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Antidiarrheal and antioxidant activities of *Ajuga iva* (L.) leave extract

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ABSTRACT

We studied the effect of *Ajuga iva* leaves extract (AIE) on the intestinal absorption, motricity and its antioxidant capacity against diarrhea. Wistar rats were divided and received either: castor oil (CO), CO and loperamide or CO and different doses of AIE. AIE prevented dose-dependently CO-induced diarrhea. AIE at 800 mg/kg showed inhibition efficiency on defecation and diarrhea. The pro-oxidant effect of the CO in the small intestine was inhibited significantly in presence of AIE: increasing glutathione peroxidase (GPx) activity and lowering oxygen free radicals (OH°, O_2°-), carbonyl protein and malondialdehyde (MDA) levels. However, co-administration of AIE in castor oil-exposed groups significantly increased the intestinal contents of calcium and magnesium. AIE exhibits significant anti-diarrheal activity, related in part to its antioxidant properties. Our investigation also provides experimental evidence for the traditional use of this medicinal plant in the treatment of diarrhea.

1. Introduction

Diarrhea is a physio-pathological mechanism which results in the emission of an important volume of saddles (more than 300 g/day for an adult) [1]. The feces are soft or liquid, which can induce a severe dehydration and even death at the time of the cholera epidemics [2]. According to the World Health Organization [3], diarrhea is the second cause (after pneumonia) of infant mortality [4], with 1.7 billion case each year in the world and 1.5 million deaths annually among children under age five in the third world countries [5]. Diarrhea seems to be a challenge for the scientific community. The main causes of acute diarrhea are the infection by rotavirus, which induces a severe dehydration among infants and young children [6], food poisonings, colitis and other bacterial infections that require immediate hospitalization. Diarrhea is said to be chronic when it persists more than one month [7]. It can be caused by infectious (Whipple) or inflammatory (Crohn) bowel diseases [8], ingestion of drugs having a laxative effect, or acceleration of the intestinal motility due to a stress or a subjacent pathological syndrome.

Today the world is suffering from increasing the resistance of currently used therapeutic agents to various common pathogens. This is why scientists resort to the discovery of drugs based on natural products mainly from medicinal plants used as traditional treatment for various diseases. These used herbs are multi-constituent safe medications. Plants are being recognized as potential sources of drug

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discovery and modern drugs are derived directly from natural sources [9]. Medicinal plants contain high amounts of active biomolecules such as: polyphenols, flavonoids, tannins, enzymes, vitamins and alkaloids. The hepatoprotective [10], neuroprotective [11], anxiolytic [12], anti-diabetic [13], anticancer [14,15] and anti-microbial [16], anti-viral [17] and anti-inflammatory [18] and antioxidant [19] activities have been reported [19].

Recently, several plants have been shown to have powerful anti-diarrheal properties such as *Moringa stenopetala* [20], *Ocimum Lamiifolium* [21], *Boswellia serrata* [22] and *Opuntia Ficus indica* [23].

However, other plants such as *Molineria capitulata*, *Holigarna*longi folia and *Steudnera colocasii folia*, had weak anti-diarrheal potentials, in spite of the abundance of antioxidant and anti-inflammatory biomolecules and the existence of interactions between the plant extracts and digestive tract enzymes [11,17,24]. Moreover, other plants induced laxative effects such as *Prunus persica, Planta goovata, Cassia auriculata* d other plants [25]. an.

The oxidative stress is an aggressive physiological imbalance of the cell components due to an increase of reactive oxygen species (ROS). ROS are in general coming from exogenous and harmful substances such as tobacco, alcohol, drugs or castor oil (CO). These substances could induce irreversible cellular damages in the long term [26]. Oxidative stress may be evaluated by numerous parameters such as antioxidant enzyme activities like superoxide dismutase (SOD) and glutathione peroxidase (GPx), free oxygen radicals (O_2°, OH°), carbonylated protein (CP) and malondialdehyde (MDA) levels [27].

