## PAPER

# Individual and synergistic protective properties of *Salvia officinalis* decoction extract and sulfasalazine against ethanol-induced gastric and small bowel injuries

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The present study was carried out to determine the phytochemical composition of *Salvia officinalis* flowers decoction extract (SOFDE) as well as its individual and/or synergistic actions with sulfasalazine against ethanol (EtOH)-induced peptic ulcer in Wistar rats. In this respect, rats were divided into six groups of eight animals each: control, EtOH, EtOH + sulfasalazine (SULF, 100 mg kg$^{-1}$, b.w., p.o.), mixture: MIX (SOFDE, 50 mg kg$^{-1}$ b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.) and EtOH + two doses of SOFDE (100 and 200 mg kg$^{-1}$ b.w., p.o.). *In vitro*, the phytochemical and the antioxidant properties were determined using colorimetric analysis. HPLC-PDA/ESI-MS assay was used to identify the distinctive qualitative profile of phenolic compounds. Our results firstly indicated that SOFDE is rich in total tannins, flavonols, anthocyanins and a moderate concentration of total carotenoids. Chromatographic techniques allowed the identification of 13 phenolic compounds and the major ones are quinic acid, protocatechuic acid, gallic acid and salviolinic acid. SOFDE also exhibited an important *in vitro* antioxidant activity using the β-carotene bleaching method. *In vivo*, SOFDE and the mixture provide significant protection against ethanol-induced gastric and duodenal macroscopic and histological alterations. Also, SOFDE alone or in combination with SULF, showed a significant protection against the secretory profile disturbances, lipid peroxidation, antioxidant enzyme activities and non-enzymatic antioxidant level depletion induced by alcohol administration. Importantly, we showed that EtOH acute intoxication increased gastric and intestinal calcium, free iron, magnesium and hydrogen peroxide (H$_2$O$_2$) levels, while SOFDE/MIX treatment protected against all these intracellular mediators' deregulation. We also showed that alcohol treatment significantly increased the C-reactive protein (CRP) and alkaline phosphatase (ALP) activities in plasma. The SOFDE and MIX treatment significantly protected against alcohol-induced inflammation. More importantly, we showed in the present work that the mixture exerted a more important effect than SOFDE and SULF each alone indicating a possible synergism between these two molecules. In conclusion, our data suggests that SOFDE and SULF exerted a potential synergistic protective effect against all the macroscopic, histological and biochemical disturbances induced by EtOH intoxication. This protection might be related in part to its antioxidant and anti-inflammatory properties as well as by negatively regulating Fenton reaction components such as H$_2$O$_2$ and free iron.

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## Introduction

Peptic ulcer is caused by the loss of balance between aggressive and defensive factors of the gastric and duodenal mucosa.[1] Also, it can be caused by *Helicobacter pylori*,[2] the use of non steroidal anti-inflammatory drugs,[3] smoking,[4] the imbalance between the secretion of hydrochloric acid as well as the production of calcium bicarbonate to buffer the pH in the gastrointestinal microbiota.[5]

Alcohol consumption is a well-known risk factor for tissue injury and gastroduodenal ulcer. It also affects other organs such as the heart, kidneys, brain, liver and pancreas.[6,7]

Moreover, previous studies have shown an inhibitory effect on the synthesis of prostaglandins leading to lesions of the gastric mucosa. This disease may also be related to neutrophil activation leading to an excessive production of reactive oxygen species (ROS).[8] Under physiological conditions, ROS are produced in small quantities during cellular respiration and metabolism, which are important for several physiological processes.[9,10] However, the intracellular imbalance between their genesis and degradation contributes to oxidative stress. This situation has been accompanied by significant damage of lipids, proteins and nucleic acids leading to cell death.[11,12]

