Antidiarrheal and antioxidant activities of chamomile (Matricaria recutita L.) decoction extract in rats

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Research Paper

Antidiarrheal and antioxidant activities of chamomile (*Matricaria recutita* L.) decoction extract in rats

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ABSTRACT

Ethnopharmacological relevance: *Matricaria recutita* L. (Chamomile) has been widely used in the Tunisian traditional medicine for the treatment of digestive system disorders. The present work aims to investigate the protective effects of chamomile decoction extract (CDE) against castor oil-induced diarrhea and oxidative stress in rats.

Methods: The antidiarrheal activity was evaluated using castor oil-induced diarrhea method. In this respect, rats were divided into six groups: Control, Castor oil, Castor oil + Loperamide (LOP) and Castor oil + various doses of CDE. Animals were per orally (*p.o.*) pre-treated with CDE during 1 h and intoxicated for 2 or 4 h by acute oral administration of castor oil.

Results: Our results showed that CDE produced a significant dose-dependent protection against castor oil-induced diarrhea and intestinal fluid accumulation. On the other hand, we showed that diarrhea was accompagned by an oxidative stress status assessed by an increase of malondialdehyde (MDA) level and depletion of antioxidant enzyme activities as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Castor oil also increased gastric and intestinal mucosa hydrogen peroxide (H_2O_2) and free iron levels. Importantly, we showed that chamomile pre-treatment abrogated all these biochemical alterations.

Conclusion: These findings suggested that chamomile extract had a potent antidiarrheal and antioxidant properties in rats confirming their use in traditional medicine.

1. Introduction

Diarrhea is a major health problem, especially for children under the age of 5 years in developing countries including Tunisia (Bryce et al., 2005). This disease is responsible for about 5 million deaths annually (Heinrich et al., 2005). Diarrhea is characterized by a discharge of semisolid or watery fecal matter from the bowel three or more times in one day (Suleiman et al., 2008) leading to inflammatory response and oxidative stress (Song et al., 2011). To protect against this disease, commercial drugs such as diaretyl are frequently used. This drug induces a severe constipation as a side effect and can also lead to colorectal cancer (Power et al., 2013). For this reason, the World Health Organization (WHO) has introduced a program for diarrheal control which involves the use of traditional herbal medicines. However, several naturally-occurring compounds are largely used to protect against digestive system diseases both in experimental and clinical situations.

Matricaria recutita L. (Chamomile) is a well-known medicinal plant species from Asteraceae family. This species is one of the most popular and widely used in traditional medicine for the treatment of gastrointestinal disorders including diarrhea (Alanís et al., 2005). However, due to its richness in therapeutically active compounds (McKay and Blumberg, 2006), this plant presents many beneficial health effects as antioxidant (Hernández-Ceruelos et al., 2010), neuro-protective (Ranpariya et al., 2011), anti-allergic (Chandrashekhar et al., 2011), anti-inflammatory (Bulgari et al., 2012), anti-microbial (Silva et al., 2012) and anti-cancer (Matić et al., 2013) activities. Chamomile is also used for its positive effects against digestive system illness (Al-Hashem, 2010).

Hence, the present study aimed to investigate the putative protective effect of CDE on diarrhea induced by castor oil administration as well as the implication of oxidative stress in such protection.

Abbreviations: CAT, catalase; CDE, chamomile decoction extract; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; LOP, loperamide; MDA, malondialdehyde; SOD, superoxide dismutase

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2. Materials and methods

2.1. Chemicals

Epinephrine, bovine catalase, 2-Thio-barbituric acid (TBA) and butylated hydroxytoluene (BHT) were from Sigma chemicals Co. (Germany). All other chemicals used were of analytical grade.

2.2. Preparation of chamomile decoction extract

Chamomile (*Matricaria recutita* L.) flowers were cultivated from the region of Beja (North-West of Tunisia) during March 2012 and identified by Mrs. Mouhiba Ben-Naceur, professor of taxonomy in the Higher Institute of Biotechnology of Béja-Tunisia. The Voucher specimens (No. M121) have been deposited with the herbarium of the Higher Institute of Biotechnology of Béja and also in our laboratory of integrated physiology in the Faculty of Sciences of Bizerta. Plant material was subsequently dried in an incubator at 50 °C during 72 h and powdered in an electric blender. The decoction was prepared with distilled water (1/5; w/v) at 100 °C during 5 min under magnetic agitation and the homogenate was filtered through a colander (0.5 mm mesh size). Finally, the obtained extract (CDE) was stored at −80 °C until use.