Ajuga iva (L.) is a Mediterranean basin plant [28] known in North Africa for its various therapeutic properties [29]. Rich in antioxidant polyphenolic compounds [30], this panacea was the object of various studies on its pharmacological role as a psychotropic [31], analgesic [32], hypoglycemic [33], antioxidant [34] and Vaso-relaxant [29],[35] plant. It is described as a "cure-all plant" [36]. *Ajuga iva* (*Ai*) is considered to promote the birth of male children while improving the fertility [37]. It was also reported that this plant was traditionally used in certain Maghreb villages to fight diarrhea [29]. However, to our knowledge, no study has yet evaluated its real potential as an anti-diarrheal capacity. The present study was conducted to evaluate the ability of the hydro-methanolic leave extract of *Ajuga iva* on castor oil-induced diarrhea, searching for effective and secure remedies against this disease. In this context, we evaluated some physiological parameters such as: the number and the weight of the defecations, the time of appearance of the diarrheal state, and biochemical parameters of diarrhea such as oxidative stress markers.

2. Materials and methods

2.1. Chemicals

All chemicals were of high grade and obtained from Sigma Aldrich, (St Louis, US-MI). Solvents were from Panreac (Barcelona, ES). Castor oil was supplied by Siphat (Tunis, TN), loperamide (Diaretyl®) was from Pasteur Institute (Tunis, TN).

2.2. Plant material and preparation of the extract

Ajuga iva was collected in March 2020 from Ain Draham (North Tunisia). A specimen was identified and authenticated by Dr Dalila Chabane (Departmentof Biology, University Saâd Dahlab-Blida, Algeria). Leaves were oven-dried at 37 °C and grounded in an electric blinder. Powder (50g) was homogenized in 300 ml of methanol and maintained on a magnetic stirring (24h, 25 °C). The mixture was centrifuged (4500 rpm/10 min) and the upper layer was lyophilized. The AIE yielded 1.06 g of yellow oily residue with a percentage yield of 2.12 % and was stored at −21 °C for further analysis.

2.3. Animals

Non pregnant female Wistar albino rats (150–200g) were purchased from Pasteur Institute (Tunisia) and cared for in compliance with the code of practice for the Care and Use of Animals for Scientific Purposes (Approval number: LNFP/Pro 152012). All animal procedures were approved by the local ethic committee of the Science Faculty of Bizerte (Ref 2021210). Rats had been given a nutritionally standard diet (rat food Nutrimix Esparto, El Badr Company, Utique-Bizerte, TN), with free access to tap water *ad libitum* and kept under controlled temperature (22 ± 2 °C) and 12/12 h light/dark cycle. To avoid interactions between sexual hormone fluctuations and digestive tract response, all the females were checked before treatment and chosen in diestrus phase [38].

2.4. Evaluation of antidiarrheal activity

2.4.1. Induction of diarrhea

Diarrhea was induced by oral administration of CO (15 ml/kg) [39]. Before experiments, rats were fasted for 18 h (absence of food but presence of water with isolation of the feces) [40] and divided in a random manner into 6 groups of 7 rats each. The day of the experiment, animals were treated by gavage as follows.

I Groups 1 (Control): received distilled water (15 ml/kg) after 30 min received the second gavage (distilled water).
II Castor Oil group (CO): received distilled water 30 min later they were individually treated with 15 ml/kg of castor oil.
III Group 3 (Loperamide): each animal treated with 10 mg/kg (*p.o.*) of loperamide (Lop), a standard anti-diarrheal agent; 30 min later they received 15 ml/kg of castor oil.

IV Groups 4–6 received orally increasing doses of AIE (200, 500 and 800 mg/kg). Half an hour after oral pretreatment, animals were treated again: every rat received 15 ml/kg of CO orally.

Thereafter, rats were individually separated in cages containing a water bottle only and then observed for 7h in order to analyze these following diarrheal parameters: the time elapsed between the CO administration and the excretion of the first diarrheal feces, the total number of fecal out-put, the number of diarrheal stools excreted by each animal in 7 h as well as the total weight of diarrheal feces [41]. The percentage of defecation and diarrhea drops inhibition were performed according to the following formula respectively [42]:

$$\%\ \text{Inhibition of Defecation (IDe)} = ([\text{CG-SG}] / \text{CG})\ \text{x}\ 100$$

Where CG is the average of defecations induced by CO and SG the average of defecations caused by the sample.

$$\%\ \text{Inhibition of Diarrhea (ID)} = ([\text{DC-DS}] / \text{DC})\ \text{x}\ 100$$

Where DC is the average of wet stools induced by CO and DS the average of wet stools caused by the sample.