The mixture of bioactive compounds from plant and synthetic drugs is an alternative for the protection and/or treatment of various pathologies and to substitute commercial drugs known for their unpredictable side effects.[13] However, in the gastrointestinal system, prolonged use of drugs (anticholinergic drugs, histamine H2-receptor antagonists, antacids) and especially with relatively high doses can exhibit toxic side effects leading to severe constipation, diarrhea, hypersensitivity reactions such as rashes, fever, central nervous system aberrations[14] and colorectal cancer.[15] In women, misoprostol can cause malformations of the embryo.[16] Importantly, we recently used a mixture between sage and loperamide as a strategy to fight against castor oil-induced diarrhea.[17]

*Salvia officinalis* (Lamiaceae family) is known as a medicinal and aromatic plant due to its richness of natural active substances.[18] Due to its antioxidant[19] and anti-inflammatory[20] properties, sage extracts exhibit many beneficial health effects such as phytoestrogenic,[21] neurprotective,[22] anti-microbial[23] and anticancer[24] activities. More importantly, this plant has been widely used in the treatment of most gastrointestinal diseases like diarrhea and dyspepsia.[17,25]

*Salvia officinalis* is an inexhaustible reservoir of chemical compounds such as alkaloids, carbohydrate, fatty acids, glycosidic derivatives (flavonoid glycosides, saponins), phenolic compounds (coumarins, flavonoids, tannins), polyacetylenes, steroids, terpenes (monoterpenes, diterpenes, triterpenoids).[26,27] However, Mansourabadi *et al.*[28] reported that flavonoids extracted from *Salvia officinalis* presented anti-inflammatory properties in carrageenan model of mouse and induced analgesic effect in a dose-dependent manner. In addition, several others molecules such as manool, carnosol and ursolic acid have been previously showed for their anti-inflammatory properties.[29,30]

Hence, the present study aimed to investigate the individual and synergistic protective properties of *Salvia officinalis* flowers decoction extract and sulfasalazine against ethanol-induced peptic ulcer in rat.

# Experimental

## Reagents and chemicals

2-Thio-barbituric acid (TBA), epinephrine, bovine catalase, trichloroacetic acid (TCA), butylated hydroxyl toluene (BHT) were from Sigma chemicals Co. (Sigma-Aldrich GmbH, Steinheim, Germany). Ethanol (EtOH), sulfasalazine, sodium chloride (NaCl 0.9%) were purchased from central pharmacy of Tunisia. All the other chemicals used were of analytical grade.

## Plant material and decoction extract

The sage flowers were cultivated in the region of Ain Draham (NW-Tunisia) during April 2018 and identified by Dr Imen Bel Hadj Ali, Associate professor in the Higher Institute of Biotechnology of Béja-Tunisia. The voucher specimens (No. SO.321) have been deposited with the Herbarium of the Higher Institute of Biotechnology of Béja. The plant material was dried in the open air and powdered in an electric blender. The decoction was made with distilled water (1/5; w/v) at 100 °C during five minutes under magnetic agitation. The homogenate was filtered by Whatman filter papers and was evaporated at 40 °C in a ventilated oven.

## Phytochemical properties and antioxidant capacity

**Mineral determination.** One gram of powder was placed in a muffle furnace for calcination process (Tony Güller Orselina Zürich MOD L 51/5) at 550 °C for 4 hours.[31] Then the magnesium, iron and calcium concentrations in the samples were determined by an atomic absorption flame spectrophotometer (SHIMADZU AA-6200).

**Identification of phenolic compounds by liquid chromatography-high resolution electrospray ionization mass spectrometry (LC-HRESIMS) assay.** The analysis for phenolic compounds was performed on a Shimadzu UFLC XR system (Kyoto, Japan), equipped with a SIL-20AXR auto-sampler, a CTO-20 AC column oven, a LC-20ADXR binary pump and a quadripole 2020 detector system. Briefly, 100 mg of the plant extract (SOFDE) were dissolved in 100 mL of 10% methanol and filtered and then 1 mL was transferred into LC-MS vials. An opposite-phase column (Pursuit XRs ULTRA 2.8, C18, 100 × 2 mm, Agilent Technologies, UK) was used to carry out HPLC investigations. 20 μL of the prepared samples were injected at a column temperature set at 30 °C. Mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). A gradient program was used for isolation at a flow rate of 1 mL min⁻¹. Mobile phases consisted of an initial composition of 100% solvent A, with a gradient of 100% solvent B over 20 minutes, held at 100% solvent B for 5 min and 100% solvent A for 25 min. The drying gas flow rate was 1 mL min⁻¹ at 320 °C. MS was operated in the positive ion mode in a mass range of 100–2000 $m/z$. High resolution mass spectral data were obtained on a Thermo Instruments ESI-MS system (LTQ XL/LTQ Orbitrap Discovery, UK) connected to a Thermo Instruments HPLC system (Accela PDA Detector, Accela PDA Autosampler and Accela Pump).[32]

