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# BIOLOGICAL EFFECT OF THE TOTAL AQUEOUS EXTRACT OF VITELLARIA PARADOXA LEAVES C.F GAERTN ON THE DIGESTIVE TRACT OF THE WISTAR STRAIN RAT

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## ABSTRACT

The antidiarrheal effects of the aqueous extract of *Vitellaria paradoxa* leaves (AEVp) were evaluated at different doses (100; 200 and 400 mg/kg BW) on the one hand, on the number of total and diarrheal stools induced by castor oil, and on the other hand, on the gastrointestinal distance traveled by a charcoal solution, in rats. The results show that AEVp, at doses of 200 and 400 mg/kg, respectively cause a significant inhibition of diarrhea of 77.58 and 77.95% of the number of diarrheal stools, compared to the control group. At the maximum dose, AEVp significantly reduces the severity of diarrhea, the volume and weight of diarrheal stools, and the secretion of electrolytes $Na^+$, $K^+$, $Cl^-$ and $Ca^{2+}$. These observations are similar to those obtained in the group of rats treated with loperamide, the reference anti-diarrheal drug. We also observe a significant reduction of 57.52% in the gastrointestinal distance induced by AEVp at the maximum dose of 400 mg/kg BW compared to the total length of the intestine. The action of atropine is similar to that of AEVp. The aqueous extract of *Vitellaria paradoxa* leaves would exert a dose-dependent anti-diarrheal effect. This effect is reflected on the one hand by a significant decrease in the number of total and diarrheal stools and on the other hand by a tendency to reduce gastrointestinal mobility. Our extract would induce a reduction in peristalsis and a decrease in intestinal secretion. The effects observed with AEVp could be due to the chemical compounds contained in *Vitellaria paradoxa* leaves.

**KEYWORDS:** Stools - Diarrhea – Gastrointestinal mobility – Rats - *Vitellaria paradoxa.*

## INTRODUCTION

According to Ambé *et al*. (2015), diarrhea is a bowel disorder characterized by loose or even liquid stools, occurring in abnormally large quantities or with increased frequency, on the order of several times per day. The most common cause is gastrointestinal infection by various types of bacteria (cholera, *Escherichia coli*), viruses (rotavirus), and parasites (WHO, 2006).

Diarrheal diseases cause approximately 1.8 million deaths each year worldwide, 90% of which are children under the age of five, mostly living in developing countries (Cazaban *et al*., 2005). Diarrhea is now the third leading cause of death from infectious diseases (Assogba *et al*., 2012) and the fifth leading cause of premature death worldwide (WHO, 2014). Africa accounts for 78% of deaths worldwide, and in Ivory Coast this disease causes approximately 1.1 million deaths per year (WHO, 2014).

In Africa, particularly in Ivory Coast, medical treatment for diarrhea is limited by the inaccessibility of certain populations to health centers and the high cost of conventional medicines (INS, 2006). These reasons have led most Ivorian populations to resort to medicinal plants to treat several illnesses, particularly diarrhea. It is therefore essential to conduct scientific research on anti-diarrheal plants in order to provide populations with improved phytomedicines that are available and at a lower cost (Dosso *et al*., 2012).

It should be noted that very few studies are available on plants sold in urban markets and used in the traditional treatment of diarrhea. This is the case of the species *Vitellaria paradoxa*, commonly used in the treatment of diarrhea by Ivorian populations through its leaves.

The main objective of this study was therefore to evaluate the potential antidiarrheal properties of the aqueous extract of *Vitellaria paradoxa* leaves.

## MATERIALS AND METHODS

### Plant Material

The plant material used consisted of *Vitellaria paradoxa* leaves.

**Animal Material**

Thirty-six normal white albino rats of the *Wistar strain* were used. They were obtained from the animal facility of the Jean Lorougnon Guédé University. They weighed between 161 and 211 g. The animals were divided into five groups of six rats each and acclimated in cages for one week prior to the experiment. The animals were fed pellets and water ad libitum and fasted for 24 hours before the experiment.