2.4.2. Intestinal fluid accumulation

After 7 h of observation, animals were sacrificed by decapitation. The small intestine was removed and weighed to quantify the intestinal content.

2.5. Biochemical analysis

Small intestine pieces (0.4 g each), were grounded in 4 ml of Tris Buffer Saline (TBS) solution (pH 7.4), homogenized on ice using a T 25 Ultra-Turrax homogenizer and centrifuged at 4 °C (9000 g/10 min). The supernatants were collected and used for further biochemical analyses. The protein content was determined according to **Lowry et al**, [43].

2.5.1. Oxidative damages

Tissue carbonylated proteins (CP) content was performed according to **Levine et al.,** [44]. The sample absorbance was measured at 366 nm. While MDA content was evaluated according to the method described by **Buege and Aust** [45]. Absorbance was recorded at 530 nm.

2.5.2. Enzymatic biomarkers

SOD activity was quantified following the procedure of **Misra and Fridovich** [46]. The sample absorbance was measured at 480 nm for 5 min. GPx activity was estimated using the method of **Sazuka *et al*** [47] and was monitored for 1 min at 412 nm.

2.5.3. Oxygen free radicals

$O_2^{\circ-}$ concentration was registered by the method of **Marklund & Marklund**, [48] with an absorbance measured at 420 nm. OH° concentration was evaluated according to the method described by **Castro and Freeman** [49] and the absorbance was measured at 532 nm.

2.5.4. Bivalent minerals

Calcium concentration was determined according to **Stern Lewis** [50]**,** using a commercial kit (Biomaghreb, Ref 20054). The absorbance was recorded at 570 nm.

Magnesium concentration was determined according to the method of **Gindler and Heth** [51] with a commercial kit (Biomaghreb, REF 2007). The absorbance was recorded at 520 nm.

2.6. Statistical analyses

The data were expressed as means ± standard error of the mean (S.E.M). Statistical analyses were performed using Two-way ANOVA followed by a Tukey post *hoc* test (GraphPad Prism, GraphPadsoftware; Inc; San Diego, US-CA). P-value of 0.05 or less was considered significant.

3. Results

3.1. Acute toxicity test

Oral administration of 800 mg/kg of AIE did not induce any mortality or visible signs of toxicity by 7 h. However, this dose seemed to cause constipation.

3.2. Effect of AIE on castor oil-induced diarrhea

Table 1 showed that CO administration produced an important watery diarrhea, with an early onset of defecation by 52.17 ± 3.64

min. AIE pretreatment significantly and dose-dependently delayed ($p < 0.05$-0.001) the onset of diarrhea, decreased the frequency of stooling (reduction in number of wet stools and total stools) as well as the weight of wet stools in comparison with the control group. A similar high inhibition of defecation (76.10 ± 6.83 %) and diarrhea (80.06 ± 1.68 %) was also noticed at the dose of 800 mg/kg of the Loperamide, the standard anti-diarrheal drug.

3.3. Effect of AIE on castor oil-induced intestinal content and fluid accumulation

To assess the preventive effect of AIE, we weighed the small intestines filled (WFI) and then emptied (WEI) of their contents. This difference in weight represents solid intestinal contents (WIC) + intestinal fluid. According to Table 2, a pretreatment with 500 mg/kg of AIE significantly inhibited the CO-induced intestinal content accumulation in comparison with that received CO only. In addition, with the higher dose (800 mg/kg), AIE pretreatment seemed to reduce in a dose-dependent manner the intestinal fluid in CO-treated rats (groups 200, 500 and 800). Loperamide also increased the intestinal content weight and fluid accumulation compared to CO and Control groups ($p < 0.001$) (Table 2).

