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Original Article

Antiulcerogenic and antioxidant activities of *Plantago ovata* ethanolic extract in rats

Atividade antiulcerogênica e antioxidante do extrato etanólico de *Plantago ovata* em ratos

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Abstract

This study aimed to determine the antiulcerogenic and antioxidant activities of Psyllium (*Plantago ovata* Forssk) seed ethanolic extract in rats. We assessed the antioxidant potential using free radical scavenging on DPPH, β-carotene bleaching activity, ferric reducing power, and hydroxyl radical scavenging activity. In the antiulcerogenic study, pre-treatment with *Plantago ovata* seeds ethanolic extract (POE) (400 mg/kg b.wt) significantly protected against ethanol-induced gastric ulcer in rats by decreasing the ulcer index value and preserving the integrity of the gastric mucosa. The oxidative stress status in the stomach tissues showed a significant increase in the antioxidant enzyme levels of superoxide dismutase, catalase, and glutathione peroxidase with a significant decrease in lipid peroxidation during pre-treatment with POE. In conclusion, the POE protects against gastric ulcer due to its antioxidant potential and presence of bioactive molecules.

Keywords: *Plantago ovata*, ulcer index, CAT, SOD, GPx, MDA, ethanol induced gastric ulcer, gastric mucosa.

Resumo

O presente estudo teve como objetivo determinar as atividades antiulcerogênica e antioxidante das sementes de Psyllium (*Plantago ovata* Forssk) em ratos. O potencial antioxidante foi avaliado utilizando o método do sequestro do radical livre DPPH, autooxidação do β-caroteno, poder redutor de ferro e atividade de sequestro do radical hidroxila. No estudo antiulcerogênico, o pré-tratamento com o extrato etanólico das sementes de *Plantago ovata* (POE) (400 mg/Kg b.wt) reduziu a úlcera gástrica induzida pelo etanol em ratos, diminuindo o valor do índice de úlcera e preservando a integridade da mucosa gástrica. O estudo do estresse oxidativo nos tecidos estomacais mostrou um aumento significativo dos níveis das enzimas antioxidantes superóxido dismutase, catalase e glutationa peroxidase, com uma diminuição significativa da peroxidação lipídica enquanto pré-tratamento com POE. Em conclusão, o POE protege contra úlcera gástrica devido aos seus potenciais antioxidantes e à presença de moléculas bioativas.

Palavras-chave: *Plantago ovata*, índice de úlcera, CAT, SOD, GPx, MDA, úlcera gástrica induzida por etanol, mucosa gástrica.

1. Introduction

Gastric ulcers constitute a major class of gastrointestinal disorder attracting global attention in health care (Choudhary et al., 2013) since about 4 million people are affected by gastric ulcers worldwide. Gastric ulcer is a lesion of the gastric mucosa resulting from an imbalance between offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors (Urs et al., 2014), characterized by necrosis, infiltration of neutrophils, blood flow reduction, oxidative stress induction, and secretion of inflammatory mediators (Amirshahrokhi and Khalili, 2015). This imbalance is often due to exogenous factors, which include infection with Gram-negative bacteria such as *Helicobacter pylori*, unhealthy diet, tobacco, excessive alcohol consumption (Laloo et al., 2013) stress, and excessive ingestion of non-steroid anti-inflammatory drugs, in addition to endogenous factors such as hydrochloric acid, pepsin, and reactive oxygen species (Rozza et al., 2012).

Among the various factors, alcohol consumption is the greatest contributor to gastric ulceration (Franke et al., 2005). Excessive alcohol consumption generally generates gastric mucosal injuries, including gastritis and ulcer, which may progress to gastric cancer (Sowndhararajan

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and Kang, 2013). Research has demonstrated that alcohol consumption leads to oxidative stress, which could result in overproduction of reactive oxygen species (ROS) that might play an important role in gastric ulcer (Chauhan and Kang, 2015; Bhattacharyya et al., 2014). Previous studies have shown that elevated lipid peroxidation and MDA, along with mucosal impairment, are associated with ethanol-induced gastric ulcer in rats (Boligon et al., 2014; La Casa et al., 2000). Otherwise, administration of antioxidants and/or increased antioxidant enzymes protect against ethanol-induced gastric ulcer via inhibiting oxidative stress-induced cell damage (Aranzales et al., 2015; El-Naga, 2015).

The treatment of ulcerative disease is still progressing and a large number of synthetic drugs have been used, such as antacids, proton pump inhibitors, anticholinergics, and antihistamines (Panda and Khambat, 2014). However, many of these drugs may produce undesirable adverse effects, such as thrombocytopenia, nephrotoxicity, hepatotoxicity, gynecomastia, and impotence in gastric ulcer patients (Martins et al., 2014). Therefore, it is necessary to discover new anti-ulcer agents that are more effective and less toxic to combat aggressive factors or stimulate mucous membrane defenses.

Phytotherapy represents a very interesting alternative therapy scope at lower cost and has never stopped evolving. It is possible to use the whole plants or their extractive products. Secondary metabolites, natural products of plant origin, emerge as a promising therapeutic resource and have been a major research object for the development of new drugs with possibly fewer side effects.

We chose the *Plantago ovata* Forssk because it is the most important genus of the Plantaginaceae family and has been long used in the traditional medicine to treat wounds, bronchitis, haemorrhagia, cystitis with hematuria, and diarrhea (Tita et al., 2009). The husk and seed of *P. ovata* are commonly known as psyllium and is widely used as a fiber supplement in the treatment of constipation (Voderholzer et al., 1997). Psyllium is classified as a mucilaginous fiber due to its powerful ability to form a gel in water. The aqueous extract of *P. ovata* seeds contain a high proportion of hemicellulose, composed of anionic polysaccharide of *L*-arabinose, *D*-xylose, and *D*-galactoronic acid (Wearnberg et al., 2009).

The actual seed has a higher amount of fermentable fiber that is effective at preventing Crohn's constipation, diarrhea, disease, obesity, hypercholesterolemia, diabetes, and atherosclerosis (Sahagùn et al., 2015). Clinical studies indicate that aqueous extract of *P. ovata* seed can increase stool weight and reduce intestinal transit time in constipated patients (Sleisenger et al., 1993; Marlett et al., 2000). Recent findings suggest that *P. ovata* husk has a protective effect on the intestinal mucosa against acetylsalicylic acid, probably due to a reduction in the absorption of acetylsalicylic acid into epithelial cells (Sahagún et al., 2015).

Thus, the aim of this study was to assess the protective effect of the *P. ovata* ethanolic extract against gastric ulcer induced by ethanol in rats.

2. Material and Methods

Firstly, we analyzed the total phenolic content, flavonoids, and tannins of POE and its antioxidant *in vitro* potential and subsequently the *in vivo* effect of POE on ethanol-induced gastric ulcer by measuring the stomach and mucus weight, gastric jus volume and pH, ulcer index (UI), oxidative stress markers, and stomach histology alterations.

2.1. Preparation of aqueous extract

Commercialized seeds of *Plantago ovata* (POE) were purchased from a traditional local market specialized in organic products and healthy foods (Origin: India; year of production: 2018; lot number: BK02/2018). The seeds were botanically identified by a Plant Biology Ph.D: Dr. Issam Saidi, who confirmed that the species purchased is *Plantago ovata* seeds.