**Extraction of *Vitellaria paradoxa* leaves**

The method of N'guessan *et al*. (2024) has been used. The harvested *Vitellaria paradoxa* leaves are washed with flowing water for ten minutes and dried in the shade at room temperature for one week. These leaves are then crushed and pulverized to carry out the extraction. One hundred (100g) grams of this powder were extracted in one and a half liters of distilled water by a 15-minute decoction. The decoction obtained was filtered through cotton and then through Whatman filter paper; The residue obtained was taken up again in one liter of distilled water and also underwent a 15-minute decoction. The filtrate obtained was evaporated at 65 °C and dried in an oven at 50 °C. A blackish-green powder is obtained which will be used to prepare the total aqueous extract of *Vitellaria paradoxa* leaves for studies of diarrhea induction and gastrointestinal motility.

**Preparation of Chemical Solutions**

Loperamide (Ciloper®) at 5 mg/kg body weight is obtained by dissolving 15 mg of loperamide in 20 ml of distilled water. The 5% charcoal solution is obtained by dissolving 5 g of charcoal in 100 ml of distilled water with 3% gum arabic (dissolving 3 g in 100 ml of distilled water). Atropine at 5 mg/kg body weight is obtained by dissolving 15 mg of atropine in 20 ml of distilled water.

**Induction of Diarrhea**

Thirty (30) rats are divided into five (5) groups of six (6). All solutions are administered by gavage. First, we administered 2 ml of saline solution to group 1, which is the control. Then, to the standard group 2 (positive control), we administered 2 ml of loperamide at a dose of 5 mg/kg body weight. Finally, to the test groups 3, 4 and 5, we administered 1 ml of TAEVp (total aqueous extract of *Vitellaria paradoxa*) respectively at doses of 100, 200 and 400 mg/kg BW. After sixty (60) minutes, 2 ml of castor oil was administered to all rats. Normal and diarrheal droppings were collected after one hour on plastics supported by white cloths. First, the severity of diarrhea was determined in the different groups followed by counting the

droppings. Subsequently, we quantified the volume of the droppings which were weighed first. The droppings are finally dried and weighed again to determine the amount of water (N'guessan et al, 2020). Biochemical studies are carried out to determine the amount of Na+, K+, Ca2+ and Cl- ions in the droppings (Dosso *et al*., 2012).

Total number of stools = normal stools + diarrheal stools. The inhibition rate of the number of diarrheal stools was determined using the formula used by Awouters *et al*. (1978):

$$A = Ct - Cd / Ct \times 100$$

A = percentage inhibition, Ct = Number of diarrheal stools in the control group, Cd = Number of diarrheal stools in the test group.

Diarrhea severity was determined using the following formula used by Dosso *et al*. (2012):

$$S = Cd/Ct \times 100$$

S = Severity of diarrhea Cd = Number of diarrheal stools Ct = Total number of stools

Water content was determined using the following formula used by Méité *et al*. (2009):

$$\text{Water content (\%)} = MH - DM / MH \times 100$$

MH: mass of wet droppings DM: mass of dried droppings

**Gastrointestinal Motility Study**

Thirty (30) rats were divided into five (5) groups of six (6). All solutions were administered by gavage. We first administered 2 ml of saline solution to group 1, which was considered the control. Then, we administered 1 ml of atropine 5 mg/kg BW to group 2, the reference group (positive control). Finally, to the three test groups 3, 4, and 5, we administered 1 ml of EAVp at doses of 100, 200, and 400 mg/kg BW, respectively. Half an hour after the administration of the first solutions, we administered 1 ml of the charcoal solution (5% charcoal and 3% gum arabic) to all rats (Garido *et al*., 2024). Thirty minutes later, the rats were sacrificed and the intestines were removed. The small intestine was removed from the pylorus to the cecum. The total length (L) of each intestine as well as the distance traveled by the coal (l) in the intestine were measured with a measuring tape. The percentage of distance traveled by charcoal relative to the total length was calculated using the following formula used by Dosso *et al*. (2012):

$$P = l / L \times 100$$

P = percentage of charcoal transit l = distance traveled by charcoal L = total intestinal length

The percentage of inhibition was calculated from the average length traveled by activated charcoal for each group of rats, using the following formula: Dosso *et al*. (2012):

$$P = D1-D2 / D2 X 100$$

P = percentage of inhibition, D1 = average distance of activated charcoal for the control group and D2 = average distance of activated charcoal for the treated group.