3.4. Effect of AIE on castor oil-induced carbonylated proteins and MDA levels

CO exposure significantly increased the intestine carbonylated protein levels compared to control group (Fig. 1a) (1622 ± 67.28 μmol/mg *vs* 372.6 ± 32.94 μmol/mg, $p < 0.001$). Most importantly, AIE pretreatment improved this increase in a dose-dependent manner (353.40 ± 33.82 *vs* 1622 ± 67.28 μmol/mg for the highest dose 800 mg/kg); $p < 0.001$) with an efficiency similar to Loperamide. CO increased also the intestinal content of MDA compared to control group ($p < 0.001$), while AIE pretreatment (200, 500 and 800 mg/kg) decreased this parameter to a lower level than that of control group. ($P < 0.001$) (Fig. 1b)

3.5. Effect of AIE on antioxidant enzyme activities

CO administration decreased SOD activity compared to control group (0.93 ± 0.04 *vs*1.44 ± 0.10 UI/min/mg, $p < 0.001$), while coadministration of AIE decreased significantly the activity of the SOD compared with all groups ($p < 0.001$). Loperamide coadministration restored the activity of this enzyme at the level of control (Fig. 1c). CO and Loperamide decreased significantly GPx activity compared to control group. However, association of AIE at all doses, increased GPx activity in comparison with control group ($p < 0.001$), while co-administration of Loparamide did not improve the decrease in this parameter(Fig. 1d).

3.6. Effect of AIE on castor oil-induced oxygen free radicals

CO increased significantly tissue $O_2^{\circ-}$concentration compared to control group (0.280 ± 0.01 *vs* 0.182 ± 0.00 UI/mg respectively, $p < 0.05$), whereas AIE co-administration decreased this parameter to a level close to the control value ($p < 0.01$). Lop at a dose of 10 mg/kg, is more efficient than the plant extract in restoring the level of this radical (Fig. 1e). In addition, CO also increased significantly OH° concentration compared to control group and unlike Loperamide, pretreatment with AIE prevented significantly this increase ($p < 0.05$) (Fig. 1f).

3.7. Effect of AIE on bivalent minerals

CO administration increased significantly the intestinal calcium concentration compared to control group ($p < 0.001$). AIE co-administration prevented effectively this effect ($p < 0.001$), while Loperamide had no effect on the CO-induced calcium increase (Fig. 2a.). Likewise, there is also no difference between control, CO and Lop groups about magnesium tissue concentrations. However, AIE increased significantly the magnesium concentration in a dose-dependent fashion when compared with the control group ($p <$

Table 1
Effect of AIE and loperamideon castor oil-induced diarrhreal parameters.

	Dose (mg/kg)	Onset of diarrhea (min)	Weight of wet stools (g)	Total weight of stools (g)	Nb. of wet Stools	Total Nb. Of Stools	IDe %	ID %
CO	**15**	52.17 ± 3.64	6.15 ± 0.86	8.0 ± 0.97	5.86 ± 0.74	7.0 ± 0.82	0.0 ± 0.00	0.0 ± 0.00
AIE	**200**	73.78 ± 4.45	5.48 ± 0.66	6.07 ± 0.43	5.33 ± 0.76	8.0 ± 0.89	20.33 ± 5.69	39.99 ± 5.45
	500	146.4 ± 20.96′	3.57 ± 0.37‴	5.49 ± 0.37′	3.86 ± 0.74′	7.17 ± 0.48	48.78 ± 9.86	46.23 ± 1.36
	800	252.7 ± 36.07‴	1.79 ± 0.22‴	4.04 ± 0.64‴	2.14 ± 0.26‴	4.25 ± 0.25′	76.10 ± 6.83	80.06 ± 1.68
Lop	**10**	Id.	0.0 ± 0.00‴	0.21 ± 0.21‴	0.0 ± 0.00‴	0.43 ± 0.43‴	100.0 ± 0.00	100.0 ± 0.00

Values are mean ± S.E.M (n = 7). **Lop**:loperamide, **CO**: castor oil, **Ide%:** % Inhibition of defecation, **ID%**: % Inhibition of diarrhea. ‘P < 0.05, “‘P < 0.01, “‘P < 0.001 *vs* Castor oil group (Two-wayANOVA, Tukey post hoc test).

Table 2
Effect of AIE and loperamideon castor oil-induced intestinal fluid accumulation.