Then, Psyllium seeds were grounded and homogenized in ethanol, then soaked for about 24 h at room temperature under continuous stirring. The mixture was filtered and centrifuged at 4500 g for 15 min, and the supernatant was dried in a rotavapor at 40 °C, in addition to lyophilized and stored at -21 °C until use.

2.2. Determination of total phenolic contents

We determined the total phenolic content of POE (1 mg/mL) using the Folin-Ciocalteu method (Tlili et al., 2013). Briefly, a volume of 250 µl of the extract was mixed with 0.125 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 1 mL of 7.5% saturated sodium carbonate (w/v). After 2 h of incubation at 45 °C, we measured the absorbance at 765 nm through Shimadzu 1240 model spectrophotometer. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/g of POE) through a calibration curve ranged 0-100 µg/mL (R^2=0.9927) and all tests were carried out in triplicate.

2.3. Determination of total flavonoid content

We applied a method described by Djeridane et al. (2006) to determine the total flavonoid content in POE (1 mg/mL). A volume of 500 µL of POE was mixed with 150 µL of $NaNO_2$ and 150 µL of $AlCl_3.6H_2O$ methanolic solution (2%). Then, after 15 min of incubation at room temperature, we measured the mixture absorbance at 430 nm. The amount of total flavonoid content is expressed as rutin equivalents (mg RE/g of POE) through the calibration curve ranged 0-400 µg/mL (R^2=0.9644) and all tests were carried out in triplicate.

2.4. Determination of total condensed tannins (Proanthocyanidin)

We determined the total condensed tannins of POE through the vanillin-H_2SO_4 method (Heimler et al., 2006). 3 mL of vanillin methanolic (4%) and 1.5 mL of concentrated sulfuric acid were added to 400 µl of POE (1 mg/mL). Subsequently, we subjected the mixture to a 15-minute incubation at room temperature and measured the absorbance at 430 nm. The amount of proanthocyanidin

is expressed as catechin equivalents (mg CE/g POE) and the calibration curve ranged 0-350 µg/mL (R^2=0.9978).

2.5. In vitro antioxidant activity of POE

2.5.1. DPPH radical scavenging activity

We applied the method reported by Grzegorczyk et al. (2007) to estimate the free radical scavenging activity of POE through the DPPH radical assay. 1 mL of various concentrations of POE (0-400 µg/mL) was mixed with 1 mL of a methanolic solution of DPPH (0.1 mM) and incubated for 30 min at 37 °C. We prepared a second range of concentrations with 1 mL of methanol to serve as a control solution. Ascorbic acid was used as a reference at the same concentration range as the test extract. Subsequently, we measured the absorbance of each sample at 517 nm. All analyses were performed in triplicate. The POE antioxidant activity was calculated as follows (Equation 1):

$$ARA\% = 1 - \left[\left(A_{sample} - A_{control} \right) / A_{DPPH} \right] \times 100 \quad (1)$$

where A_{DPPH} is the absorbance of DPPH solution without sample extract, A_{sample} is the absorbance of sample extract mixed with DPPH solution, and $A_{control}$ is the absorbance of the sample extract tested without DPPH. The IC_{50} value is the concentration of POE capable of scavenging 50% of the DPPH radical.

2.5.2. Ferric reducing antioxidant power

We assessed the capacity of the POE, at different concentrations (0-500 µg/mL), to reduce the ferric ion (Fe^{3+}) present in the $K_3[Fe(CN)_6]$ complex to ferrous ion (Fe^{2+}) using the method described by Chu et al. (2000). Briefly, 2.5 mL of potassium phosphate buffer (0.1 M, pH 6) and 2.5 of potassium ferricyanide (1% w/v) were mixed to 1 mL of the *P. ovata* extract (0-500 µg/mL). We subjected the reaction mixture to a 20-minutes incubation at 50 °C in a water bath, and subsequently added 2.5 mL of trichloroacetic acid solution (10%, w/v) for the mixture to be centrifuged at 3000 rpm for 10 min. Then, 2.5 mL of supernatant was mixed to 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ and finally incubated at 20 °C during 30 min. We measured the absorbance of the samples at 700 nm. Ascorbic acid was used as standard for comparison purposes and the tests were carried out in triplicate.

2.5.3. Determination of antioxidant activity using β-carotene bleaching (BCB) assay

We followed the experimental protocol by Ozsoy et al. (2008). 2 mg of β-carotene were dissolved in 10 mL of chloroform, and 1 mL of this solution was mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40. The chloroform was then evaporated under reduced pressure by a rotavapor at 40 °C and the resulting residue was taken up by 50 mL of ultra pure oxygenated water. We prepared tubes containing 5 mL of this emulsion adding 200 µL of POE samples or standard antioxidants (BHT) at different concentrations (2-10 mg/mL). We agitated the mixture and measured the absorbance at 470 nm immediately at t_0 against a blank containing the emulsion without the β-carotene. Covered tubes were incubated at 50 °C and absorbance reading is performed after 120 min. A control is prepared in parallel containing 5 mL of β-carotene emulsion and 200 µL of ethanol.

The relative antioxidant capacity was calculated according to the Formula 2:

$$Inhibition\% = \left[1 - \left(AS_0 - AS_{120} \right) / \left(AC_0 - AC_{120} \right) \right] * 100 \quad (2)$$

where AS_0 is the absorbance of the sample at t = 0 min; AS_{120} is the absorbance of the sample at t = 120 min; AC_0 is the absorbance of the control at t = 0 min, and AC_{120} is the absorbance of the control at t = 120 min. All determinations were performed in triplicate.

2.5.4. OH• scavenging activity

We assessed the OH• radical scavenging activity of the extract according to the Wang et al. (2008) method with few modifications. The reaction mixture (3 mL) consists of 1 mL of $FeSO_4$ (1.5 mM), 0.7 mL of H_2O_2 (6 mM), 0.3 mL of sodium salicylate (20 mM) and different concentrations of the POE (0-250 µg/mL). BHT was used as standard. After incubation at 37 °C for 1 h, the absorbance was measured at 562 nm.

The scavenging effect of the hydroxyl radical is calculated according to the following Equation 3:

$$\%\ Scavenging = \left[1 - \left(A_1 - A_2 \right) / A_0 \right] * 100 \quad (3)$$

where A_0: negative control absorbance; A_1: test absorbance, and A_2: absorbance of the test without H_2O_2.

2.6. In vivo antiulcerogenic activity

2.6.1. Animals

Adult male Wistar rats, weighing 150 g, were obtained from the Faculty of Sciences of Gabes (Tunisia). They were fed with standard diet (SNA, Sfax, Tunisia) and water *ad libitum*, under standard conditions of temperature (22 ± 2 °C), relative humidity (70 ± 4%), and 12 h light/dark cycle. All animals were subjected to a 24-hour fast before the experiment and kept in cages with raised floors of wide mesh to prevent coprophagia.

The experimental protocol was approved by the Faculty of Ethics Committee in our institution under ethics approval number 1204. Animals were maintained in accordance with international guidelines for care and use of living animals in scientific investigations (Council of the European Communities, 1986).

