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Role of laxative and antioxidant properties of *Malva sylvestris* leaves in constipation treatment

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ABSTRACT

Introduction: Mallaw (*Malva sylvestris* L.) is a medicinal plant who is traditionally being used as an antiulcer, laxative and anti-hemorrhoid, besides of its culinary use as a food in Tunisian cuisine. The present study was carried out to evaluate the protective effect of *Malva sylvestris* aqueous extract (MSAE) on constipation- induced by loperamide in male *Wistar* rats.
Methods: Rats were divided into six groups of six animals each: Control, Loperamide (LOP), LOP+ yohimbine (2 mg/kg *b.w, i.p.*) and LOP+ various doses of MSAE (100, 200 and 400 mg/kg *b.w, p.o.*). The laxative activity was determined based on the weight, frequency and water content of the feces matter. The effects of MSAE were also evaluated on normal gastric emptying and gastro-intestinal transit as well as loperamide-induced serum metabolic parameters changes and colon oxidative stress.
Results: The MSAE (100, 200 and 400 mg/kg; *b.w, p.o.*), significantly reduced loperamide-induced constipation in a dose-dependent manner. Our extract improves also the gastrointestinal motility, mitigates colon lipid peroxidation and H_2O_2 colon overload, preserve normal antioxidant enzymes activities and non-enzymatic antioxidant levels, as well as provides stability of lipid profile, hepatic (AST and ALT) and renal (urea and creatinine) levels changed by loperamide intoxication.
Conclusion: We suggests in the present work that MSAE had a potent effect against Loperamide- induced constipation through in part to an increase of gastrointestinal motility, astimulation of water intestinal secretion as well as its antioxidant properties.

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1. Introduction

The term "constipation" has several meanings and how to use it can differ not only among patients but also between different cultures and regions. Functional constipation is generally described as an affection characterized by persistent difficulty with defecation or a sensation of incomplete exemption associated with infrequent bowel movements (once every 3–4 days or less) [1,2]. This disease has many different origins, ranging from dietary changes, physical activity or life style, until primary movement disorders caused by myopathy or colic neuropathy. Chronic constipation causes fecal impaction, leading to intestinal obstruction, stercolar ulcerations and/or bowel perforation [2]. Constipation can also be secondary to a disorder of the stool exemption. Such disorder can be associated with a paradoxical anal contraction or involuntary anal spasm,which in two thirds of patients can be an acquired behavioral disorder [3,4]. It was estimated that constipation has a prevalence range from 8.75% in the Asian Pacific region to 27% in Western countries [2]. Constipation is common worldwide, affecting all ages, with prevalence of 0.7–29.6% in children and adolescentsand 15–50% in the elderly [2].

Therefore, the treatment of constipation and its prevention have become one of the current problems. Indeed, research is always oriented to find drugs that have the same properties as the marketed drugs, but without side effects that serve as powerful laxative agent. In this context, the World Health Organization (WHO) has strongly encouraged the investigations based on the medicinal plants for the constipation treatment.

Malva sylvestris L., known as common mallow (Malvaceae) is an herbaceous plant, robust, localized mainly in Europe (France),

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North Africa, Southwest Asia and throughout the Mediterranean basin [5]. In Tunisia it covers the entire North. This species has a long history of use as food, and because of its therapeutic relevance, many parts of this plant have been used in traditional medicines and also in ethno-veterinary medicine [5]. Leaves, in particular, have been reported to have potent antioxidant, anti-inflammatory, anticancer and also in maintaining the integrity of the skin tissue [6,7]. In pharmaceutical preparations, it can be used to treat conditions such as gastrointestinal disorders, abdominal pain and constipation [8,9].

The present work was conducted to investigate the possible usefulness of the mallow leaves aqueous extract in maintaining of intestinal comfort and constipation treatment.

2. Materials and methods

2.1. Chemicals

Epinephrine, bovine catalase, butylated hydroxytoluene (BHT), 2-Thio-barbituric acid (TBA), yohimbine, charcoal meal, gum Arabic, red phenol, methyl cellulose, and trichloroacetic acid were from Sigma chemicals Co (Germany). All other chemicals used were of analytical grade.

2.2. Plant collection and MSAE preparation

Mallow (*Malva sylvestris* L.) leaves were collected during March 2016 from the area of Béja (North-West of Tunisia) and identified by the laboratory of taxonomy in the Faculty of Sciences of Tunis (FST)-Tunisia. The Voucher specimens (No. MSB01) has been deposited with the herbarium of the Higher Institute of Biotechnology of Béja. The Mallow leaves were dried in an incubator at 40 °C during 72 h and powdered in an electric blender (MOULINEX Ovatio 2, FR). The leaves powder was then dissolved in distilled water and incubated at room temperature for 24 h in a shaking bath. Sample was centrifuged at 10,000*g* for 10 min and the supernatant was lyophilized, aliquoted and stored at −80 °C until use.