2.3. Animals and treatment

Adult male Wistar rats (weighing 200–220 g; housed five per cage) and adult male Swiss Albino mice (weighing approximately 25 g; housed ten per cage) were purchased from Pasteur Institute of Tunis and used in accordance with the local ethic committee of Tunis University for use and care of animals in conformity with the NIH recommendations. They were provided with food (standard pellet diet- Badr Utique-TN) and water *ad libitum* and maintained in animal house at controlled temperature (22 ± 2 °C) with a 12 h light-dark cycle.

2.4. Acute toxicity study

The chamomile decoction extract (CDE) in the dose range of 12.5, 25, 50 ,100, 200, 400, 800, 1600 and 3200 mg/kg was orally administered to different groups of mice ($n=10$). The animals were examined every 30 min up to a period of 4 h and then, occasionally for additional period of 8 h. After2 4 h , the mortality was recorded. The mice were also observed for other signs of toxicity, such as motor co-ordination, righting reflex and respiratory changes.

2.5. Treatment and evaluation of antidiarrheal activity

Rats were divided into six groups of 10 animals each. Groups 1 and 2 served as controls and received bidistilled water (5 mL/kg, *b.w.*, *p.o.*). Groups 3, 4, and 5 were pre-treated with various doses of chamomile decoction extract (25, 50 and 100 mg/kg, *b.w. p.o.*), while group 6 was pre-treated with loperamide (20 mg/kg, *b.w. i.p.*).

After 60 min, each animal, except group 1, received castor oil (5 mL/kg, *b.w.*) by gavage and placed in a separate cage for antidiarrheal activity evaluation.

The antidiarrheal activity of chamomile was evaluated according to the method of Awouters et al. (1978) modified by Mukherjee et al. (1998). Briefly, after castor oil administration, animals were observed for defecation up to 4 h. Transparent plastic dishes were placed beneath each cage and the characteristic diarrheal droppings were noted.

The intestinal fluid accumulation was determined according to Dicarlo et al. (1994), with some modifications. Briefly, 2 hafter castor oil administration, animals were anesthetized with urethane (1.25 g/kg, *i.p.*). Laparotomy was performed and the small intestine was removed, after ligation at the pyloric end and ileocaecal junction, and weighed. The intestinal content was then expelled into a graduated tube and the volume was determined. The small intestine was reweighed and the difference between full and empty intestine was calculated.

2.6. Lipid peroxidation measurement

The lipid peroxidation was determined by MDA measurement according to the double heating method (Draper and Hadley, 1990). Briefly, aliquots from gastric and intestine mucosa homogenates were mixed with BHT-TCA solution containing 1% BHT (w/v) dissolved in 20% TCA (w/v) and centrifuged at 1000*g* for 5 min at 4 °C. Supernatants were blended with 0.5 N HCl, 120 mM TBA in 26 mM Tris and then heated at 80 °C for 10 min. After cooling, absorbance of the resulting chromophore was determined at 532 nm using a UV–visible spectrophotometer (Beckman DU 640B). MDA levels were determined by using an extinction coefficient for MDA–TBA complex of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.7. Antioxidant enzyme activity assays

SOD activity was determined by using modified epinephrine assay (Misra and Fridovich, 1972). At alkaline pH, superoxide anion O_2^- causes the autoxidation of epinephrine to adenochrome, while competing with this reaction, SOD decreased the adenochrome formation. One unit of SOD is defined as the amount of the extract that inhibits the rate of adenochrome formation by 50%. Enzyme extract was added in 2 ml reaction mixture containing 10 µl of bovine catalase (0.4 U/µl), 20 µl epinephrine (5 mg/ml) and 62.5 mM sodium carbonate/bicarbonate buffer pH 10.2. Changes in absorbance were recorded at 480 nm.