2.6.2. Toxicity study of the extract

The study was carried out using 24 Wistar rats divided randomly into 4 groups orally supplemented with gradual concentrations of *Plantago ovata* ethanolic extract (100 to 1000 mg/kg bw). The animals were kept directly under observation for toxic symptoms, after the first 4 h of dosing. 24 h later, the surviving animals were maintained under daily observations for two weeks to have their general behavior studied, as well as discomfort nervous manifestation and any other signs of toxicity and mortality.

2.6.3. Animal treatment

The animals were divided into 4 groups, each containing 6 rats. All animals were treated by gavage as follow:

- Control group (T): received distilled water 0.5 mL/rat for 14 days orally by gavage. On the last day, 1 h after the first administration, they orally received a second administration of distilled water;
- Ethanol treated group (E): received 0.5 mL of distilled water for 14 days orally by gavage. On the last day, 1 h after the first administration, they orally received 10 mL/kg of absolute ethanol (Zheng et al., 2016);
- Omeprazole treated group (OMP): received omeprazole at 20 mg/kg bw (El-Naga, 2015) by gavage for 14 days followed by absolute ethanol to induce gastric ulcer;
- POE treated group (POE): received POE at 400 mg/kg by gavage for 14 days followed by absolute ethanol.

The animals were sacrificed by decapitation one hour after ethanol or distilled water administration. We collected the stomachs, ligated the pyloric canal, collected and centrifuged the gastric juice to measure its volume and pH.

The stomachs were weighed then opened along the great curvature; the gastric wall mucus was gently scraped with a glass slide and weighed.

2.6.4. Determination of the ulcer index and the protection index

We visualized the mucosal lesions of the collected stomachs through magnifying glass to be scored according to selected scale, as follows: (Gul et al., 2016)

0 = Normal coloration
0.5 = Red coloration
1 = Spot ulcer
1.5 = Hemorrhagic stress
2 = Deep ulcer
3 = Perforations

Ulcer index was calculated according to the Formula 4 below:

$$U_I = \left(U_n + U_s + U_p\right)/10 \quad (4)$$

where U_n is the average of number of ulcers per animal, U_S is the mean severity of ulcer score, and U_p is the percentage of animals with ulcer incidence (Umamaheswari et al., 2007).

Anti-ulcer activity was calculated by applying the Formula 5:

$$Anti-ulcer\ activity = \left[\left(U_{IE\ group} - U_{ITreated\ group}\right)/U_{IE\ group}\right]*100 \quad (5)$$

where $U_{IE\ group}$ is the ulcer index of rats treated with ethanol and $U_{ITreated}$ group is the ulcer index of rats pre-treated with POE (P) or omeprazole (Omp).

2.6.5. Biochemical assays

Subsequent to the macroscopic analyses, we grounded the stomach tissues (1 g) in Tris buffer saline solution (1 mL) (pH 7.4) using a crusher (homogenizing Ultra-Turax) to be centrifuged with 9000 tr/min for 15 min at 4 °C. Supernatants were collected and used for the biochemical assays. The protein content was determined through Lowry's method (Lowry et al., 1951). MDA was estimated using the thiobarbituric acid test (Ohkawa et al., 1979). We estimated the SOD activity according to the method described by Misra and Fridovich (1972), and measured CAT activity using the Aebi's (1984) method. GPx activity was determined according to the method described by Sazuka et al. (1989).

2.6.6. Histopathological examination

Formalin-fixed stomachs were processed according to the routine, embedded in paraffin, sectioned at 3-4 µm, and stained with hematoxylin and eosin (H&E). An expert in histopathological assessment (Gabe, 1968) examined the slides.

2.7. Statistical analysis

We performed two independent experiments. Data were expressed as means ± standard deviation (SD). Statistical significance was assessed using Student's t-test. $p < 0.05$ was considered significant.

3. Results

3.1. Phytochemical studies of Plantago ovata ethanolic extract

The phytochemical studies of *Plantago ovata* ethanolic extract (POE) revealed a high number of polyphenols (286.8 ± 6.05 mgGAE/gDW of POE), a significant number of flavonoids, and a lower amount of condensed tannins (101.83 ± 12.9 mgRE/gDW POE and 39.33 ± 1.52 mgCE/gDW POE, respectively) (Table 1).

3.2. In vitro antioxidant capacity

3.2.1. DPPH radical scavenging activity

Figure 1 illustrates the *in vitro* antiradical activity of the POE against the DPPH radical. Indeed, the higher the extract concentration the higher the anti-DPPH activity, until reaching the maximum concentration of 400 µg/mL. We determined the antioxidant capacity from IC_{50}, which corresponds to the concentration required to reduce

Table 1. The *Plantago ovata* ethanolic extract phytochemical composition.

	Total phenolics (mgGAE/gDW)[a]	Total flavonoids (mgRE/gDW)[b]	Total condensed tannins (mgCE/gDW)[c]
Plantago ovata	286.8 ± 6.05	101.83 ± 12.9	39.33 ± 1.52

[a]Total phenolic content as the gallic acid equivalent; [b]Total flavonoid content as the rutin equivalent; [c]Condensed tannin as the catechin equivalent. Values are expressed as mean ± SD ($n = 3$).

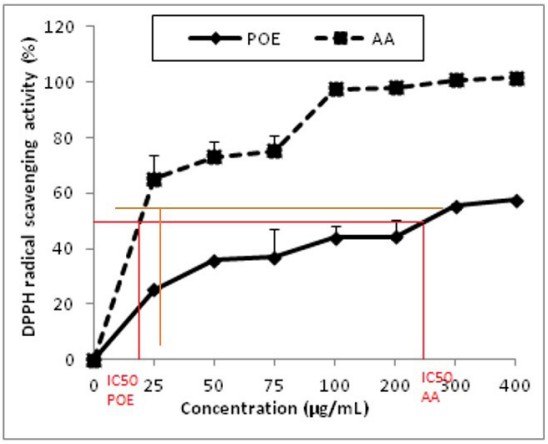

Figure 1. The antiradical activity of *Plantago ovata* against the radical DPPH. Values are represented as mean ± SD (n = 3).

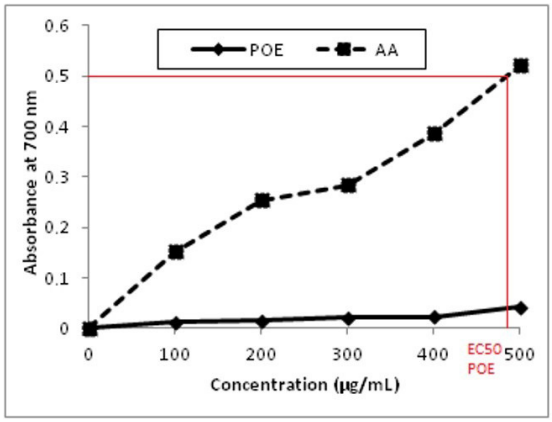

Figure 2. The reducing power of *Plantago ovata* and ascorbic acid by the FRAP assay. Values are expressed as mean ± SD (n = 3).

50% of the DPPH radical. The IC_{50} of *P. ovata* extract is mathematically calculated and valued as 228.69 ± 3.3 µg/mL, which is significantly lower than the ascorbic acid used as a positive control (19.41 ± 2.71 µg/mL) (Table 2).

