikiema et al., 2001), alkaloids and saponosides (Meité et al., 2010).

Conclusion

This work was initiated to justify the laxative properties of *C. febrifuga* used in traditional Congolese medicine. The results show that the aqueous extract of the leaves has sedative properties with high dose toxicity (5000 mg/kg). Moreover, by rectal administration at the doses used (100, 200 and 400 mg/kg), the aqueous extract exhibits laxative properties such as normacol* (reference laxative). Phytochemically, these properties are due to the presence of secondary metabolites highlighted in this plant. These results could justify the use of *C. febrifuga* leaves in the traditional treatment of constipation.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

Bennini A et Merdaci H (2016). Etude de l'effet anti-diarrhéique et apéritif de Nigella Sativa. Mémoire présenté en vue de l'obtention du Diplôme de Master, Université des Frères Mentouri Constantine-Faculté des Sciences de la Nature et de la Vie 71 p.

Bouquet A (1969). Féticheurs et médecines traditionnelles du Congo (Brazzaville), ORSTOM. Paris 36:238-288.

Camara BM (1999). La constipation. Médecine d'Afrique Noire 46(4):38-43.

Chatsri D, Sutthasnee P, Watchareewan T, Nateep K (2005). Barakol extracted from cassa samea stimulates chloride secretion in rat colon, The Journal of Pharmacology and Experimental Therapeutics, 314(2):737-737

Cirillo C, Capasso R (2015). Constipation and Botanical Medicines:une vue d'ensemble. Phytotherapy Research 29(10):1488-1493.

Clarke EGC. (Ed) (1975). Isolation and Identification of Drugs. Pharmacentical Press, London 2:905.

Dimidi E, Christodoulides S, Fragkos KC, Scott SM, Whelan K (2014). The effect of probiotics on functional constipation in adults: a systematic review and meta-analysis of randomized controlled trials. American Journal of Clinical Nutrition 100(4):1075-1084.

Dumont A, Chambin O, Pillon F (2010). The proper use of laxatives, Pharmaceutical News 492:12-25

Faures S (2012). Laxatives, Actualités pharmaceutiques 51(513):49-52.

Johanson JF, Kralstein J (2007). Chronic constipation a survey of the patient perspective. Alimentary pharmacology & therapeutics 25(5):599-608.

Kim JE, Lee YJ, Kwak,M.H, Ko J, Hong JT, Hwang DY (2013). Aqueous extracts of *Liriope platyphylla* induced significant laxative effects on loperamide-induced constipation of SD rats. BMC Complementary and Alternative Medicine 2(13):333.

Meité S, Calixte B, Dodéhé Y, Jacques YD, Djaman JA,'guessan DJN. (2010). Laxative activity of *Mareya micrantha* (Benth) Mull. Arg. (Euphorbiaceae) leaf aqueous extract in rats BMC Complementary and alternative Medicine 10:7 doi:10.1186/1472-6282-10-7

Nikiema JB, Vanhaelen FR, Vanhaelen M (2001). Effects of anti-inflammatory triterpenes isolated from *Leptadenia hastate* latex on keratinocyte proliferation. Phytotherapy Research 15(2):131-134.

Niwa T, Nakao M, Hoshi S, Yamada K, Inagaki K, Nishida M, Nabeshima T (2002). Effectof dietary fiber on morphine-induced constipation in rats. Bioscience, Biotechnology, and Biochemistry 66(6):1233-1240.

OECD (2001). Acute Oral Toxicity-Acute Toxicity Class Method. OECD guideline for the testing of chemicals. OECD N°423, 14 p.

Pasricha PJ (2006). Goodman's and Gilman's. Treatment of motility disorders and water flow. *The pharmacological basis of therapeutics*. 11th edition. NewYork: McGraw-Hill pp. 983-1008.

Salawu OA, Chindo BA, Tijani AY, Adzu B (2008). Analgesic, anti-inflammatory, antipyretic and antiplasmodial effects of the methanolic extract of *Crossopteryx febrifuga.* Journal of Medicinal Plants Research 2(8):213-218.

Shimotoyodome A, Meguro S, Hase T, Tokimitsu I, Satake T (2000). Decreased colonic mucus in rats with loperamide-induced constipation. Comparative Biochemistry and Physiology 126(2):203-211.

Wintola OA, Sunmonu TO, Afolayan AJ (2010). The effect of *Aloe ferox* Mill. in the treatment of loperamide-induced constipation in the Wistar rats. BMC Gastroentérologie 10(1):1-5.

Zimmermann M (1983). Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16(2):109-110.