CAT activity was assayed by measuring the initial rate of H_2O_2 disappearance at 240 nm (Aebi, 1984). The reaction mixture contained 33 mM H_2O_2 in 50 mM phosphate buffer pH 7.0 and CAT activity calculated using the extinction coefficient of 40 $mM^{-1}\ cm^{-1}$ for H_2O_2.

GPx activity was measured by the procedure of Flohé and Günzler (1984). Briefly, 1 mL of reaction mixture containing 0.2 mL of sample, 0.2 mL of phosphate buffer 0.1 M pH 7.4, 0.2 mL of GSH (4 mM) and 0.4 mL of H_2O_2 (5 mM) was incubated at 37 °C for 1 min and the reaction stopped by addition of 0.5 mL TCA (5%, w/v). After centrifugation at 1500*g* for 5 min, aliquot (0.2 mL) from supernatant was mixed with 0.5 mL of phosphate buffer 0.1 M pH 7.4 and 0.5 mL DTNB (10 mM) and absorbance recorded at 412 nm. GPx activity was expressed as nmol of GSH consumed/min/mg protein.

2.8. H_2O_2 determination

Gastric and intestine mucosa H_2O_2 levels were performed according to Dingeon et al. (1975). Briefly, in the presence of peroxidase, the hydrogen peroxide reacts with p-hydroxybenzoic acid and 4-aminoantipyrine leading to a quantitative formation of a quinoneimine which has a pink color detected at 505 nm.

2.9. Iron measurement

Gastric and intestine mucosa non heme iron were measured colorimetrically using ferrozine as described by Leardi et al. (1998). Briefly, the iron dissociated from transferrin–iron complex by a solution of guanidine acetate and reduced by ascorbic acid reacts with ferrozine to give a pink complex measured at 562 nm.

2.10. Protein determination

Protein concentration was determined according to Hartree (1972) which is a slight modification of the Lowry method. Serum albumin was used as standard.

2.11. Statistical analysis

Data were analyzed by unpaired Student's *t*-test or one-way analysis of variance (ANOVA) and were expressed as means ± standard error of the mean (SEM). Data are representative of ten independent experiments. All statistical tests were two-tailed, and a *p* value of 0.05 or less was considered significant.

3. Results

3.1. Acute oral toxicity of CDE

In the acute oral toxicity study, neither abnormal behavior nor mortality was detected during the observation period. The LD50 value was greater than 3200 mg/kg *b.w.* for the decoction extract of *Matricaria recutita*.

3.2. Effects of CDE on castor oil-induced diarrhea

We firstly demonstrated in the present study that, 4 h after castor oil (5 ml/kg, *b.w., p.o.*) administration, all rats produced copious diarrhea (Table 1). However, pre-treatment with various doses of CDE (25, 50 and 100 mg/kg, *b.w., p.o.*) significantly and dose-dependently reduced the number of defecations. Administration of loperamide (20 mg/kg, *b.w., p.o.*), a standard antidiarrheal molecule, produced a more marked antidiarrheal effect but less than the high dose of CDE.

3.3. Effects of CDE on castor oil-induced enteropooling

The effects of CDE on castor oil-induced fluid accumulation are presented in the Table 2. As expected, castor oil per se significantly increased the volume and the weight of intestinal fluid when compared to control group while loperamide reduced it to near control level. Interestingly, CDE pre-treatment inhibited significantly and dose dependently castor oil-induced enteropooling.

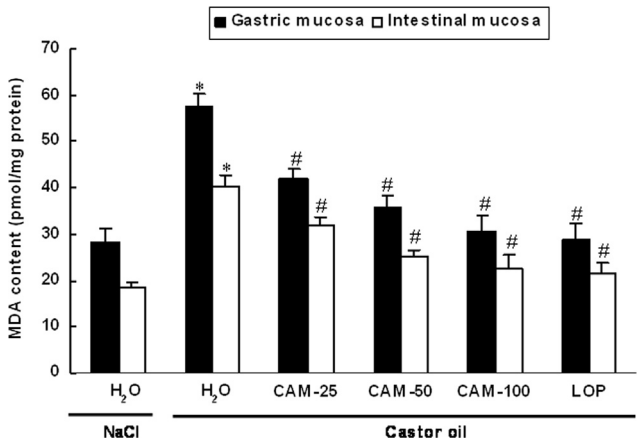

Fig. 1. Effect of chamomile decoction extract (CDE) and loperamide (LOP) on castor oil-induced changes in stomach and intestinal mucosa MDA level. Animals were pre-treated with various doses of CDE (25, 50 and 100 mg/kg, *p.o.*), reference molecule (LOP, 20 mg/kg, *b.w., i.p.*) or vehicle (NaCl 0.9%). One hour after, animals received castor oil (5 ml/kg *b.w.*) by gavage for 2 h. Assays were carried out in triplicate. $^{*}p < 0.05$ compared to control group and $^{\#}p < 0.05$ compared to castor oil group.