3.2.2. Ferric reducing antioxidant power

As shown in Figure 2, Psyllium is able to reduce Fe^{3+} to Fe^{2+} at different concentration ranges. The reducing power of POE and its concentration are dose-dependent. It was found to be 0.042 ± 0.008 of absorbance unit at 500 µg/mL at the effective EC_{50} concentration of 59.97 ± 1.03 mg/mL. Such activity appeared significantly ($p < 0.001$) lower in relation to the positive control (ascorbic acid), which was 0.52 ± 0.003 of absorbance unit at 500 µg/mL with EC_{50} of 0.47 ± 0.002 mg/mL (Table 2).

3.2.3. Antioxidant activity using the β-carotene assay

In this assay, linoleic acid oxidation produced hydroperoxyl radicals. We tested the antioxidant activity through the b-carotene/linoleate model system of POE and established a comparison with BHT (Figure 3). In the linoleic emulsion system, the oxidation of β-carotene was effectively inhibited by the POE, achieving values of IC_{50} up to 10.56 ± 3.11 mg/mL (Table 1). A significant difference was found between the antioxidant activity of POE and BHT ($p < 0.05$).

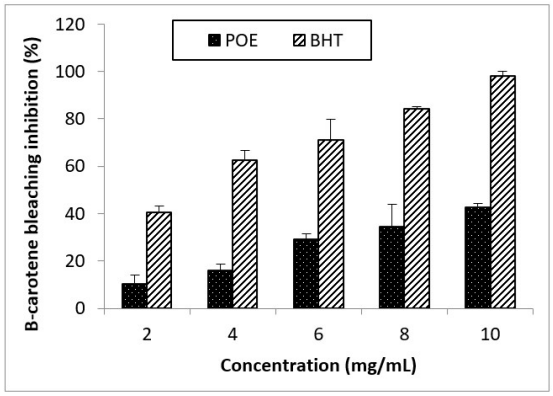

Figure 3. The inhibition of β-carotene potential of *Plantago ovata.* Values are expressed as mean ± SD (n = 3).

3.2.4. OH• scavenging activity

As shown in Figure 4, the POE presented an important potential to scavenge the OH• radicals. The IC_{50} of POE (75.3 ± 0.42 µg/mL) is highly significant comparing with BHTs IC_{50} (27.24 ± 0.54 µg/mL) (Table 2).

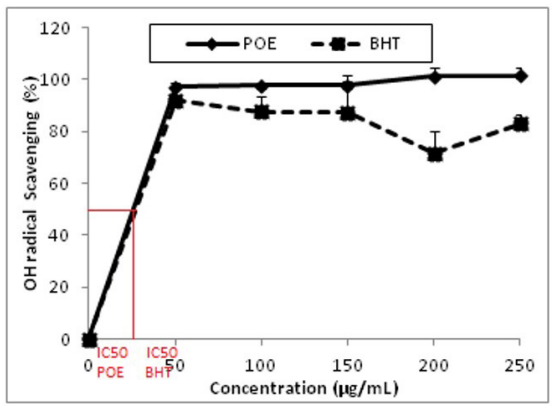

Figure 4. The radical OH• scavenging activity of *Plantago ovata.* Values are expressed as mean ± SD (n = 3).

3.3. Antiulcerogenic activity

3.3.1. Acute toxicity

The tested animals received different doses of Psyllium extract (100-1000 mg/kg). The data of the acute toxicity test showed no toxicity or lethality up to 1000 mg/kg. In this study, we chose to use a dose of 400 mg/kg BW to investigate the antioxidant and antiulcerogenic activities of the ethanolic extract of *P. ovata* in experimental animals.

3.3.2. Effect of POE on various gastric physiochemical and morphological parameters in ethanol-induced gastric ulcer

The stomach weight in ethanol-treated rats increased significantly by 38.54% ($p < 0.001$), while for the control group, the gastric mucus secretion has significantly decreased from 67.66 ± 10.01 mg to 39.5 ± 10.19 mg ($p < 0.001$). POE administration induced an increase of mucus weight (SMW) from 39.5 ± 10.19 mg for the (E) group to 79.5 ± 9.54 ($p < 0.001$), in addition to a significant decrease of the stomach weight by 15.03% ($p < 0.05$). These results are comparable to those of the OMP group (1.04 ± 0.08 vs 1.13 ± 0.08 g to POE group ($p > 0.05$) for stomach weight, and 81.66 ± 7.55 vs 79.5 ± 9.54 mg to the POE group ($p > 0.05$) for SMW).

The gastric juice volume (GJV) in the stomachs of rats treated with ethanol increased significantly in relation to the control group (3.23 ± 0.45 vs 0.66 ± 0.24 mL; $p < 0.001$) (Table 3), leading to greater gastric volume (Figure 5). It is worth emphasizing that the pre-treatment of rats with

Table 2. The *Plantago ovata* ethanolic extract antioxidant capacity.

	DPPH (IC_{50},µg/mL)	FRAP (EC_{50},mg/mL)	β-carotene inhibition (IC_{50}, mg/mL)	OH• radical scavenging (IC_{50}, µg/mL)
Plantago ovata	228.69 ± 3.3	59.97 ± 1.03	10.56 ± 3.11	25.72 ± 0.42
Ascorbic acid	19. 41± 2.71	0.47 ± 0.002	_	_
BHT	_	_	3.09 ± 1.63	27.24 ± 0.54

Values are expressed as mean ± SD ($n = 3$).

Table 3. Effect of POE and OMP on various gastric physicochemical parameters in gastric ethanol-induced ulcer.

Groups	Stomach weight (g)	SMW (mg)	GJ Volume (mL)	GJ pH
T	0.96 ± 0.12	67.66 ± 10.01	0.66 ± 0.24	4.25 ± 0.42
E	1.33± 0.08 ++	39.5 ± 10.19 ++	3.23 ± 0.45 ++	1.83 ± 0.4 ++
OMP	1.04 ± 0.08 **	81.66 ± 7.55 **	1.21 ± 0.49 **	4.83 ± 0.75 **
POE	1.13 ± 0.08 *	79.5 ± 9.54 **	1.42 ± 0.5 **	3.66 ± 0.81 **

SMW = stomach mucus weight; GJ = gastric juice. Values correspond to the mean of 6 measurements ± SD compared with t-test. * $p \leq 0.05$; ** $p \leq 0.001$ compared to ethanol treated group (E); ++ $p \leq 0.001$ compared to control group (T).