**Statistical Analyses**

In both pharmacological experiments, results are expressed as means ± SEM (Standard Error of Mean). Comparison of means across batches was possible using the Student t-test. A difference is considered significant when $p < 0.05$. Graphpad Prism 8 and Excel software were used to perform these statistical tests.

## RESULTS

**Study of Diarrhea Induction with Castor Oil**

**Effects of TAEVp on the Number of Feces Emitted by Rats**

The total aqueous extract of Vitellaria paradoxa leaves (TAEVp) significantly reduced diarrheal feces at doses of 200 and 400 mg/kg BW. Only 4.17 ± 0.60** and 4.1 ± 1.08** remained at doses of 200 and 400 mg/kg BW, respectively, compared to the control group, which was 18.6 ± 0.76. EAVp inhibited castor oil-induced diarrhea by 7.52%, 77.58%, and 77.95% at doses of 100, 200, and 400 mg/kg BW, respectively. Our extract has the same kinetics of action as the reference product, loperamide. The results are shown in Table 1.

**Table 1: Effect of TAEVp on the number of droppings in rats treated with castor oil.**

| Treatments | Normal droppings | Diarrheic droppings | Inhibition of diarrhea (%) |
|---|---|---|---|
| Saline water (0,09% NaCl) | 1,67 ± 0,11 | 18,6 ± 0,76 | -- |
| Loperamide (5 mg/kg) | 11,67 ± 0,32* | 3,6 ± 0,77** | 80,64 |
| TAEVp 100 mg/kg | 5,17 ± 0,46 | 17,12 ± 1,5 | 7,52 |
| TAEVp 200 mg/kg | 8,11 ± 0,31* | 4,17 ± 0,60** | 77,58 |
| TAEVp 400 mg/kg | 10,21 ± 0,42* | 4,1 ± 1,08** | 77,95 |

Results are expressed as means ± SEM, * $p < 0.05$; ** $p < 0.01$ compared to the control group

**Effects of EAVp on Diarrhea Severity**

The severity of diarrhea was very high in rats treated with saline, reaching 91.76%. Diarrhea severity decreased very rapidly with TAEVp at doses of 76.80%, 33.95%, and 28.65%, respectively, at doses of 100, 200, and 400 mg/kg BW. Loperamide also decreased diarrhea severity by 23.57%. Table 2 below presents the results.

**Table 2: Effect of TAEVp on the severity of diarrhea in rats treated with castor oil.**

| Treatments | Normal droppings | Diarrheic droppings | Severity of diarrhea (%) |
|---|---|---|---|
| **Saline water (0,09% NaCl)** | 1,67 ± 0,11 | 18,6 ± 0,76 | 91,76 |
| **Loperamide (5 mg/kg)** | 11,67 ± 0,32* | 3,6 ± 0,77** | 23,57 |
| **TAEVp 100 mg/kg** | 5,17 ± 0,46 | 17,12 ± 1,5 | 76,80 |
| **TAEVp 200 mg/kg** | 8,11 ± 0,31* | 4,17 ± 0,60** | 33,95 |
| **TAEVp 400 mg/kg** | 10,21 ± 0,42* | 4,1 ± 1,08** | 28,65 |

Results are expressed as means ± SEM, * $p < 0.05$; ** $p < 0.01$ compared to the control group

**Effects of TAEVp on the Volume and Water Mass of Rat Feces**

**Effects of TAEVp on Fecal Volume**

One hour after castor oil administration, fecal excretion was quantified in small test tubes. The control group in the TAEVp studies had a higher fecal volume (4.1 ± 0.13 mL). This volume was significantly reduced to 2.5 ± 0.03 and 1.80 ± 0.13 mL, respectively, in rats treated with doses of 200 and 400 mg/kg BW of our plant extracts. The reduction in fecal volume was dose-dependent. Loperamide reduced fecal volume to 1.54 ± 1.16 mL. Figure 1 below shows the results.