Table 1
Effect of chamomile decoction extract (CDE) and loperamide (LOP) on castor oil-induced diarrhea.

Group	Onset of diarrhea (min)	Total number of stools	Number of wet stools	Percentage of wet stools (%)	Percentage protected (%)
Control	–	2.76 ± 0.19	0	0	–
Castor oil	78 ± 4.86	13.5 ± 1.21*	13.01 ± 0.39*	96.37	0
Castor oil + CAM-25	98 ± 5.01#	9.23 ± 0.74#	7.54 ± 0. 55#	81.69	42.04
Castor oil + CAM-50	147 ± 8.78#	7.36 ± 0.49#	4.57 ± 0.6#	62.09	64.87
Castor oil + CAM-100	191 ± 10.63#	4.21 ± 0.32#	3.26 ± 0.31#	77.43	74.94
Castor oil + LOP	209 ± 6.17#	3.9 ± 0.66#	2.1 ± 0.57#	53.84	83.85

Animals were pre-treated with various doses of CDE (25, 50 and 100 mg/kg, *p.o.*), reference molecule (LOP, 20 mg/kg, *b.w., i.p.*) or vehicle (NaCl 0.9%). One hour after, animals received castor oil (5 ml/kg *b.w.*) by gavage and observed for defecation up to 4 h.

* $p < 0.05$ compared to control group.
\# $p < 0.05$ compared to castor oil group.

Table 2
Effect of chamomile decoction extract (CDE) and loperamide (LOP) on castor oil-induced enteropooling.

Group	Volume of intestinal content (ml)	Percentage protected (%)	Weight of intestinal content (g)	Percentage protected (%)
Control	00	–	00	–
Castor oil	4.9 ± 0.27*	00	5.42 ± 0.59*	00
Castor oil + CAM-25	3.55 ± 0.19#	27.55	4.01 ± 0.41#	26.01
Castor oil + CAM-50	2.47 ± 0.24#	49.59	2.84 ± 0.33#	47.60
Castor oil + CAM-100	1.75 ± 0.16#	64.28	1.98 ± 0.15#	63.46
Castor oil + LOP	1.55 ± 0.15#	68.36	1.77 ± 0.13#	67.34

Animals were pre-treated with various doses of CDE (25, 50 and 100 mg/kg, *p.o.*), reference molecule (LOP, 20 mg/kg, *b.w., i.p.*) or vehicle (NaCl 0.9%). One hour after, animals received castor oil (5 ml/kg *b.w.*) by gavage for 2 h.

* $p < 0.05$ compared to control group.
\# $p < 0.05$ compared to castor oil group.

3.4. Effects of CDE on castor-oil induced gastric and intestinal lipoperoxidation

To investigate the implication of oxidative stress in the anti-diarrheal effect of CDE, stomach and intestinal mucosa were firstly assessed for MDA determination. As expected, castor oil administration significantly increased stomach and intestinal mucosa MDA levels. Castor oil-induced lipoperoxidation was reduced by loperamide or chamomile pre-treatment in a dose dependant manner (Fig. 1).