Groups	Dose (mg/kg)	WFI (g)	WEI (g)	WIC (g)	Intestinal fluid (ml)
Control		4.81 ± 0.24	3.54 ± 0.08	1.28 ± 0.20	0.88 ± 0.15
CO	15	5.18 ± 0.27	3.28 ± 0.18	1.89 ± 0.16	2.20 ± 0.11
AIE	200	5.85 ± 0.48	3.70 ± 0.13	2.13 ± 0.15	2.03 ± 0.37
	500	5.32 ± 0.21	3.69 ± 0.12	0.98 ± 0.14’	1.79 ± 0.20
	800	5.69 ± 0.21	3.75 ± 0.13	0.98 ± 0.15′	1.67 ± 0.21
Lop	10	9.39 ± 0.22***’’’	4.41 ± 0.15***’’’	4.98 ± 0.33***’’’	4.64 ± 0.43***’’’

Values are mean ± S.E.M (n = 7). **Lop**:loperamide,**CO**: Castor oil, **WFI**: Weight of the filled intestines, **WEI**: Weight of the emptied intestines, **WIC**: Weight of intestinal content. *P < 0.05, **P < 0.01, ***P < 0.001 *vs* Control group. ‘P < 0.05, ‘‘P < 0.01, ‘‘‘P < 0.001 *vs* Castor oil group (TowwayANOVA, Tukey post hoc test).

0.001) (Fig. 2b.).

4. Discussion

Diarrhea is a common disorder characterized by the frequent emission of liquid feces [41]. It implies an increase of the volume, fluid, and frequency of the stools which become wet. The patient suffers from dehydration and abdominal pains. Physiologically, diarrhea is caused by an increase in fluid secretion combined with a reduction of the fluid absorption in the intestinal lumen, leading to a water loss [52].

Diarrhea can be caused by microbial or parasitic infection, while the non-infectious form can be induced by antibiotics, toxins or chronic diseases [8].

Prescribed drugs for diarrhea are generally of synthetic origin. Nevertheless, these very effective drugs present some harmful and undesirable effects in case of a long-term use as bacteria resistance [53]. Recent studies are looking for remedies from natural origins to counteract these non-negligible side effects.

In the present work, we proposed an ethnopharmalogical study of a medicinal plant called *Ajuga iva* (L.) or more commonly "*Chandgoura*" in Maghreb countries. This panacea was recognized as a traditional medicinal herb very much used for its antidiarrheal virtues [29].

Non pregnant female Wistar rats were used for the experiments of diarrhea. The hormonal factor of these rats did not constitute a physiological constraint on the digestive tract. Rats did not suffer from digestive disorders before the experiments. In line with previous data [54], our study showed that castor oil-induced copious watery diarrhea and intestinal fluid storage, with early appearance of wet stools.

Castor oil derived from the seeds of a tropical shrub called *Ricinus communis* (*Euphorbiaceae*) and it is known to cause water and electrolyte permeability alteration in the intestinal mucosal membranes leading to the increase in the watery luminal content that flow rapidly across the small and large intestines [41]. The ricinoleic acid contained in this oil would also act like an agonist of the prostaglandins EP3 receptors at the level of the epithelial cells of the intestine. The action of ricinoleic acid consists in contracting the epithelial cells of the small intestine, which leads to stretching of the tight junctions [55]. This mechanism facilitates the entry of electrolytes in the intestinal lumen and allows the hydration of the food contents, thereby making a pro-diarrheal effect. Diarrhea induction by CO can also be related to the liberation of prostaglandins by intestinal cells [42].

Overall, AIE administration exhibited a significant antidiarrheal activity against castor oil-induced diarrhea in a dose-dependent fashion. Thus, it significantly delayed the onset of diarrhea and reduced the number and weight of diarrheal stools. In addition, AIE decreased the percentage of total and diarrheal defecations. Similar results were observed with the use of the whole plant of *Mezoneuron bethamianum* [41] and leaf extracts of *Ocimum Lamiifolium* [21], *Moringa stenopetala* [20] and *Avicennia alba* [13] in Mice. In terms of physiological actions, a pretreatment with 200 mg/kg of AIE dehydrated the food contents at the small intestine level. This dehydration declined with a dose of 500 mg/kg of AIE, while facilitating the intestinal motility, which is characterized by a transfer of the food contents of the small intestine to the large one [41]. However, this mode of action is not found in *Mezoneuron bethamianum*. Thus, with a dose of 800 mg/kg of AIE, a major part of the food contents is localized in the large intestine in order to undergo an additional dehydration. Moreover, a dose of 800 mg/kg of AIE seemed to slow down the intestinal motility at this level of the digestive tract. The same antidiarrheal mechanism is found with the use of *Sanseviera liberica* [56]. The involvement of the large intestine in the antidiarrheal mechanism may be explained by the fact that small intestines of the 500 and 800 mg/kg of AIE groups presented a similar weight. However, loperamide-administred rats had a high difference of weight due to thr inhibitory effect on the intestinal motility.