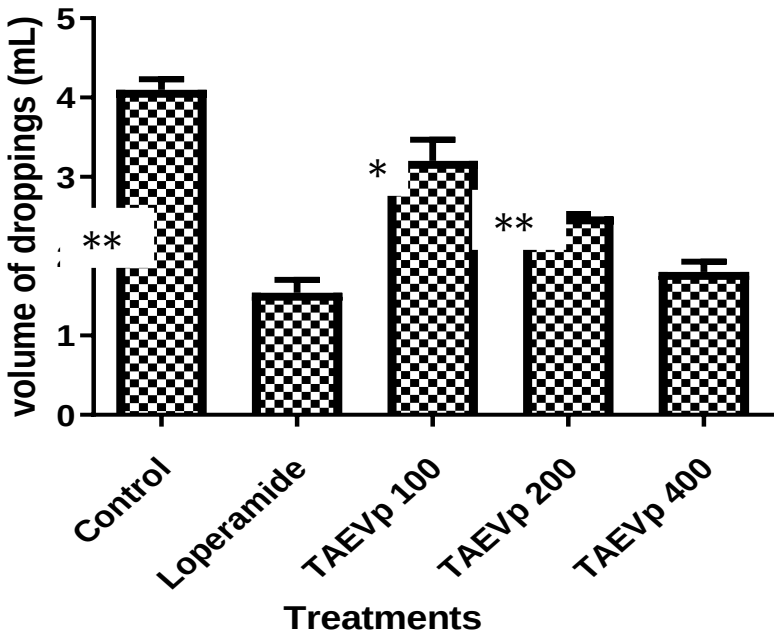


**Figure 1: Changes in fecal volume in diarrheal rats treated with TAEVp.**

Results are expressed as means ± SEM, *$p < 0.05$; **$p < 0.01$ compared to the control group.

**Effect of TAEVp on Fecal Water Mass**

One hour after castor oil administration, fecal masses were assessed using an electric scale. In studies with TAEVp, the control group had a water mass of 3.5 ± 0.11 g. This water mass was significantly reduced to 0.61 ± 0.20 g in the group of rats treated with loperamide and to 1.1 ± 0.16 g in the group treated with TAEVp p at 400 mg/kg BW. The amount of water remaining was only 2.4 ± 0.11 g and 2.2 ± 0.12 g, respectively, in rats treated with doses of 100 and 200 mg/kg BW of TAEVp. The reduction in water mass by TAEVp was dose-dependent. Figure 2 below shows the results.

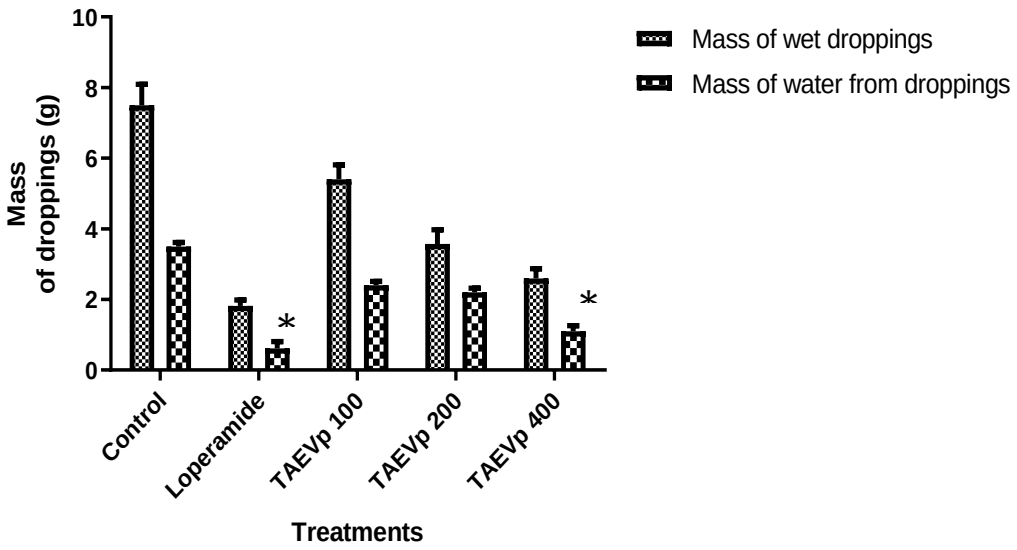


**Figure 2: Evolution of fecal water masses in diarrheic rats treated with TAEVp. Results are expressed as means ± SEM, * p < 0.05 compared to the control group.**