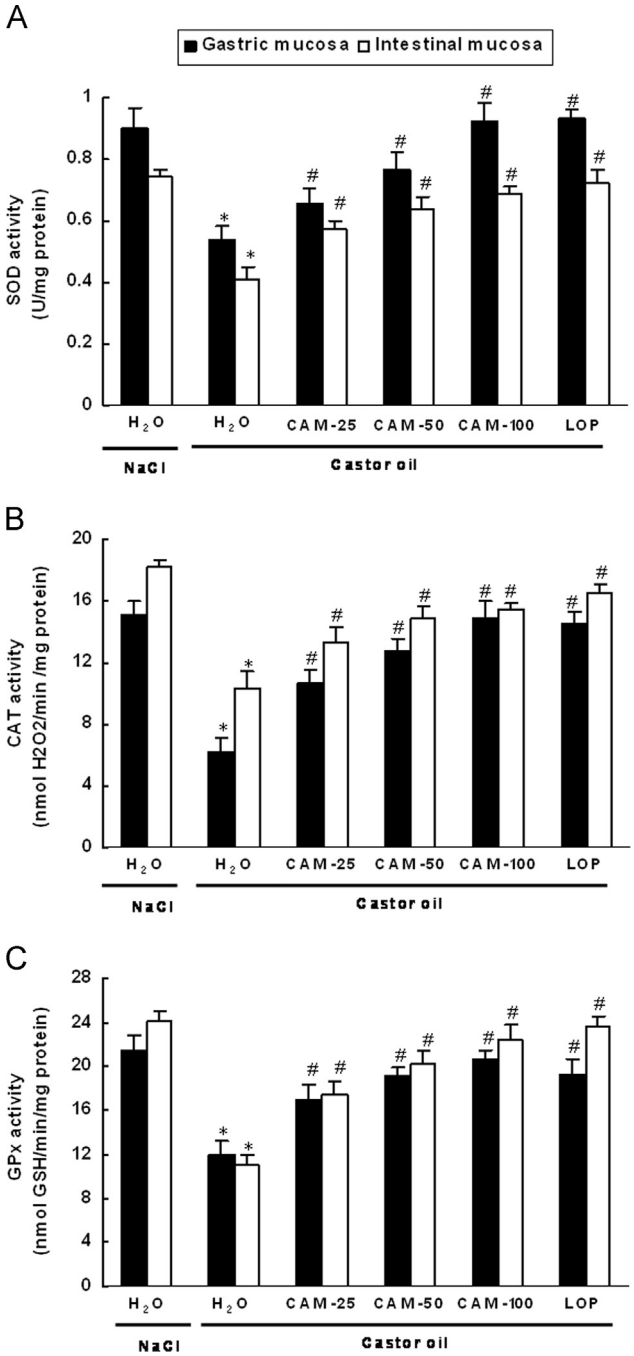

Fig. 2. Effect of chamomile decoction extract (CDE) and loperamide (LOP) on castor oil-induced changes in stomach and intestinal mucosa antioxidant enzyme activities: SOD (A), CAT (B) and GPx (C). Animals were pre-treated with various doses of CDE (25, 50 and 100 mg/kg, *p.o.*), reference molecule (LOP, 20 mg/kg, *b.w., i.p.*) or vehicle (NaCl 0.9%). One hour after, animals received castor oil (5 ml/kg *b.w.*) by gavage for 2 h. Assays were carried out in triplicate. $^{*}p < 0.05$ compared to control group and $^{\#}p < 0.05$ compared to castor oil group.

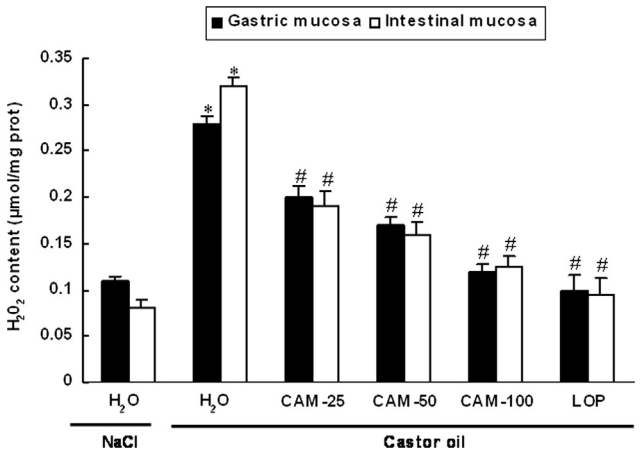

Fig. 3. Effect of chamomile decoction extract (CDE) and loperamide (LOP) on castor oil-induced changes in stomach and intestinal mucosa hydrogen peroxide level. Animals were pre-treated with various doses of CDE (25, 50 and 100 mg/kg, *p.o.*), reference molecule (LOP, 20 mg/kg, *b.w., i.p.*) or vehicle (NaCl 0.9%). One hour after, animals received castor oil (5 ml/kg *b.w.*) by gavage for 2 h. Assays were carried out in triplicate. $^{*}p < 0.05$ compared to control group and $^{\#}p < 0.05$ compared to castor oil group.