AIE decreased the intestinal motility by an agonist effect on the central α2 adrenergic receptors that inhibited the acetylcholine release which is known to facilitate the intestinal motility by contracting the smooth muscle cells [57,58].

According to the results on the food dehydration in the different groups, it appears that loperamide and *Ajuga iva* do not have clearly the same mechanism of action. Loperamide is known to stimulate absorption and reduce motilty in the small intestine [59]. However, AIE increases the dehydration of the food contents and decreases secretion in the large intestine due to its potential adrenergic action like *Sansevier aliberica* [56].

The acute exposition to AIE revealed that this extract is safe and did not induce any obvious abnormality or toxic effects even at the higher dose of 800 mg/kg. This observation is in agreement with the high value of its lethal dose 50 (LD_{50}) (4500 mg/kg) [31].

Oxidative stress corresponds to an imbalance between ROS generation and antioxidant defenses of the organism. Tobacco

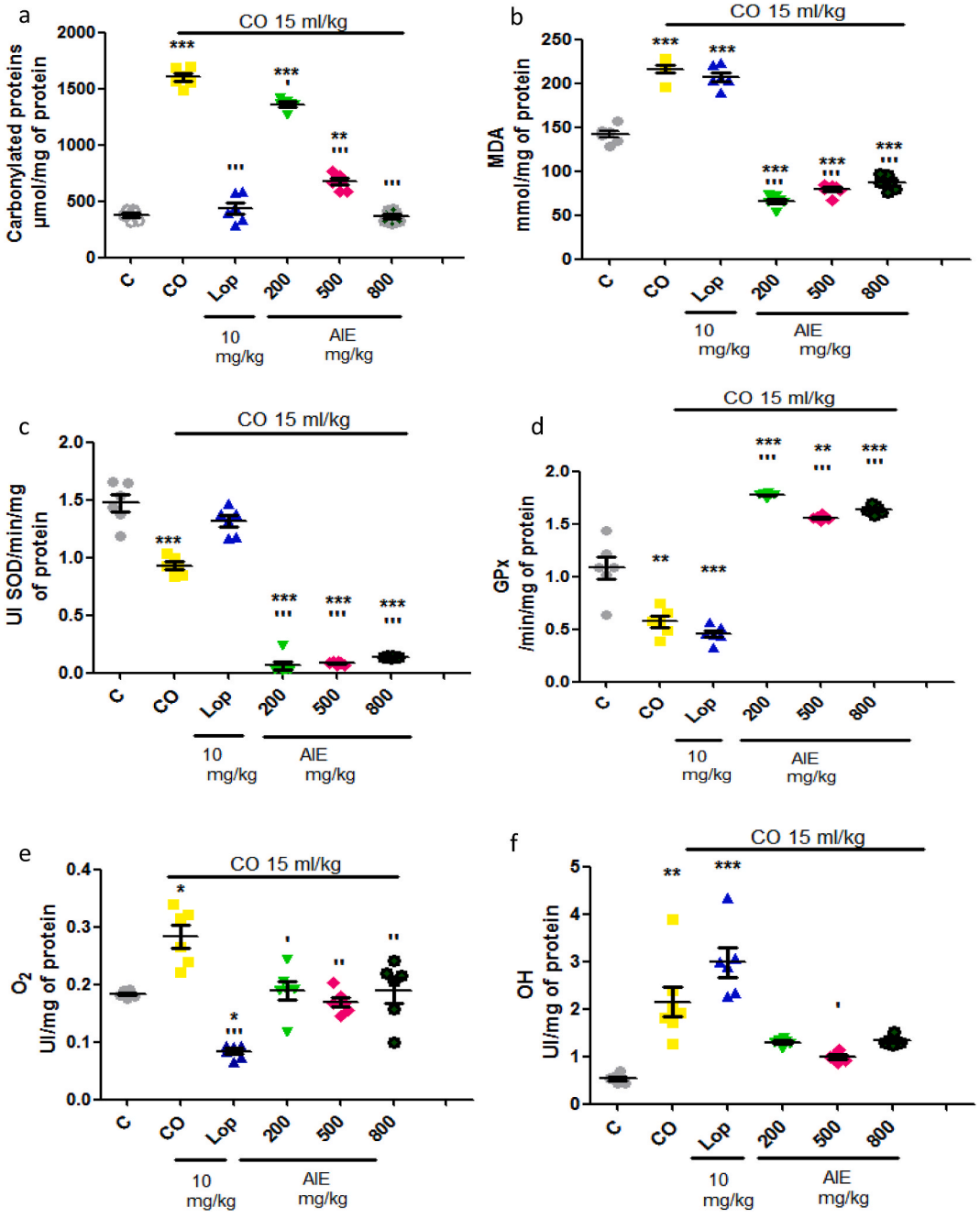

Fig. 1. Effect of AIE and loperamide on castor oil-induced oxidative stress markers in small intestine
aCarbonylated proteins (µmol/mg), **b** Malondialdehyde (mmol/mg).**c** Superoxide dismutase (UI/min/mg), **d**Gluthatione peroxidase (mol/min/mg), **e** Superoxide anion (UI/mg), **f** Hydroxyl radical (UI/mg).
Values are mean ± SEM (n = 7). **C**: Control, **CO**: Castor oil, **Lop**: loperamide. *P < 0.05, **P < 0.01, ***P < 0.001 *vs* Control group. ‘P < 0.05, “‘P < 0.01, “‘P < 0.001 *vs* Castor oil group (Two-way ANOVA, Tukey *post hoc test*).