**Study of the effect of TAEVp on electrolyte secretion (Na+, K+, Cl-, and Ca2+)**

**Effect of TAEVp on sodium secretion**

The results show that the inhibition of sodium secretion by TAEVp is dose-dependent. The sodium concentration in rats treated with saline was 6.1 ± 0.21 mg/L. Loperamide, the reference product, significantly inhibited sodium ion secretion with a concentration reduced to 3.4 ± 0.4 mg/L. The extract at a dose of 400 mg/kg BW significantly inhibited sodium ion secretion, leaving only 3.6 ± 0.32 mg/L. Figure 3 below presents the results.

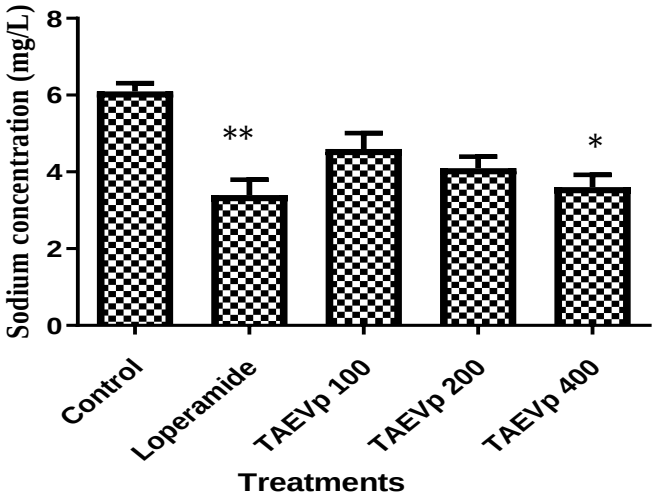


**Figure 3: Evolution of sodium concentration in diarrheal rats treated with TAEVp. Results are expressed as means ± SEM, * p < 0.05; ** p < 0.01 compared to the control group.**

Effect of TAEVp on Potassium Secretion Potassium concentration in rats treated with saline was 1.1 ± 0.03 mg/L. Loperamide significantly inhibited potassium secretion with a value of 0.25 ± 0.03 mg/L. For the extracts, the inhibition of potassium ion secretion was dose-dependent. Potassium concentration decreased significantly and corresponded to 0.5 ± 0.04; 0.49 ± 0.02 and 0.31 ± 0.03 mg/L for doses of 100, 200 and 400 mg/kg, respectively. Figure 4 below shows the results of this study.

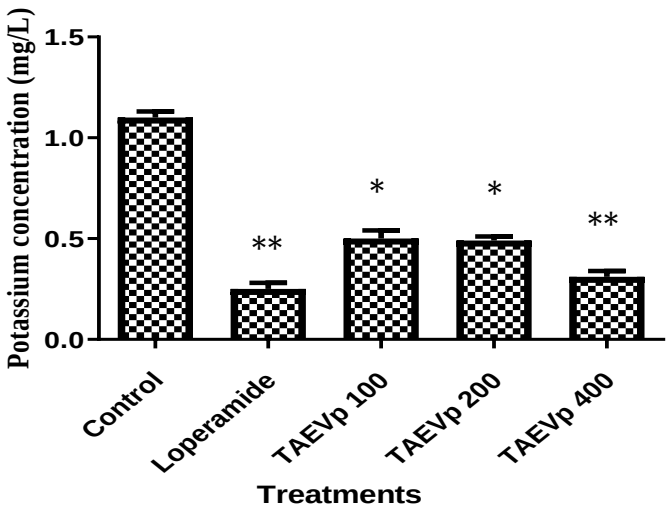


**Figure 4: Evolution of potassium concentration in diarrheal rats treated with TAEVp. Results are expressed as means ± SEM, * p < 0.05; ** p < 0.01 compared to the control group.**

**Effect of TAEVp on Chlorine Secretion**

The chlorine concentration in rats treated with saline water is very high, at 27.2 ± 0.4 mg/L. Chloride ion secretion is significantly inhibited in rats treated with loperamide, at 14.4 ± 0.5 mg/L. The inhibition of chlorine secretion is dose-dependent. The extract, at the maximum dose of 400 mg/kg BW, inhibits the secretion of chloride ions, the concentration of which remains at only 15 ± 0.4 mg/L. This inhibition is not significant at doses of 100 and 200 mg/kg. Figure 5 below presents the results of the study.

