

## Original Research Article

# Antidiarrheal Activity of Aqueous Extract of the Stem Bark of *Sapium Ellipticum* (Euphorbiaceae)

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### Abstract

**Purpose:** To investigate the antidiarrheal activity of the aqueous extract of the stem bark of *S. ellipticum* (Euphorbiaceae) (AESE).

**Methods:** AESE was prepared by decoction of the powder from the dry stem bark of *S. ellipticum*. Its oral antidiarrheal effect was evaluated *in vivo* at the doses of 5.2, 10.4 and 20.8 mg/kg on castor oil induced diarrhea and on gastro-intestinal transit. AESE was also evaluated *in vitro* (0.125 – 4 mg/ml) on ileal smooth muscle motility. The acute oral toxicity of AESE (5, 10, 15, 20 and 25 mg/kg) was also assessed in mice.

**Results:** AESE significantly and dose dependently delayed the time of appearance of the first stools, decreased the frequency of defecation and the intestinal transit with respective percentage of 319.14, 62.50 and 36.51 % at the dose of 20.8 mg/kg in comparison with negative control (distilled water). AESE, in a concentration-dependent manner, reduced the tone and amplitude of spontaneous contractions of the ileal smooth muscle with EC<sub>50</sub> of 33.29 and 45.43 µg/ml, respectively. None of the doses used in acute toxicity test induced any significant behavioral changes or mortality.

**Conclusion:** These results suggest that AESE possesses antidiarrheal properties mediated at least partially by the inhibition of intestinal motility and may be devoid of acute toxicity.

**Keywords:** *Sapium ellipticum*, Antidiarrheal, Intestinal transit, Spasmolytic, Acute toxicity.

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## INTRODUCTION

Diarrhea can be defined as an alteration in the normal bowel movement, characterized by a situation in which an adult daily stools exceeds 300 g and contains 60 – 95 % water [1]. Diarrhea can cause severe dehydration that can lead to death. In fact, it represents the second most common cause of infant mortality after pneumonia in developing countries, and it is

responsible for the death of about 2 - 4 million children every year, especially in Africa [2].

Although a diarrhea disease control programme (DDC) has been launched by the World Health Organization (WHO), diarrhoea is still a big public health challenge in developing countries. In Cameroon, for example, diarrhea remains the number one killer disease among children under five years, while babies between the ages of 7 - 12 months remain susceptible [3]. In addition,

many rural populations live very far away from health centers, thus limiting their access to medication. In these conditions, medicinal plants appear as an alternative and available health care solution. Hence, some African medicinal plants have been reported for their efficiency in the treatment of diarrhea, thanks to the contribution of many researchers [4].

*Sapium ellipticum* (Hochst.) Pax is a medicinal plant belonging to the family Euphorbiaceae. It is locally called Fai'ngou in Bamoun or Oseimvot in Owondo tribes. The plant is widely spread in the tropical region of Africa but especially found in areas with altitude varying between 1000-2450 m with a mean annual rainfall of about 1200 - 2000 mm [5]. The methanolic extract of *S. ellipticum* was shown to possess antioxidant activity [6]. The decoction of the leaves or the stem bark of *S. ellipticum* has been used in Ethiopia, Congo, Cameroon and Burundi traditional medicine to treat gastrointestinal disorders, particularly diarrhea [7]. To the best of our knowledge, there is no scientific report ascertaining the claimed property, the mechanism of action as well as the toxicity of these extracts. Castor oil-induced diarrhea, activated charcoal meal and isolated intestinal assays are currently used as useful tools for the identification and evaluation of antidiarrheal substances [8]. Thus, the present study sought to evaluate the *in vivo* and *in vitro* antidiarrheal activity of AESE, as well as its acute toxicity.

## EXPERIMENTAL

### Reagents

Diphenoxylate and atropine sulphate were purchased from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany). Charcoal meal (Carbophos, Tradiphar, France) was bought from a local pharmacy.

### Animals

Three months old Swiss albino mice (*Mus musculus*) of both sexes weighing 20-30 g, were used for acute toxicity study whereas Wistar albino rats aged 7-9 weeks and weighing 140-170 g of either sex were used for antidiarrheal activity. These animals were raised in the animal house of the Laboratory of Animal Physiology and Phytopharmacology of the University of Dschang (Cameroon) under natural conditions and had free access to water and food.

The protocols were approved by the Laboratory committee (Laboratory of Animal Physiology and

Phytopharmacology, Department of Animal Biology, University of Dschang, Cameroon) according to the standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive [9].