addiction, alcoholism, obesity, intense physical exercise, and also bad dietary habits, increase in an abnormal way the ROS production in the cells. In the long term, oxidative stress can contribute to the appearance of various age-related illnesses like cancers or cardiovascular diseases [60].

Castor oil to be a is shown pwerfull antioxidant agent causing oxidative damages by the production of ROS and stimulating the nitric oxide release that potentializes the diarrhea [61]. On the other hand, CO induces the carbonylation of proteins, stimulates lipidic peroxidation and causes at long term chronic diseases [62,63].

In the present study, castor oil decreased the SOD and GPx activities in the small intestine tissue. It is also noted that castor oil

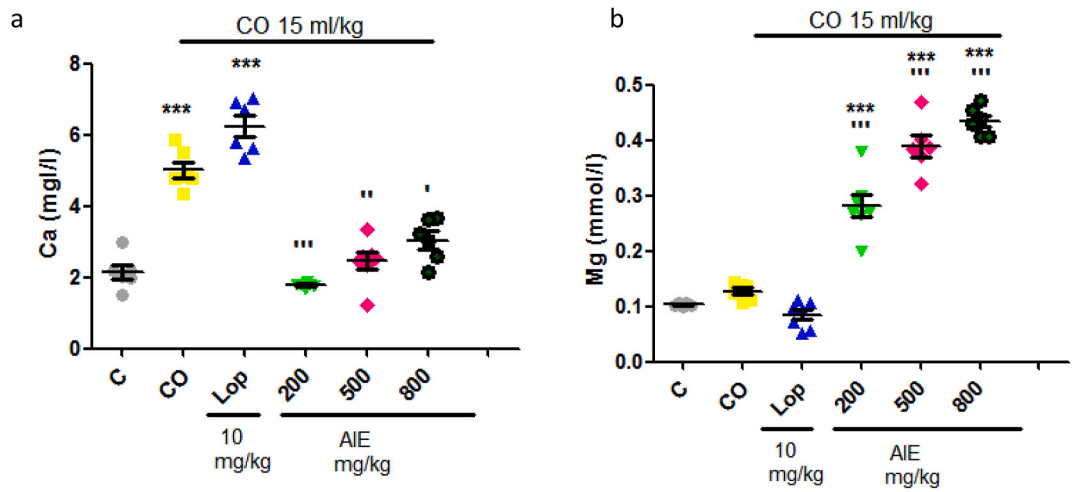

Fig. 2. Effect of AIE and loperamide on castor oil-induced bivalent mineral concentration changes in small intestine
a Calcium (mg/l),**b** Magnesium (mg/l)
Values are mean ± SEM (n = 7). **C**: Control, **CO**: Castor oil, **Lop**: loperamide. *P < 0.05, **P < 0.01, ***P < 0.001 *vs* Control group. ‘P < 0.05, “‘P < 0.01, “‘P < 0.001 *vs* Castor oil group (Two-way ANOVA, *Tukey post hoc test*).

increased the calcium rate compared to control group,but did not change the magnesium concentration. An increase in the intracellular calcium allows the cellular contraction, a stretching of the tight junctions and thus a facilitation of the passage of water flux in the lumen of the intestinal tube [64]. This constitutes an additional and a solid argument concerning the castor oil-induced diarrhea mechanism.