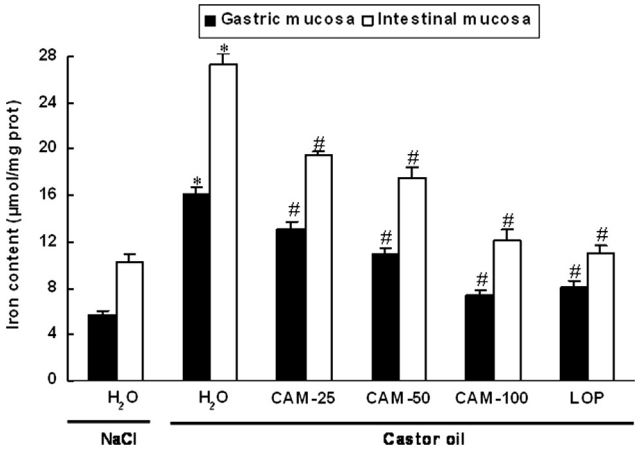

Fig. 4. Effect of chamomile decoction extract (CDE) and loperamide (LOP) on castor oil-induced changes in stomach and intestinal mucosa free iron level. Animals were pre-treated with various doses of CDE (25, 50 and 100 mg/kg, *p.o.*), reference molecule (LOP, 20 mg/kg, *b.w., i.p.*) or vehicle (NaCl 0.9%). One hour after, animals received castor oil (5 ml/kg *b.w.*) by gavage for 2 h. Assays were carried out in triplicate. $^{*}p < 0.05$ compared to control group and $^{\#}p < 0.05$ compared to castor oil group.

3.5. Effects of CDE on castor-oil induced mucosa antioxidant enzymes depletion

We further looked at the effect of castor oil and CDE on antioxidant enzymes activities (Fig. 2). Castor oil treatment drastically decreased gastric and intestinal antioxidant enzyme activities as SOD (A), CAT (B) and GPx (C). Pre-treatment with CDE significantly reduced all castor oil-induced decrease in antioxidant enzyme activities in a dose-dependant manner. Loperamide, a standard antidiarrheal molecule, also protected against castor oil-induced antioxidant enzymes activities depletion.

3.6. Effects of CDE on castor-oil induced mucosa elevation of iron and H_2O_2 levels

We also studied the variation of hydrogen peroxide and free iron levels in stomach and intestinal mucosa. Castor oil per se significantly increased mucosal levels of H_2O_2 and labile iron both

in stomach and small intestine. However, acute pre-treatment with loperamide or CDE significantly reduced the castor oil-induced increase of H_2O_2 and labile iron in a dose-dependant manner (Fig. 3 and Fig. 4)

4. Discussion

In the present study, we evaluated the protective effects of chamomile decoction extract against castor oil-induced diarrhea in adult healthy rats as well as the implication of oxidative stress in such a protection.

We firstly showed that the LD50 value was greater than 3200 mg/kg *b.w.* for the CDE. However, neither mortality nor behavior impairment were noted during the observation period. Chamomile methanol extract, has also been shown to have any evidence of toxicity (Chandrashekhar et al., 2011).

We demonstrated in the present investigation that acute pre-treatment with CDE protected against castor oil-induced diarrhea by inhibiting the number of defecations when compared to untreated group. Castor oil induced diarrhea by causing increased mucosa secretion of fluid and electrolytes into the bowel lumen, resulting in fluid accumulation and a watery luminal content that flowed rapidly through the small and large intestines (Burks, 1991). The active component of castor oil has been demonstrated to be the ricinoleic acid (Karim et al., 2010), which stimulated the production of several mediator substances that include prostaglandins, nitric oxide, platelet activating factor, cAMP and tachykinins (Izzo et al., 1999). Bacterial infection could also impair bowel function and give rise to digestive system disorders as diarrhea (Ojewole et al., 2008). In this context, chamomile extracts have been widely studied for their inhibitor effects against some bacterial strains such as *Arcobacter butzleri* (Cervenka et al., 2006) and *Helicobacter pylori* (Shikov et al., 2008). We also showed in the present study that CDE pre-treatment inhibited castor oil-induced enteropooling in a dose-related manner. However, it is well-known that drugs affecting frequency, and consistency of diarrhea also affected secretion and fluid accumulation in small intestine (Hsu, 1982; Amresh et al., 2004).