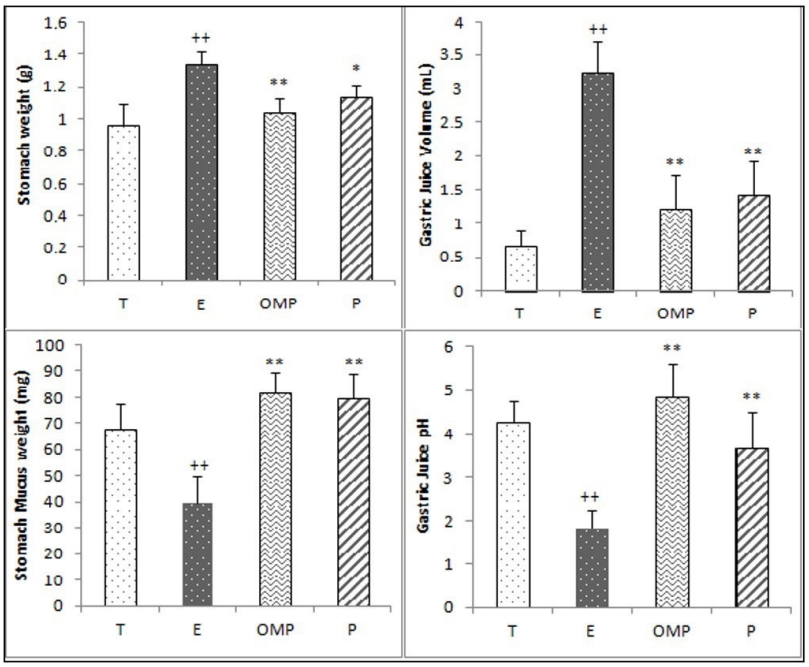

Figure 5. Effect of POE and OMP on stomach and mucus weight and on gastric juice volume and pH. T = control group, E = ethanol treated group, OMP = omeprazole pretreated group (20 mg/kg), P = POE pretreated group (400 mg/kg). Values correspond to the mean of 6 measurements ± SD compared with t-test. * $p \leq 0.05$. ** $p \leq 0.001$ compared to ethanol treated group (E). + $p \leq 0.05$. ++ $p \leq 0.001$ compared to control group (T).

POE significantly decreased the stomach changes induced by alcohol intoxication, a decrease of around 56.03% ($p < 0.001$) comparing with the (E) group (1.42 ± 0.5 vs 3.23 ± 0.45 mL for (E) group) (Figure 5). Importantly, these results are comparable to those of the OMP group (1.21 ± 0.49 vs 1.42 ± 0.5 mL; $p > 0.05$).

In contrast, gastric juice pH significantly decreased in ethanol-treated rats in relation to the control group (1.83 ± 0.4 vs 4.25 ± 0.5; $p < 0.01$) (Table 3). Interestingly, POE pretreatment significantly increased the gastric juice pH (3.66 ± 0.81 vs 1.83 ± 0.4 for (E) group) at a rate of 100% (Table 3). Omeprazole (OMP), used as reference molecule, markedly increased the gastric juice pH from 1.83 ± 0.4 to 4.83 ± 0.75 ($p < 0.001$).

3.3.3. Effect of POE on the gastric macroscopic damage induced by ethanol and protection indexes

As shown in Figure 6, no macroscopic lesions were observed in the control group. Intragastric administration of ethanol induced macroscopic morphological changes, such as hemorrhagic streaks and ulceration spots in the mucosal layer (Figure 6) with a significant increase in the ulcer index (U_I) (Figure 7, Table 4). These changes are attenuated with the administration of OMP (20 mg/kg bw) to reach an UI = 8.62 ± 0.03 vs 11 ± 0.01 mm^2 for the E group ($p < 0.001$). However, pretreatment with POE considerably reduced areas of gastric damage in the ethanol-treated group (Figure 6) and decreased the U_I by 50% (from 11 ± 0.01 mm^2 in the (E) group to 10.49 ± 0.009; $p < 0.001$) (Figure 7, Table 4). Furthermore, the values of protection percentage (PP) recorded were 4.62% ± 0.21 for POE pre-treatment and 21.61% ± 0.34 for OMP (Table 4).

3.3.4. Effect of POE on stomach oxidative stress biomarkers

Ethanol-exposed group showed significant oxidative changes with an increase in lipid peroxidation as shown by the increase in the MDA levels in relation to the control group (8.2 ± 1.97 vs 2.98 ± 0.38 nmol/mg protein; $p < 0.001$) (Figure 8). Interestingly, POE pretreatment significantly protected the gastric mucosa from lipid peroxidation. The MDA level was 4.26 ± 0.31 vs 8.2 ± 1.97 nmol/mg protein for the (E) group ($p < 0.01$).

The activity of gastric CAT was 8.31 ± 0.61 µmoles H_2O_2/min/mg protein and decreased by 65.34% in ethanol-treated rats (2.88 ± 0.87 µmoles H_2O_2/min/mg protein) ($p < 0.001$). The animals pre-treated with POE exhibited a restoration in CAT activity (Figure 8), which was 4.03 ± 0.45 µmoles H_2O_2/min/mg protein ($p < 0.01$) vs 2.88 ± 0.87 µmoles H_2O_2/min/mg protein for the (E) group). OMP restored CAT activity to a level of 6 ± 0.68 vs 8.31 ± 0.61 µmoles H_2O_2/min/mg protein for the control group ($p < 0.001$).

Table 4. Effect of POE and OMP on Ulcer index (UI) and Protection index (PI) in gastric ethanol-induced ulcer.

Groups	UI (mm^2)	PI (%)
T	0.0 ± 0.0	_
E	11 ± 0.01 $^{++}$	_
OMP	8.62 ± 0.03 **	21.61 ± 0.34
POE	10.49 ± 0.009 **	4.62 ± 0.21 **

Values correspond to the mean of 6 measurements ± SD compared with t-test. ** $p \leq 0.001$ compared to ethanol treated group (E); ++ $p \leq 0.001$ compared to control group (T); "" $p \leq 0.001$ compared to OMP group.

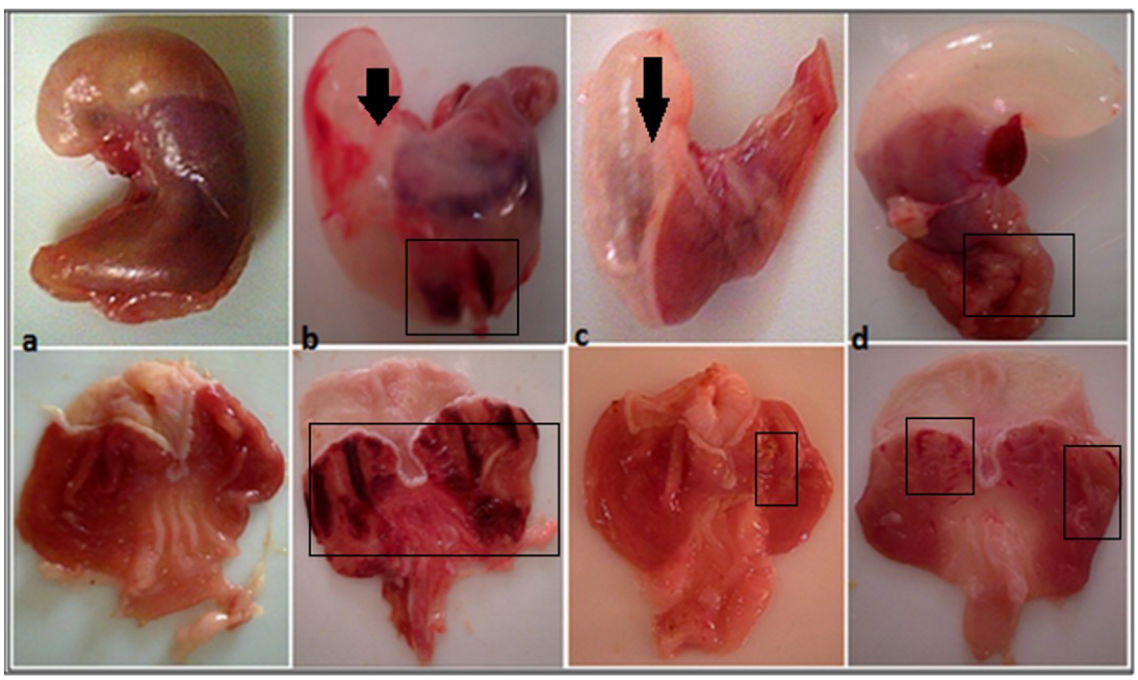

Figure 6. Gastroprotective effect of POE and OMP against ethanol-induced ulcer on rats. (a) control group (T); (b) E group; (c) OMP group (20 mg/kg); (d) POE group (400 mg/kg). arrows = gastric juice accumulation; rectangles= gastric wall ulcerations.