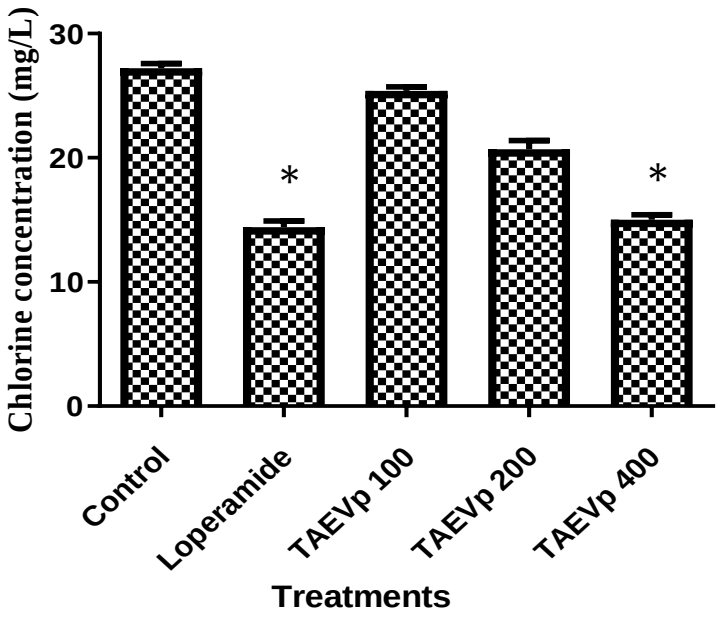


**Figure 5: Evolution of chlorine concentration in rats treated with castor oil. Results are expressed as means ± SEM, * p < 0.05 compared to the control group**

**Effect of TAEVp on Calcium Secretion**

The saline-treated rat group had a high calcium ion concentration of 0.95 ± 0.02 mg/L. In the loperamide-treated rat group, this concentration decreased significantly to 0.3 ± 0.02 mg/L. The inhibition of calcium ion secretion by the extract was significant and dose-dependent. Calcium ion concentrations decreased to 0.4 ± 0.03, 0.36 ± 0.02, and 0.32 ± 0.02 mg/L at doses of 100, 200, and 400 mg/kg BW, respectively. Figure 6 below presents the results of the study.

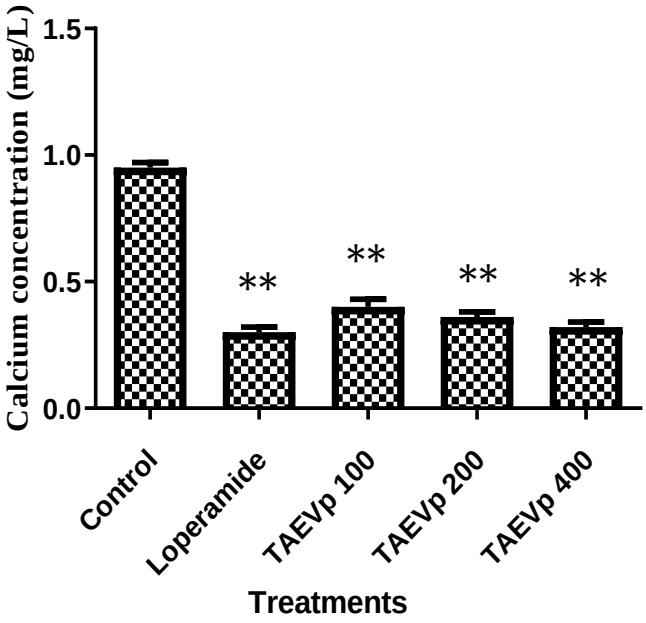


**Figure 6: Evolution of calcium concentration in rats treated with castor oil. Results are expressed as means ± SEM, * p < 0.05; ** p < 0.01 and *** p < 0.001 compared to the control group.**