Ajuga iva is composed of several active ingredients like flavonoids, tannins and tannic acid [65]. Overall, our results indicated that AIE presented a better antioxidant capacity than loperamide. Indeed, AIE protected proteins from carbonylation in the same way as for the seeds of *Vigna radiata* (green soybean) [66], while reducing the lipidic peroxidation revealed by the reduction in tissue MDA content, in accordance with previous data following oral treatment by *Atriplex halimus* or *Atriplex canescens* leaves [27].

However, AIE unexpectedly, strongly decreased the intestine activity of the SOD. The same effect was found in the kidneys of rats when testing *Ajuga iva* as a hypocholesterolemic agent [67]. The reduction in the SOD activity may be explained by the inhibiting effect of AIE activity [68]. In our work AIE increased significantly the GPx activity compared to CO and control group ($p < 0.001$).

AIE decreased both the $O_2^{\circ-}$ and OH° tissue levels, compared to the CO group. This finding corroborated the previous report [69], indicating that *Ajuga iva* decreased the intestine OH °content. As regards to the decrease of SOD activity, the depletion of the $O_2^{\circ-}$ level, may be related to the antioxidant potential of flavonoid compounds of *Ajuga iva* [68]. In this context, tannins and tannic acid are shown to reduce intestinal secretion by complexing proteins that coat intestinal mucosa [70]. Tannnins and flavonoids also reduced the intestinal motility and irritability [71]. The anti-diarrheal activity of flavonoids has been associated with their capacity to inhibit intestinal peristalsis and ion secretions [72] and by increasing colonic reabsorption and water filling [70]. Polyphenol compounds play a major role in absorbing and neutralizing ROS free radicals and decomposing peroxides [73] and present beneficial effects on human health [74].

Castor oil increased the intestinecalcium rate, which could lead to a prodiarrhoeal process. AIE administration prevented this effect, namely with the dose of 200 mg/kg. These results confirm the antidiarrhoeal property of AIE evidenced by the reduction of the hydration of the food contents in the lumen of the small intestine and also the intestinal motility. This effect was also reported for *Fagopyrum cymosum,* whose active compounds are capable of blocking the calcic channels which results in the inhibition of the intestinal muscle cell contractility [75] and for the Bromide of Pinaverium, the antispasmodic active ingredient of Dicetel® (drug used against the irritable bowel syndrome) [75]. Magnesium constitutes one of the most significant antioxidant cofactor [76]. In presence of AIE, there is a significant dose-dependent increase of the magnesium concentrationin the intestinal tissue. These additional data constitute an additional antioxidant mechanism which couldbe attributed to *Ajuga iva* [77]. Our research on this particular plant has yielded promising results in terms of its potential as a therapeutic tool for combatting diarrhea. However, further investigations are needed to fully isolate and identify the specific active compounds responsible for these effects.

5. Conclusion

These findings suggest that methanolic extract of *Ajuga iva* leaves exhibited a powerful anti-diarrheal activity mainly through its properties and provides experimental evidence for the traditional use of *Ajuga iva* in the treatment of diarrhea as a new useful therapeutic alternative which could satisfy certain patients insensitive or allergic to loperamide.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Mohamed H. Ladjimi: Data curation, Methodology, Project administration, Validation, Writing – review & editing. **Zaineb Ben Barka:** Data curation, Methodology, Project administration, Validation, Writing – review & editing. **Karima Lahbib:** Data curation, Methodology, Project administration, Validation, Writing – review & editing. **Hanène Ben Miled:** Data curation, Methodology, Project administration, Validation, Writing – review & editing. **Khemais Ben Rhouma:** Data curation, Methodology, Project administration, Validation, Writing – review & editing. **Mohsen Sakly:** Data curation, Methodology, Project administration, Validation, Writing – review & editing. **Olfa Tebourbi:** Data curation, Methodology, Project administration, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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