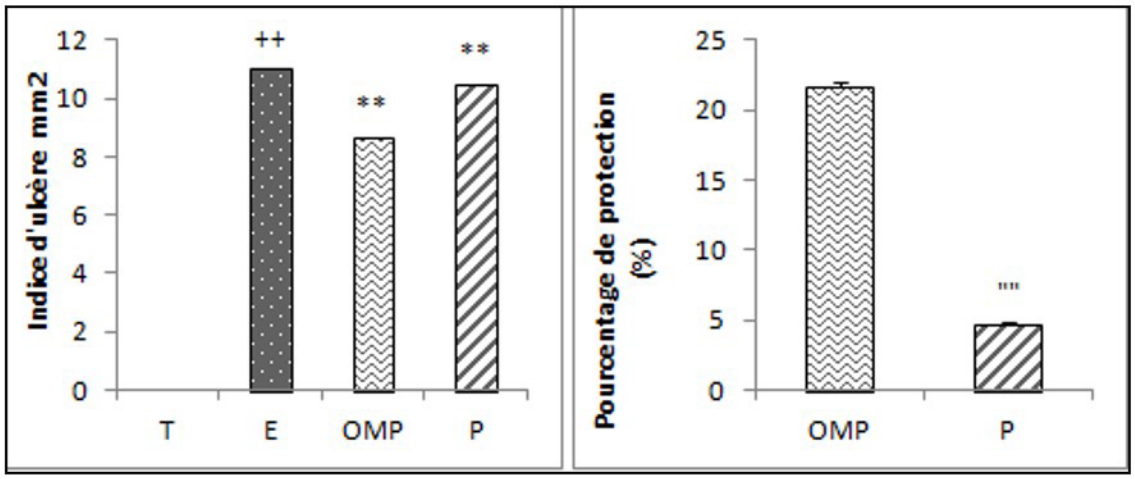

Figure 7. Ulcer index and Percentage of protection. Values correspond to the mean of 6 measurements ± SD compared with t-test. ** $p \leq 0.001$ compared to ethanol treated group (E). ++ $p \leq 0.001$ compared to control group (T). "" $p \leq 0.001$ compared to OMP group.

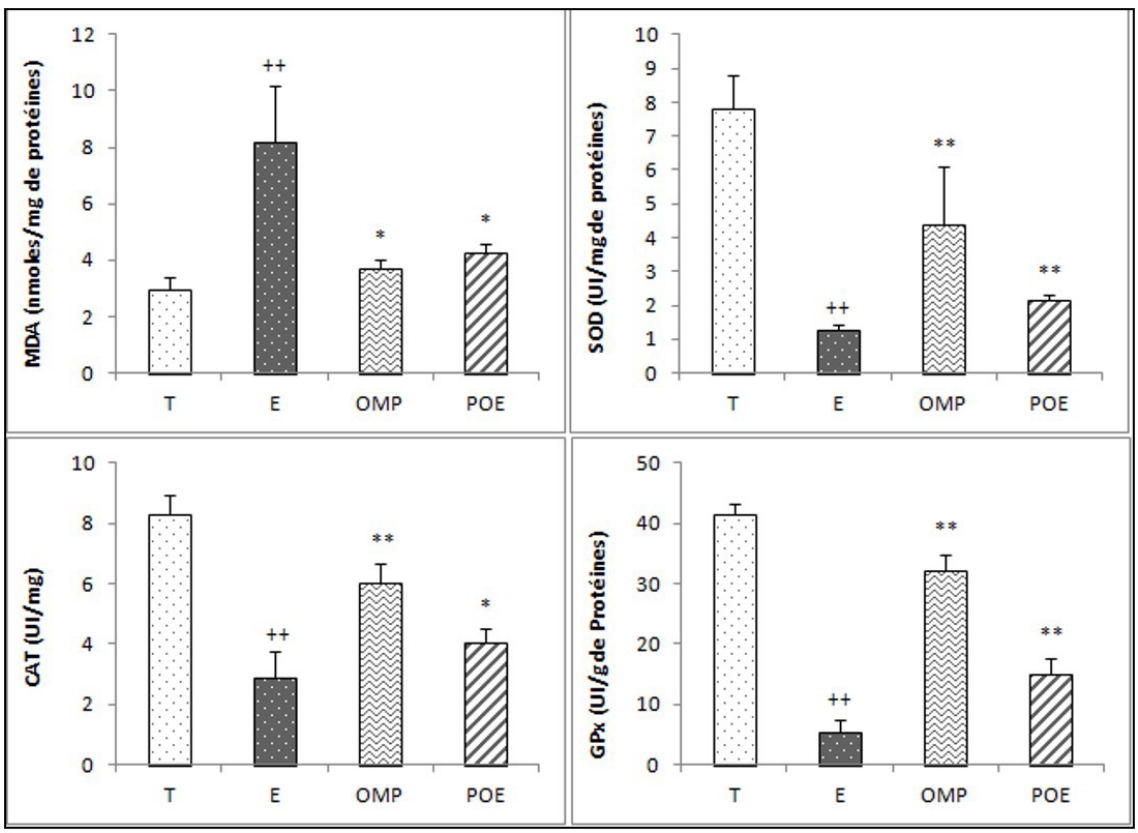

Figure 8. Effect of ethanolic extract of *P. ovata* (POE) and omeprazole (OMP) treatments on lipid peroxidation (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) activities. E = ethanol; OMP = omeprazole (20 mg/kg); POE = *P. ovata* seeds ethanolic extract (400 mg/kg). All drugs were administered by oral way. Values are expressed as mean ± SEM for six animals in each group compared with t-test. * $p \leq 0.05$. ** $p \leq 0.001$ compared to ethanol treated group (E). + $p \leq 0.05$. ++ $p \leq 0.001$ compared to control group (T).

Similarly, ethanol treatment induced a significant decrease in SOD activity from 7.81 ± 0.97 to 1.27 ± 0.13 UI/min/mg protein ($p < 0.001$) (Figure 8), which was inhibited by POE. The high significant inhibition was recorded for the POE and OMP pretreatments, in which SOD activity was maintained at a value of 2.16 ± 0.17 UI/min/mg protein

for POE ($p < 0.001$) and 4.38 ± 1.69 UI/min/mg protein for the OMP group ($p < 0.001$).

GPx activity was 41.53 ± 1.87 nmol/min/mg protein in the control group and decreased with ethanol administration, reaching 5.33 ± 2.25 nmol/min/mg protein ($p < 0.001$) (Figure 8). Pre-treatment with POE significantly reversed the ethanol action ($p < 0.001$) by maintaining GPx activity at a value of 15.11 ± 2.63 nmol/min/mg protein. This protective activity also appeared in the OMP group (32.24 ± 2.7 nmol/min/mg protein; $p < 0.001$).