### Plant collection and extraction

The plant materials of *S. ellipticum* were collected in Fouban (West Region of Cameroon) in September 2009 and identified by Dr G Achoundong at the Cameroon National Herbarium by comparison to an existing voucher specimen number SRFC/49462. The stem bark was washed, cut into small pieces, dried at room temperature and ground into a powder. The obtained powder (500 g) was boiled with 2.5 L of distilled water for 20 min, filtered with a Whatman paper no. 3 and evaporated at 40 °C in a ventilated oven. This procedure yielded 165.5 g of the dry aqueous extract. This extract was dissolved in distilled water prior to administration. Following the prescriptions of the traditional healer, the therapeutic dose was determined to be 10.4 mg/kg. The two other doses were determined by dividing and multiplying the therapeutic dose by a factor of 2.

### Acute toxicity test in mice

In order to study any possible toxic effect or changes in normal behaviour, 6 groups of 10 mice (5 males and 5 females) were used in this experiment. Eighteen hours before the experiment, food was withheld, but animals had free access to water [10]. Five doses of AESE (5, 10, 15, 20 and 25 g/kg) were administered orally as a single bolus. Symptoms such as mobility, aggressiveness, sensitivity to pain, sensitivity to noise, the broadcast of stools and mortality were noticed for four hours post treatment. Animals were kept under observation for 7 days and were monitored daily for changes in body weight, food and water consumption and for any sign of toxicity.

### *In vivo* antidiarrheal activity

#### Castor oil-induced diarrhea in rats

Diarrhea was induced according to the method described by Teke *et al* [11], with some modifications. Animals were fasted for 24 h prior to the experiment, but had free access to water. Rats were randomly assigned to one of the following groups (n=10): Group 1 served as control and received distilled water (10 ml/kg), group 2 received the reference drug, diphenoxylate at a dose of 5.26 mg/kg, groups 3

to 5 received AESE at the respective doses of 5.2, 10.4 and 20.8 mg/kg. All drugs were administered by gavage as a single bolus. One hour after administration of the above drugs, 10 ml/kg of castor oil were orally administered to all groups. Animals were kept in separate metabolic cages with transparent plastic container beneath the cage and lined with Whatmann paper to collect faeces. Following castor oil administration, parameters such as latency time, frequency of defecation, total surface of impregnation and fresh total stools weight were measured for an 8 h period and compared with those of the control. Fresh stools were then dry overnight in an oven to determine water content.

### Gastrointestinal motility test

Gastrointestinal motility test was performed according to the method described by Abdullahi *et al* [2]. 24 h fasted rats were randomly assigned to the following groups: Group 1 received distilled water (10 ml/kg, p.o.), group 2 received Atropine (5 mg/kg, i.p.) and groups 3-5 received AESE at the doses of 5.2, 10.4 and 20.8 mg/kg per os respectively. Immediately after the administration of the above treatments, all animal received 400 mg/kg of charcoal meal per os. One hour after, rats were sacrificed and the small intestine was removed from the pyloric sphincter to the ileo-coecal junction. Thereafter, the distance covered by charcoal was measured and expressed as a percentage of the overall length of the small intestine using the equation below.

Intestinal transit (%) =  $(D/L) \times 100$  ..... (1)

where D = distance covered by charcoal (in meters) and L = intestinal length (in meters).

### *In vitro* antidiarrheal activity

This test was carried out following the protocol described by Wansi *et al* [4]. Rats were fasted for 24 h, sacrificed by cervical dislocation and exsanguinated. The ileum was rapidly removed and free from fats and connective tissue. Fragments of about 1.5 cm were suspended in a 20 ml organ bath containing Tyrode's solution at 37 °C (pH=7.4) and aerated. The composition of the Tyrode solution in mmol was as follow: [NaCl (136.75), NaHCO<sub>3</sub> (11.90), MgSO<sub>4</sub> (1.06), NaH<sub>2</sub>PO<sub>4</sub> (0.42), KCl (3.60), CaCl<sub>2</sub> (1.80) and glucose (5.56)]. An initial tension of 1 g was applied and responses of the fragments were recorded isometrically with a Ugo Basile 7006 isotonic transducer, coupled to a one channel unirecord Ugo Basile 7050 recorder. Fragments were allowed to equilibrate for 60 min during

which the physiological solution was changed every 15 min. After the equilibration period, concentration-response curve for the plant extract (0.125 to 4 mg/ml) was constructed.

