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Original article

Phytochemical properties and pharmacological effects of *Quercus ilex* L. aqueous extract on gastrointestinal physiological parameters *in vitro* and *in vivo*

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ABSTRACT

Introduction: Several research studies have reported on the pharmacological relevance of the medicinal plants used for treating various gastrointestinal disorders and controlling the dietary glucose uptake in the intestinal tract.
Methods: Male rats were used to investigate the pharmacological effects of green oak acorn aqueous extract (GOAE) on gastrointestinal physiological parameters *in vivo* and *in vitro*. In this respect, the gastrointestinal motility and hypersecretion essays were evaluated using a simple test meal (10% charcoal in 5% gum arabic) and castor oil induced diarrhea. However, the effect of GOAE on glucose absorption and homeostasis was assessed by the Ussing chamber system and oral glucose tolerance test (OGTT) measures.
Results: Various doses of the *Quercus ilex* aqueous extract (125, 250 and 500 mg kg^{-1}) administered orally produced a significantly dose-related inhibition of gut meal travel distance in normal rat. The highest intestinal transit reduction of 49.34% was obtained with 500 mg kg^{-1} compared to 58.33% caused by reference drug (clonidine, 1 mg kg^{-1}). In castor oil induced diarrhea in rat, Q. ilex extract reduced the frequency of defecation, fluid accumulation and electrolyte transport. These effects were associated with decreased histopathological damage and regulation of intracellular mediators disturbance in the intestinal mucosa. In addition, GOAE treatment improved glucose tolerance and significantly and dose-dependently reduced (>50%) the glucose absorption via intestinal epithelium. Phytochemical screening revealed the presence of many bioactive natural compounds.
Conclusion: These results suggest that the extract was effective towards reducing diarrhea, fluid accumulation, electrolyte transport and glucose absorption, and no toxic effects of the GOAE presented on this study.

1. Introduction

Intestinal transport of water and electrolytes is carried out largely across the villous epithelium [1]. Fluid and electrolyte imbalance can produce numerous perturbations such as diarrhea. This pathophysiology is a dominant factor in morbidity and mortality worldwide, especially among children in developing countries resulting in a major health care problem [2]. Moreover, the pathogenesis of diabetes mellitus, a chronic metabolic disorder, is characterized by deregulation of glucose transport and metabolism. Indeed, the absorption of dietary glucose through intestinal mucosa is made essentially by the Na^+/glucose co-transporter (SGLT1) and the glucose transporter type 2 (GLUT2). In many recent studies, to control hyperglycemia, SGLT1 activity has

Abbreviations: CO, Castor oil; DM, Dry matter; DNS, Dinitrosalicylic acid; GAE, Gallic acid equivalent; GIT, Gastrointestinal transit; GOAE, Green oak acorns aqueous extract; Isc, Short circuit current; LOP, Loperamide; NaCl, Sodium chloride; OGTT, Oral glucose tolerance test; QE, Quercetin equivalent; TAE, Tannic acid equivalent.

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to be regulated via numerous bioactive compounds to monitor glucose transport and to prevent against diabetes [3].

Currently, there is no prophylactic therapy to ameliorate or prevent the gastrointestinal disorders. Therefore, the ability of bioactive compounds to dampen or inhibit nutrient absorption and hypersecretion may represent an attractive new therapeutic strategy in the treatment of motility disorders and diabetes. It has been reported that plant secondary metabolites include flavanoids, phenolics acid and tannins display a significant antisecretory effect and inhibition of intestinal glucose absorption [4–6].

Quercus trees, commonly known as oaks, belong to the family Fagaceae. Acorns, the fruit of oak trees, have long been employed as a source of tannin, oil, and especially food because of the content of carbohydrates, amino acids, proteins, lipids, and various sterols [7]. In North Africa, notably in Tunisia, large areas of unexploited oak acorn forest have raised the interest of scientists mainly due to their large availability and their resistance to drought. Oak kernels were traditionally used in medicine, particularly roasted ones as astringents and antidiarrheals [8,9]. However, to the best of our knowledge, there are no scientific data concerning the effect of *Quercus ilex* fruit on gastrointestinal disorders. The aim of this study was to examine the phytochemical properties and the pharmacological effects of *Quercus ilex* L. aqueous extract on gastrointestinal physiological parameters *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals and reagents

Sodium pentobarbital, D-mannitol, D-glucose, charcoal meal, gum Arabic, clonidine, loperamide, gallic acid, quercetin, tannic acid, Folin–Ciocalteu, dinitrosalicylic acid, castor oil, haematoxylin and eosin were from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). All other chemicals and reagents used were of analytical grade.