**Effect of TAEVp on Gastrointestinal Mobility in Rats**

The results obtained in this study show that the distance traveled by charcoal is greater in control rats (111.6 ± 2.3 cm) and corresponds to 92.46% of intestinal transit compared to the total intestinal length. This distance decreases significantly in atropine-treated rats, leaving only 29.97%, or 35.4 ± 2.4 cm, of intestinal transit. The inhibition of intestinal transit by TAEVp is dose-dependent. A decrease in intestinal transit is observed with TAEVp at 100 mg/kg BW, but this decrease is not significant. On the other hand, TAEVp at doses of 200 and 400 mg/kg BW inhibited intestinal transit by respective percentages of 50.35 and 57.52%, compared to the control group with distances traveled by the coal of 55.4 ± 2.4 and 47.4 ± 2.2 cm. The results are presented in Table 3 and Figure 7.

**Table 3: Effect of TAEVp on gastrointestinal motility.**

| Treatments | Total length (cm) | Distance traveled by coal (cm) | Transit percentage (%) | Inhibition (%) |
|---|---|---|---|---|
| **Saline water (0,09% NaCl)** | 120,7 ± 1,2 | 111,6 ± 2,3 | 92,46 | - |
| **Atropine (5 mg/kg)** | 118,1 ± 1,4 | 35,4 ± 2,4** | 29,97 | 68,27 |
| **TAEVp 100 mg/kg** | 118,3 ± 1,6 | 87,4 ± 3,2 | 73,88 | 21,68 |
| **TAEVp 200 mg/kg** | 119,4 ± 2,1 | 55,4 ± 2,4* | 46,39 | 50,35 |
| **TAEVp 400 mg/kg** | 118,6 ± 1,3 | 47,4 ± 2,2* | 39,96 | 57,52 |

Results are expressed as means ± SEM, * p < 0.05; ** p < 0.01 compared to the control group

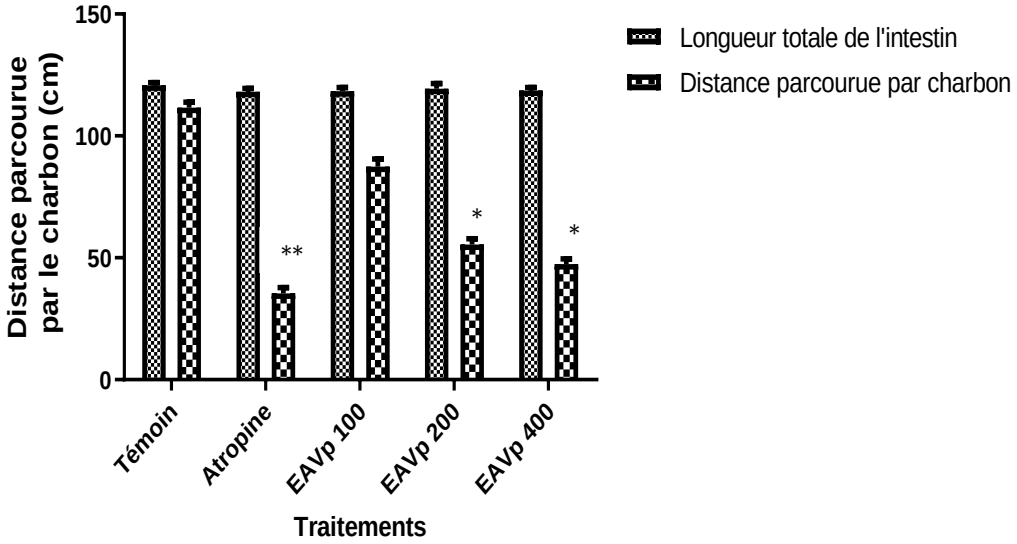


**Figure 7: Evolution of the intestinal distance traveled by charcoal in rats treated with TAEVp. Results are expressed as means ± SEM, * p < 0.05; ** p < 0.01 compared to the control group.**

## DISCUSSION

Several animal models for studying the antidiarrheal activity of plants are possible with different pharmacological agents such as castor oil (Dosso *et al*., 2012; N'guessan *et al*., 2020), magnesium sulfate (Mujumdar et al., 2005), or serotonin (Rouf et al., 2003). In our study, the castor oil diarrhea induction model (the most commonly used) was chosen because its diarrhea induction mechanism is more similar to acute secretory and infectious diarrhea, which is very common in Africa (Abdullahi *et al*., 2001).