### Statistical analysis

Results were expressed as mean  $\pm$  SEM. Statistical differences among the experimental groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey-Kramer as post hoc test. The level of significance was set at  $p < 0.05$ . The data were analyzed using Graphpad prism version 5.0. for Windows XP. EC<sub>50</sub> (concentration that induced 50 % of the maximal activity) was calculated with the same software.

## RESULTS

### Acute toxicity

The results obtained within the 7 days of observation in acute toxicity showed neither mortality, nor any signs of behavioral changes independently of the dose of extract administered. However, at doses of 20 and 25 g/kg, a decrease in locomotion, aggressiveness and sensitivity to the noise was noticed. These changes disappeared within 48 h post treatment. The body weight of mice treated with the aqueous extract of *S. ellipticum* for 7 days did not undergo significant variation as compared with animals of the control group. The average food consumption tended to increase in all batches while the state of the stools remained granulous along the observation period. The evolution of water consumption after administration of a single dose of AESE varies in an irregular manner although a significant reduction was observed only on days 3 (42.69, 38.78 %) and 7 (28.33, 37.34 %) in animals treated with the extract at respective doses of 5 and 10 g/kg as compared with the control (Table 1).

### *In vivo* antidiarrheal activity

As shown in table 2, AESE induces a dose dependent and significant increase of the latency time of the onset of diarrhea (319.14 % at 20.8 mg/kg) and a significant decrease in the frequency of defecation of diarrheal stools compared to the control. Also, the plant extract provoked a significant decrease in a dose-dependent manner on the surface of impregnation, the fresh weight and the water content of stools at all the doses compared with the control.

**Table 1:** Effect of *Sapium ellipticum* on food consumption, water consumption and body weight during acute toxicity

Parameter	Time (day)	Doses (g/kg)					
		Control	5 g/kg	10 g/kg	15 g/kg	20 g/kg	25 g/kg
Food consumption (g/mice/day)	1	20.00 ± 2.21	16.50 ± 1.13	17.37 ± 0.94	17.00 ± 0.35	17.25 ± 0.92	29.75 ± 0.76
	3	25.25 ± 1.61	22.75 ± 0.43	25.87 ± 0.65	26.25 ± 0.47	26.12 ± 0.42	32.62 ± 1.47
	5	26.87 ± 1.42	24.50 ± 0.89	26.87 ± 0.89	28.75 ± 1.98	29.00 ± 0.54	33.75 ± 0.95
	7	34.62 ± 3.35	24.37 ± 1.03	28.37 ± 0.80	29.50 ± 1.51	31.12 ± 0.71	35.95 ± 0.76
Water consumption (ml/mice/day)	1	18.62 ± 2.04	13.12 ± 0.43	14.75 ± 1.03	18.37 ± 0.75	16.75 ± 1.20	17.12 ± 0.43
	3	22.25 ± 3.11	12.75 ± 0.66 <sup>a</sup>	13.62 ± 0.82 <sup>a</sup>	19.00 ± 2.56	18.37 ± 1.56	15.87 ± 0.74
	5	18.50 ± 0.54	13.25 ± 0.92	10.75 ± 0.85	18.62 ± 2.08	18.87 ± 0.80	15.87 ± 0.62
	7	20.75 ± 1.01	14.87 ± 0.42 <sup>a</sup>	13.00 ± 0.41 <sup>c</sup>	18.62 ± 1.91	17.87 ± 0.95	16.87 ± 0.89
Body weight (%)	1	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	3	108.85 ± 1.42	110.61 ± 1.02	114.07 ± 1.81	113.84 ± 1.81	109.94 ± 1.46	109.29 ± 0.96
	5	119.86 ± 2.74	119.91 ± 1.36	123.08 ± 2.87	123.60 ± 1.83	119.50 ± 1.74	121.61 ± 1.58
	7	131.27 ± 4.07	130.17 ± 1.54	137.83 ± 5.21	131.60 ± 1.54	129.06 ± 2.07	133.13 ± 1.67

Values are mean ± SEM (n = 10), <sup>a</sup>p < 0.05; <sup>c</sup>p < 0.01; significantly different with respect to the control