2.2. Green oak acorn collection and aqueous extract preparation

Green oak acorns were collected from the area of Fernana (North-West of Tunisia) during the month of December 2014 and identified by the laboratory of taxonomy in the Faculty of Sciences of Tunis (FST)-Tunisia. Oak acorns were subsequently dried in an incubator at 50 °C for 72 h and powdered in an electric mixer. The pulp powder was dissolved in double-distilled water. The water extracts were prepared (1/5;w/v) under magnetic for 24 h and the homogenate was filtered through a colander (0.5 mm mesh size). Finally, the supernatants were lyophilized (extraction yield = 10%) and used.

2.3. Phytochemical screening

GOAE phenolic content was examined using the Folin-Ciocalteau reagent method [10]. The results were expressed as gallic acid equivalence (GAE) in mg/g of dry matter (DM). The total flavonoid content was estimated by the method developed by Jia et al., [11]. It was reported in terms of mg of quercetin equivalents (QE)/g of DM. Tannins were determined spectrophotometrically using the method of Folin–Ciocalteu reaction using tannic acid as a standard [12]. The results were expressed as tannic acid equivalence (TAE) in mg/g of dry matter.

We used dinitrosalicylic acid (DNS) as a reagent for the determination of total and reducing sugars [13]. The fiber contents in the samples were analyzed using enzymatic-gravimetric analysis according to AOAC International Official Method 991.42. [14]. All experiments were performed in triplicates at each concentration.

2.4. Animals used for experimentations

Wistar male rats weighing 200–230 g and male mice weighing 20–30 g (SIPHAT, Ben-Arours, Tunisia) were kept in cages under standard laboratory conditions with tap water and standard ad libitum, in a 12-h/12-h light/dark cycle at a temperature between 21 and 23 °C. The experimental protocol was realized in accordance with the local ethics committee of Tunis University for the use and care of animals in accordance with the NIH recommendations [15].

2.5. Acute toxicity study

Orally administration of Q. ilex aqueous extract at various doses ranging from 0.05 to 5 g kg^{-1} was used to assess acute toxicity in mice (n = 10). The animals were observed every 30 min during 4 h and then, occasionally for additional period of 8 h. Mortality was recorded 24 h after the treatment. The mice were also examined for other signs of toxicity, such as motor co-ordination, righting reflex and respiratory changes.

2.6. Test of motility and movement of electrolytes across the epithelium

Rats fasted for 16 h (were used and given by oral administration NaCl (0.9%, control group) or various doses of green oak acorn aqueous extract (125, 250 and 500 mg kg^{-1}), the testing of these doses has shown clear changes in the parameters studied). One group of animals was received the reference drug (clonidine, 1 mg kg^{-1}). 2 h later, all animals were given a test meal. 30 min later, rats were anaesthetized, a laparotomy was performed and the distance traveled by the meal compared to the total length of the small intestine was measured according to the following rule:

% GIT = Distance moved by charcoal (cm)/total intestinal length (cm) x 100

To clarify the effects of castor oil (5 mL kg^{-1}), various doses of green oak acorn aqueous extract and reference drug (10 mg kg^{-1}, b. w., *p.o.*) on small intestinal physiological parameters, animals fasted for the same period were randomly allocated to two groups (six animals for each). Group I received 10 mL kg^{-1} of saline solution, group II treated orally with castor oil. The other three groups (III, IV and V) were pretreated with the extract at various doses in combination with castor oil. However, the last group (VI) was pretreated with reference drug in association with castor oil. The number of defecations was counted during 4 h. For fluid accumulation, after 2 h, animals were anaesthetized by intraperitoneal administration of sodium pentobarbital (40 mg kg^{-1} b.w.) and sacrificed by decapitation (Fig. 1). The small intestine was removed and the content was milked into a graduated tube and measured. After centrifuging of the intestinal fluid, the electrolytes concentrations in the supernatants were measured by flame photometry. The characteristic diarrheal droppings were noted in the absorbent paper placed beneath the individual rat perforated cages [16,17].

2.7. Determination of intracellular mediators

Hydrogen peroxide level in small intestinal mucosa was performed according to Dingeon et al., [18]. Briefly, the hydrogen peroxide reacts with p-hydroxybenzoic acid and 4-aminoantipyrine in the presence of peroxidase leading to the formation of quinoneimine that has a pink color detected at 505 nm. Results were expressed as μmol of H_2O_2 per mg of protein.

Free iron was determined by the FerroZine method using a commercially available kit from Biomaghreb, Tunisia. Briefly, at