3.3.5. Effect of POE on histopathological gastric injuries

The microscopic study of the histological sections proved that the gastric mucosa of rats treated with ethanol showed morphological changes, such as erosive lesions (Figure 9, B and b). The surface epithelial cells

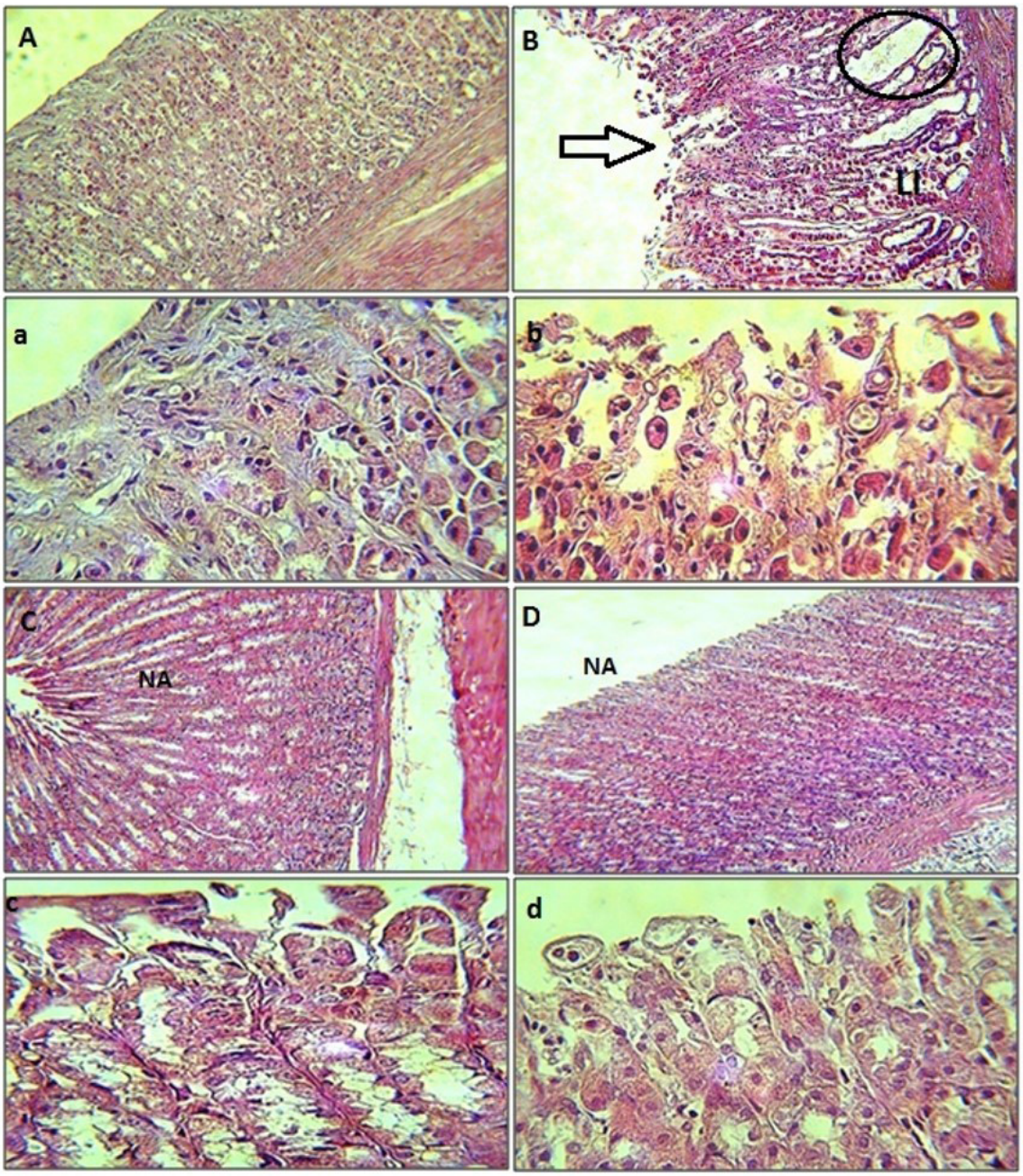

Figure 9. Histological photographs of rat gastric mucosa (sections was stained with H&E and magnification was ×20 for capital letter and x40 for small letter). (A, a) Stomach from control rat; (B, b) Stomach from rat treated with ethanol showed inter-glandular space dilatation (circle), exfoliation of epithelial cells (arrow) and leucocytes infiltration (LI); (C, c) Stomach from ulcerated rats pretreated with omeprazole; and (D, d) Stomach from ulcerated rat pretreated with POE showed regular gastric layer and normal architecture (NA).

were disrupted and became more reddish. Inflammatory cells infiltrated in the gastric mucosa. The inter-glandular spaces were dilated and became deeper. However, rats pretreated with POE showed a marked attenuation of histopathology changes induced by ethanol. The gastric layer became more regular in the "lamina propria" of gastric mucosa (Figure 9, D and d). The rats treated with omeprazole and distilled water (T group) presented an apparently intact gastric mucosa, in addition to showing a continuous epithelial surface and a thin layer of mucus in the neck cells (Figure 9, C and c).

4. Discussion

In order to investigate the *Plantago ovata* protective effect against ethanol-induced ulcer, we studied the POE following three steps: exploring its polyphenols, flavonoids, and tannin contents, assessing its antioxidant potentials *in vitro*, and studying its anti-ulcer activity *in vivo*.

The phytochemical screening of the POE showed high numbers of polyphenols, flavonoids, and tannins, which could be responsible for its anti-oxidative and anti-ulcer efficacy (Chaouche et al., 2014). Species of *Plantago* are known for the presence of phenolic compounds with antioxidant activity (Pereira et al., 2017). In fact, these compounds play important roles in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Pareek et al., 2011; Stagos et al., 2012). Phenolics, flavonoids and condensed tannins have demonstrated an ideal structural chemistry for free radical scavenging activity derived from the following features: high reactivity as hydrogen or electron donors, ability to stabilize and delocalize the unpaired electron (chain-breaking function), and potential to chelate metal ions (termination of the Fenton reaction) (Chanda and Dave 2009; Zhong et al. 2010).

Therefore, this study prioritized investigating the antioxidant activities of POE *in vitro*. The DPPH radical scavenging power (IC_{50} = 228.69 ± 3.3 µg/mL) and ferric reducing test (EC_{50} = 59.97 ± 1.03 mg/mL) showed a low potential in Psyllium extract in relation to the ascorbic acid. However, the β-carotene inhibition (IC_{50} = 10.56 ± 3.11 mg/mL) and $OH^{\cdot}$ radical scavenging (IC_{50} = 25.72 ± 0.42 µg/mL) tests showed a significant potential comparing with BHT. In this context, studies demonstrated that the reducing activity of plant extract could be largely attributed to flavonoids content due to their chelating ability for metal ions, such as iron and copper (Chanda and Dave, 2009; Alimi et al.,2013). Compounds with reducing power indicate an electron donor characteristic and ability to reduce the oxidized intermediates of lipid peroxidation processes to act as primary and secondary antioxidants (Alimi et al., 2013).