Castor oil-induced diarrhea and fluid accumulation have been shown to be attenuated by many plant extracts as *Strychnos potatorum* (Biswas et al., 2002), *Amaranthus spinosus* (Hussain et al., 2009), *Ixora Coccinea* (Maniyar et al., 2010), *Pyrenacantha staudtii* (Awe et al., 2011), *Ficus bengalensis* (Patil et al., 2012) and *Punica gratum* (Das et al., 1999) or isolated molecules as ternatin (Rao et al., 1997) and piperine (Bajad et al., 2001).

Particularly, our investigation revealed that acute administration of castor oil (5 ml/kg *b.w., p.o.*) for 2 h increased the formation of MDA in the stomach and intestine mucosa indicating an increase in lipid peroxidation and depletion of antioxidant activities of SOD, CAT and GPx. CDE pre-treatment prevented all the alterations induced by castor oil in a dose-dependent manner and returned their levels to near-normal with the highest dose. Our results are in line with previous reports demonstrating that castor oil-induced toxicity can be accompanied by an oxidative stress status in the intestinal fluid (Rao et al., 2008). However, it is generally accepted that reactive oxygen species-mediated lipid peroxidation that resulted in extensive subcellular damage and played a major role in the pathogenesis of gastrointestinal disorders (Halliwell et al., 1992).

Previous studies have well shown the richness of extracts as well as essential oil of chamomile in phenolic compounds (Guimarães et al., 2013). These molecules such as quercetin and cafeic acid previously identified by Nováková et al. (2010), are the primal source of antioxidant ability of this plant, by scavenging free radicals as hydroxyl radical (OH•) which is the major cause of lipid peroxidation (Kogiannou et al., 2013). In addition, chamomile extracts are especially known for their richness in tannins (Schulz and Albroscheit, 1988). Besides their antioxidant activities, these molecules are implicated in the regulation of diarrhea (Biswas et al., 2002). Indeed, tannins are present in many plants and can denature protein to form protein tannate complex which makes the intestinal mucosa more resistant reducing its secretion (Kouitcheu et al., 2006). However, apigenin and later apigenin-7-glucoside were the first flavonoid compounds isolated from chamomile (Nováková et al., 2010). These flavonoids are previously shown for their negative effect on both small and large intestinal transit time in mice with castor oil-induced diarrhea (Di Carlo et al., 1993). Castor oil-induced oxidative stress has also been shown to be attenuated by *Cinnamomum tamala* ethanol extract (Rao et al., 2008). Moreover, recent findings indicated that chamomile extracts protected against oxidative stress were induced by cisplatin (Salama, 2012), hydrogen peroxide (Bhaskaran et al., 2013), streptozotocin (Cemek et al., 2008) and ischemia injury (Chandrashekhar et al., 2010).

More importantly, our data showed also that chamomile pre-treatment abolished acute castor oil-induced increase in H_2O_2 and free iron levels in gastric and intestine mucosa. Furthermore, both iron deficiency and iron excess can lead to cellular dysfunction, since maintaining normal iron homeostasis is crucial (Andrews, 1999). Iron accumulation catalyzed hydroxyl radical-mediated oxidative injury via its participation in the Fenton pathway. As previously, proposed for other extracts rich in phenolic compounds as carob (Souli et al., 2013) or grape seeds and skin extracts (Charradi et al., 2011; Hamlaoui-Gasmi et al., 2011), it is tempting to speculate that CDE is capable of chelating free iron and scavenging H_2O_2.

5. Conclusion

In conclusion, our data clearly demonstrated the protective effects of CDE against castor oil-induced diarrhea and fluid accumulation in rats as well as the implication of oxidative stress and Fenton pathway in such protection. These findings confirmed the basis for the use of chamomile extracts in traditional medicine for the treatment and/or management of digestive system disorders as diarrhea.

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