**Table 2:** Effects of the aqueous extract of *Sapium ellipticum* (AESE) on castor oil-induced diarrhea

Treatment	Dose (mg/kg)	Latency time (min)	Frequency of defecation (stools/8h)	Inhibition of defecation (%)	Total surface of impregnation (cm <sup>2</sup> )	Total weight expense of deposit (g)	Water content (%)
Water	10ml/kg	70.62 ± 5.63	4.00 ± 0.50	0.00	98.11 ± 25.39	6.12 ± 0.68	87.10 ± 4.95
Diphe	5.26	147.62 ± 5.28 <sup>a</sup>	1.37 ± 0.46 <sup>c</sup>	65.62	27.85 ± 12.38 <sup>c</sup>	2.07 ± 0.49 <sup>c</sup>	58.66 ± 9.49 <sup>c</sup>
	5.2	113.5 ± 26.12	2.25 ± 0.80 <sup>b</sup>	43.75	56.37 ± 8.34 <sup>b</sup>	3.68 ± 0.39 <sup>c</sup>	69.25 ± 6.34 <sup>a</sup>
AESE	10.4	293.37 ± 0.37 <sup>c</sup>	2.12 ± 0.90 <sup>c</sup>	46.87	37.76 ± 9.52 <sup>c</sup>	3.50 ± 0.90 <sup>c</sup>	64.99 ± 6.54 <sup>b</sup>
	20.8	296.00 ± 21.50 <sup>c</sup>	1.50 ± 0.50 <sup>c</sup>	62.50	18.69 ± 6.89 <sup>c</sup>	2.48 ± 0.43 <sup>c</sup>	54.47 ± 5.04 <sup>c</sup>

Each value represents the mean ± SEM of 10 animals; <sup>a</sup>p < 0.05, <sup>b</sup>p < 0.01, <sup>c</sup>p < 0.001, significantly different compared to negative control group (distilled water); Diphe = Diphenoxylate

**Table 3:** Effect of aqueous extract of the stem bark of *Sapium ellipticum* on gastro-intestinal motility

Treatment	Dose (mg/kg)	Distance travelled by charcoal (cm)	Intestinal transit (%)
Distilled water	10 ml/kg	83.93 ± 4.17	92.24 ± 2.79
Atropine	5	57.88 ± 4.03 <sup>c</sup>	68.67 ± 6.88 <sup>c</sup>
Aqueous extract	5.2	63.79 ± 7.46 <sup>c</sup>	76.22 ± 5.17 <sup>c</sup>
Aqueous extract	10.4	56.24 ± 5.95 <sup>c</sup>	72.44 ± 3.12 <sup>c</sup>
Aqueous extract	20.8	53.29 ± 1.61 <sup>c</sup>	68.99 ± 3.48 <sup>c</sup>

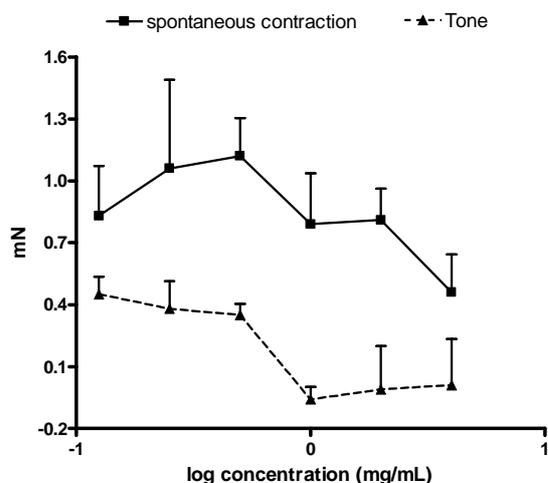
Each value represents the mean ± SEM of 10 animals; <sup>c</sup>p < 0.001 statistically significant compared to negative control group (distilled water)

The extract of *S. ellipticum* caused a dose-dependent reduction of the progression of charcoal meal in the gastro-intestinal tract and also a reduction in the percentage of the intestinal motility. This activity is significant (p < 0.001) at all the doses compared to the control (Table 3).

#### In vitro antidiarrheal activity

AESE tested in vitro on the ileal smooth muscle induced a concentration dependent reduction in both the amplitude of the spontaneous contraction and the tone with respective EC<sub>50</sub> of 45.43 mg/ml and 33.29 µg/ml. The tone of the

muscle was completely abolished in present of extract at the concentration of 1 mg/ml (Fig 1).