The redox properties of polyphenols are believed to be the main driver of their antioxidant mechanism, allowing them to act as reducing agents, hydrogen donors, free radical quenchers, metal chelators, and decomposers of peroxides (Itagaki et al., 2009). Besides, tannins are known to have styptic properties due to their ability to react with the proteins of the tissue layers with which they come into contact. Tannins are known to 'tan' the outermost layer of the mucosa rendering it less permeable and more resistant to injury or irritation. Applying tannins to the mucosa at a low concentration leads to precipitation of micro-proteins at ulcer sites forming a protective layer that makes it less susceptible to biological and chemical irritation (De Jesus et al., 2012).

The investigation of gastric physicochemical and morphological parameters (stomach weight, stomach mucus weight, gastric juice volume and pH, ulcer index, and protection percentage) illustrated the protective efficacy of POE on ethanol-induced ulcer. Ethanol administration has induced an increase in stomach weight (1.33 ± 0.08 g), gastric juice volume (3.23 ± 0.45 mL), and ulcer index (11 ± 0.01), possibly due to a stomach inflammation resulting from an erosion of the gastric mucosa. Inas et al. (2011) have also attributed gastrointestinal injury to eroded mucin content. This erosion is facilitated by onslaughts of both internal (pepsin and oxidants produced in the gastric lumen) and external (drugs and chemicals) aggressive agents on mucosal epithelia (Sabiu et al., 2015). In correlation with our results, a study on indomethacin-induced ulcer explained that the significant increase in ulcer index and gastric volume following oral administration of indomethacin in the ulcerated rats may be attributed to either free radical formation or inhibition of prostaglandin synthesis.

The decreased prostaglandin level has been attributed to impaired gastroprotection and increased gastric acid secretion, which are important events in the etiology of mucosal ulceration (Sabiu et al., 2015). However, a decrease in SMW and gastric juice pH appeared in the ethanol-treated group. In fact, pH gives an idea of the level of acidity and volume of gastric secretions. Low pH value is a manifestation of increased hydrogen ion concentration in gastric juice. This has been linked to the pathogenesis of ulcer and gastric damage in experimental animals (Lüllmann et al., 2000) through histamine stimulation by alcohol administration, leading to a continuous secretion of chloride acid by stomach parietal cells.

Histologically, ethanol caused tissue damage in the stomach: exfoliation of epithelial cells, enlargement of the inter-glandular space, and infiltration of neutrophils in ulcerated areas. These manifestations have improved in the groups pretreated with the POE. Other studies (Bagheri et al., 2018) indicated absence of gastric pathological changes, such as leucocytes infiltration, in rats pretreated with aqueous extract of *P. ovata* seeds (AEPOS), in addition, the tissue was able to maintain its regular architecture. Such beneficial effects may be related to a highly hydro soluble fiber of AEPOS that provides markedly viscous solutions and increases the thickness of the gastrointestinal diffusion barrier. This effect could help to maintain the morphology and functional integrity of the liver and stomach by preventing indomethacin penetration and trapping in the gastric mucosa layer.

The action developed by the Psyllium fibers would not only be protective against deleterious effects of acetylsalicylic acid, but also restorative, thus favoring epithelial turnover and regeneration. In this sense, fiber was able to stimulate proliferation in the gastric glands

directly (Goodlad et al., 1995), while fractions of *Plantago ovata* strongly stimulated the proliferation of keratinocytes (Deters et al., 2005). Such protective effect results from the ability of this fiber to fix organic and inorganic substances, their absorption by the intestinal epithelium is delayed, reduced or even avoided, protecting this epithelium from a potential damage (Leng-Peschlow, 1991; Wilpart and Roberfroid, 1987).

In contrast, the gastric physicochemical and morphological parameters were ameliorated in the POE pretreated group, and the lower ulcer index (10.49 ± 0.009) illustrated the protective anti-ulcer activity of Psyllium. These results corroborate a study by Bagheri et al. (2018) demonstrating that the aqueous extract of *Plantago ovata* seed significantly decreased the macroscopic and microscopic ulcer indices. High fiber intake, especially soluble fibers, would have a mucosa-protective action, reducing the risk of promoting a faster healing of duodenal ulcers (Sahagún et al., 2015). Indeed, Psyllium seed produces large amounts of butyrate and acetate. Butyric acid exhibits anti-neoplastic activity against colorectal cancer and may be helpful in the treatment of ulcerative colitis (Nordgaard et al., 1996). These protective effects are associated with the antioxidant activities and antiradical power of POE, in addition to the richness of POE in bioactive molecules such as phenols, flavonoids, tannins, and fibers. This plant is classified as a bulk-forming laxative, which means a mucilage of Psyllium seeds in the presence of water swells forming a gel layer (Hosseini et al., 2015). Moreover, the *Plantago ovata* husk is a higher hydro soluble fiber that provides a markedly viscous solution. Such viscosity is associated with prolonged gastric emptying (Marciani et al., 2000), increasing the retention of ethanol in the stomach, and consequently delaying the drug access to the gastric mucosa. Additional mechanisms may include an anti-inflammatory action, reducing pro-inflammatory biomarkers such as C-reactive protein, IL-6, IL-12 or tumor necrosis factor-α (Galisteo et al., 2010; Kumar et al., 2012).

La Casa et al. (2000) suggested that free radicals could be the main causes of gastric ulcer development, while Hamauzu et al. (2007) demonstrated that the free radicals generated by neutrophil cells have a significant role in the development of gastric ulcer registered in ethanol-treated rats.

Our study has shown that the administration of ethanol significantly ($p < 0.001$) decreased the activities of SOD, CAT, and GPx enzymes comparing with the control rats. In contrast, pretreatment of ethanol-ulcerated rats with POE preserved the activities of the same antioxidant enzymes nearly to normal, in addition to reducing the lipid peroxidation product (MDA). Ethanol has the ability to degrade mucous membrane proteins and induce local inflammation. This event calls upon the activated neutrophil migration, in which they can produce and release hydrogen peroxide (H_2O_2) and superoxide radicals (O^{2-}) worsening and deepening the ethanol-initiated ulcer (Cunningham and Van Horn, 2003).

Plant derived antioxidants, especially phenolic compounds, have been ascribed to various properties, like anti-cancer, anti-ulcer, anti-inflammatory, anti-diabetic, anti-aging, and prevention of cardiovascular diseases (Dixon et al., 2005). Flavonoids are antioxidant compounds that efficiently remove superoxide anion, hydroxyl, peroxyl, and alcoxyl radicals (Mota et al., 2011), while the removal of these same ROS along with peroxynitrite radicals has been described for chlorogenic and caffeic acids as well (Amaral et al., 2013). Since superoxide anion and hydroxyl radical are the ROS involved in oxidative stress caused by ethanol and peroxyl, and alcoxyl radicals are the major products in the LPO process (Laine et al., 2008). The scavenging of these species explains the protective effects of *P. ovata* against gastric injury induced by ethanol. In addition, some flavonoids also interfere with inflammation process and increase mucus content in gastric mucosal, resulting in cytoprotective effects (Shaker et al., 2010; Amaral et al., 2013).

5. Conclusion

Our results allow to conclude that POE could prevent gastrointestinal lesions because of the fiber solubility and viscosity less ethanol absorption, or the antioxidant potential of its active compounds. Further studies should be conducted to determine the active constituents of aqueous extract of *P. ovata* seeds, providing the extract with anti-ulcer properties, and elucidate the mechanisms underlying the phenomena of inflammation and ulceration.

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