2.3. Animals

Adult male Wistar rats (weighing 200–220 g, 15 weeks old and housed five per cage) were purchased from Pasteur Institute of Tunis and used in accordance with the local ethics committee of Tunis University for the use and care of animals and in accordance with the NIH recommendations [10]. They were provided with standard food (BADR, Utique, TN) and water *ad libitum* and maintained in animal house under controlled temperature (22 ± 2 °C) with a 12/12 h light–dark cycle.

2.4. Induction of constipation in the rats

Constipation was induced in the animals by oral administration of 1 mL loperamide (3 mg/kg body weight in 0.9% sodium chloride for 5 days) at 09:00 and at 18:00 h, while the control rats were administered with the normal saline only [11,12].

The *Malva sylvestris* aqueous extract was administered at a dose of (100, 200 and 400 mg/kg, *b.w. p.o*) daily, one hour after loperamide administration during the experiments. The latter group was treated with yohimbine (2 mg/kg, *b.w. p.i.*), used as standard drug. Preliminary experiment indicated that 100, 200 and 400 mg/kg *b.w* MSAE were the lowest doses that give a significant protective effect. We measured water intake, food intake, body weight throughout the experiment. The excreted fecal pellets were daily collected throughout the experiment, and total number, weight and fecal water content were determined.

At the end of experiments, the animals were sacrificed, blood was collected and plasma processed for lipid profile, urea, creatinine, glucose and transaminases determinations. The distal portions of the colon for each animal, were removed and cut longitudinally, then cleaned with physiological saline to remove fecal residues and homogenised in phosphate buffer saline. After centrifugation at 10,000*g* for 10 min at 4 °C, supernatant was used for biochemical determination of protein, –SH groups, reduced glutathione (GSH), calcium, free iron, H_2O_2 and MDA levels as well as antioxidant enzyme activities.

2.5. Measurement of gastro-intestinal propulsion (GIP)

Five groups of 10 animals each were fasted for 16 h and treated as follows: Group 1 served as a control and received 1 mL of physiological solution (NaCl, 0.9%, p.o.); Group 2 received yohimbine (2 mg/kg, b.w. i.p.) and groups 3, 4 and 5 were treated with various doses of MSAE (100, 200 and 400 mg/kg, *b.w. p.o.*). GIP was measured using the charcoal meal test as previously described by Ali and Bashir [13]. Briefly, two hours after treatment, all animals received the standard charcoal meal (10% charcoal in 5% gum Arabic). 30 min later, animals were anesthetized and the small intestine was rapidly dissected out and put on a clean surface. The distance traveled by the charcoal meal from the pylorus was measured. The gastrointestinal propulsion was calculated according to the following rule:

GIP = Distance moved by charcoal (cm)/total intestinal length (cm) × 100

2.6. Measurement of gastric emptying

The gastric emptying rate was determined by the red phenol method [14]. Five groups of 10 animals each were used: Group 1 served as a control and received 1 mL of physiological solution (NaCl, 0,9%, *p.o.*), groups 2, 3 and 4 were pre-treated with various doses of MSAE (100, 200 and 400 mg/kg, *b.w. p.o.*). and group 5 was pre-treated with yohimbine (2 mg/kg, *b.w. p.i.*), 1 h prior to test meal (1,5 mL/rat) consisting of a solution of 50 mg phenol red in 100 mL aqueous methyl cellulose (1.5%). Sixty min later, the animals were killed. The stomach and its contents were homogenised with 100 mL of 0.1 N NaOH. The suspension was allowed to settle for 1 h at room temperature, and 5 mL of the supernatant was added to 0.5 mL of 20% (w/v) trichloroacetic acid and then centrifuged at 1800*g* for 20 min. The supernatant was mixed with 4 mL of 0.5 N NaOH and the absorbance of the sample read at wavelength of 560 nm. Phenol red recovered from animals killed immediately after the administration of the test meal was used as standard (0% emptying). Percent gastric emptying was calculated according to the following formula:

Gastric emptying rate (%) = (1 − absorbance of treated/absorbance of standard) × 100

2.7. Biochemical estimations

SOD activity was estimated according to the method described by Misra and Fridovich [15]. GPx activity was determined according to the method described by Flohé and Günzler [16]. CAT activity was measured using Aebi's method [17]. Thiol groups (–SH) was performed according to Ellman's method [18] and GSH level according to Sedlak and Lindsay method. MDA was estimated

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