**Figure 1:** Effect of the aqueous extract of *Sapium ellipticum* on the tone and amplitude of contraction of the ileum smooth muscle. n = 5; mN = millinewton

## DISCUSSION

The results of acute toxicity showed that the plant extract did not cause any death at the end of the 7 days of experimentation. Moreover, some behavioral modifications that were observed after administration of AESE at higher doses (20 and 25 g/kg), returned to normal after 48h. According to Loomis and Hayes [12], any product with LD<sub>50</sub> higher than 5 g/kg is regarded as non toxic supporting the hypothesis that the extract might not be toxic. The drop in sensitivity and social interaction could be related to a depressive effect caused at the level of the central nervous system as previously mentioned by Atsamo *et al* [10] while using aqueous extract of *Erythrina segalensis*. In addition to the above results, it should be noticed that in the control group and even in all test groups, the state of the stools remained granulous. This could justify the antidiarrheal property of the plant. The variation of body weight is used as an indicator of the harmful or toxic effects of plants' extracts or the active biological molecules [13].

In the present study, AESE does not cause significant variation in body weight, but induced a significant decrease in water consumption at lower doses (5 and 10 g/kg) throughout the experiment and a significant increase in food consumption at the highest dose used. The increase in food consumption could be due to the fact that the extract may contain compounds likely to stimulate food ingestion. Concerning the drop in water consumption, AESE active

compounds might act on the central nervous system by decreasing the activity of the hypothalamus which is known as the regulatory center of thirst. The study of acute toxicity indicated that, AESE could be regarded as a relatively non-toxic substance and therefore could be use as pharmacological agent.

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry, leading to an excess loss of fluid in the feces [14]. The results of this study revealed that AESE produced statistically significant protection against diarrhea, and was found to be comparable to diphenoxylate, a drug widely used against diarrhea disorders which effectively antagonizes diarrhea induced by castor oil, prostaglandin and cholera toxin [15]. Indeed, the oral administration of AESE provoked, like diphenoxylate, a significant dose-dependent increase in the latency time, a significant decrease in the frequency of defecation with a subsequent increase in the percentage of inhibition of defecation in castor oil treated animals.

Diarrhea induced by castor oil results from the action of ricinoleic acid which causes the irritation and inflammation of the intestinal mucosa leading to prostaglandins (PGE<sub>2</sub>α) release. The released PGE<sub>2</sub> stimulates gastrointestinal motility and secretion of water and electrolytes [16], thus inducing an increase in the peristalsis and an intestinal hyper secretion of fluid. The inhibition of prostaglandins biosynthesis prolongs the time of induction of diarrhea by castor oil [17]. In addition to the increase in the latency time and a decrease in the frequency of defecation, the administration of AESE to rats also caused a significant reduction of total fresh weight of deposit, of water content and of the surface of impregnation of deposit. These results are similar to those obtained with diphenoxylate used as standard drug and suggest that AESE might act as diphenoxylate. In fact, the antidiarrhoeal activity of diphenoxylate results from its antispasmodic and antisecretory properties on the intestine [18].

In addition, the administration of the aqueous extract of *S. ellipticum* in rats caused a significant reduction in the progression of charcoal meal and in the intestinal transit time. This activity is comparable to that of atropine used here as reference drug and which is known to reduce intestinal motility [19]. The antidiarrheal effects of AESE could thus result from a reduction of

intestinal motility and an increase in the intestinal absorption of water and electrolytes. In fact, many previous studies have shown that drugs and natural products as well, can induce their antidiarrheal effect through antispasmodic activity [20].

To assess this hypothesis, AESE was evaluated *in vitro* on isolated ileal smooth muscle. Interestingly and consistently, AESE reduced both the amplitude of spontaneous contractions and the tone of the muscle. The effect of AESE was more important on the tone than on the amplitude of contraction. These results confirmed our hypothesis and clearly indicate that the antidiarrheal effects of AESE are due, at least partially to its spasmolytic properties on the intestinal smooth muscle. Previous study of the phytochemical analysis of the aqueous extract of *S. ellipticum* (unpublished work) revealed the presence of tannins, sterols, triterpenes, saponins and of coumarins. Indeed, Longanga *et al* [19] screened a number of medicinal plants and showed that antidiarrhoeal activities of these plants were due to tannins, alkaloids, saponins, flavonoids, steroids, terpenes and glycosides contained in them. The presence of some of these phytochemical constituents in *S. ellipticum* may be responsible for the antidiarrheal effect.

## CONCLUSION

The plant extract contains pharmacologically active substances with antidiarrheal properties. This antidiarrheal activity probably results from the spasmolytic or may be due to a possible antisecretory effect of the plant extract on the intestinal smooth muscle. Thus, this lends some credence to its widespread traditional use by the Cameroon local population as an antidiarrheal agent. The plant seems safe based on the results of acute toxicity testing.

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