According to Wendel *et al*. (2007), castor oil-induced diarrhea is due to hypermotility of the gastrointestinal tract and hypersecretion of the intestinal mucosa. Ammon *et al*. (1974) reported that hydrolysis of castor oil releases ricinoleic acid, which causes irritation and

inflammation of the intestinal mucosa. This inflammation is responsible for the release of prostaglandins according to Galvez *et al*. (1993), which contribute to pathophysiological functions in the gastrointestinal lumen. The released prostaglandins also stimulate gastrointestinal motility and secretion, thereby preventing the reabsorption of NaCl and H2O (Galvez *et al*., 1993; Dosso *et al.*, 2012). Castor oil therefore induces diarrhea by increasing the volume of intestinal contents by preventing intestinal reabsorption (Chitme *et al*., 2004). Also, the released fatty acid increases peristaltic activity and hypersecretion by promoting the exchange of electrolytes and water in the intestinal membrane (Mujumdar *et al*., 2005). Secretory diarrhea caused by castor oil would also be due to the activation of sodium channels leading to a massive secretion of sodium ions and water (Chitme *et al*., 2004).

For the study of diarrhea induction, our results showed that the aqueous extract of *Vetellaria paradoxa* leaves (TAEVp) causes a significant inhibition of diarrheal droppings. These are consistent with literature data obtained for other plants (Gabriel *et al.*, 2004; N'guessan *et al*., 2020). Diarrhea is clinically apparent in all rats. TAEVp significantly reduces diarrhea severity, water content, and dropping mass at doses of 200 and 400 mg/kg BW. Also, TAEVp significantly reduces the secretion of Na+, K+, Ca2+, and Cl- ions at doses of 200 and 400 mg/kg BW. These results are consistent with those obtained by (Kouitcheu *et al*., 2006; Dosso, 2014). Our studies also showed a significant reduction in gastrointestinal distance induced by EAVp at doses of 200 and 400 mg/kg BW compared to the control group.

These results are consistent with studies by authors such as Rouf *et al*. (2003); Chitme *et al*. (2004); and Méité *et al*. (2009) with other plant extracts.

Phytochemical studies conducted by Kamagaté *et al*. (2021) showed the presence of tannins, polyphenols, sterols, anthraquinones, alkaloid polyterpenes, and saponins in *Vitellaria paradoxa* leaves. The work of Houekou *et al*. (2016) demonstrated the antidiarrheal properties of tannins and saponins. Also, diarrhea inhibition was attributed to polyphenols, polyterpenes, and saponins in the studies of Biswas *et al*. (2013).

According to the studies of Méité *et al* (2009), reported by Dosso *et al*. (2012), tannins would denature proteins by the formation of tannate proteins which would make the intestinal mucosa more resistant and reduce secretion. Other studies such as those of Dosso *et al*. (2012) have shown that galloyl-tannins (RG-tannin) inhibit cholera toxins which are found in the accumulation of ileal fluid of rabbits or mice. The inhibition of the cytochrome P450

system is attributed to the anti-diarrheal property of polyphenols. This action has an impact on the drugs metabolized by this system according to N'guessan *et al*. (2020).

## CONCLUSION

This study shows that *Vitellaria paradox*a possesses antidiarrheal effects, significantly inhibiting both castor oil-induced diarrhea and gastrointestinal motility. This observed antidiarrheal and antimotility activity is believed to be due to the chemical constituents contained in *Vitellaria paradoxa* leaves. This work therefore provides scientific justification for the traditional use of Vitellaria paradoxa leaves in the treatment of diarrhea.

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