**Total tannins, flavonols, total carotenoids and total anthocyanins in SOFDE.** Total tannins were evaluated by the method using the Folin-Ciocalteu reagent.[33] In fact, 500 μL of Folin-Ciocalteu (50%) was added to 500 μL of extract followed by 1 mL of $Na_2CO_3$ (20%). Absorbance of the supernatant was measured using an ultraviolet (UV)-visible spectrophotometer (DU 640B, Beckman Coulter) at 730 nm.

The quantification of flavonols was determined as previously described by Rigane et al.[34] Briefly, 1 mL $AlCl_3$ (20%) was added to 1 ml of the extract and 3 mL of sodium acetate (50 mg mL$^{-1}$). After incubation for 2 hours and 30 minutes, the absorbance was read at 440 nm. Results were expressed as mg of rutin equivalents per 100 gram of dry matter (mg RE/100 g DM).

Total carotenoids in SOFDE was evaluated by the procedure described by Marina et al.[35] Firstly, 1 mL of the extract was mixed with 1 mL of distilled water and 2 mL of extraction solvent (hexane/acetone/ethanol; 50/25/25%; v/v/v) and the mixture was vortexed for 1 min and centrifuged at 6000$g$ at 5 °C for 10 min. The upper layer of hexane containing the pigments was recovered and expelled to a 25 mL volumetric flask. The remaining layer was subjected to a second extraction (same procedure as the extract) and the hexane layers were combined and adjusted to 25 mL. The total carotenoid content was evaluated by adding 1 mL of hexane extract by measuring the absorbance at 450 nm and was expressed in β-carotene using the absorbance coefficient of 2500 according to the following formula:

$$\text{Total carotenoids } (\mu g \text{ mL}^{-1}) = A450 \times \text{volume (mL)} \times 1000/2500 \times \text{weight of the sample (g)}$$

Total anthocyanin compounds were evaluated according to the differentiation and using two buffers: KCl at pH 1.0 (0.025 M) and $CH_3COONa$ (0.025 M) pH 4.5 (0.4 M). 400 μL of the extract were mixed with 3.6 mL of the buffer (1), followed by 400 μL of the other, in buffer (2). The reaction mixture was incubated during 30 minutes in the dark and the absorbances are then read at 510 and 700 nm. The concentration of anthocyanin pigment in the extract is expressed as mg cyanidine equivalent glucosyl-3/g dry matter (mg ECy/g DM).[36]

**Antioxidant activity by the β-carotene bleaching inhibition method.** The antioxidant activity was performed by the method of β-carotene bleaching inhibition according to Kulisica et al.[37] Indeed, the discoloration of β-carotene can be slowed down in the presence of antioxidant, which blocks the formation of free radicals. 0.2 mg of β-carotene, 20 mg of linoleic acid and 200 mg of tween 40 are dissolved in 0.5 mL of chloroform. The solvent was then evaporated and the mixture obtained was diluted with 50 mL of water bubbled with oxygen. 4 mL of the obtained emulsion was expelled into tubes respectively containing 0.2 mL of the extract or studied molecule, 0.2 mL of BHT: synthetic antioxidant for the comparative test and 0.2 mL of the solvent used which will serve as a negative witness. The blocked tubes were kept out of the light and at 50 °C in a water bath. The absorbance of the samples is measured at 470 nm at initial time and every 15 minutes during 120 minutes. The blank test is an emulsion prepared as above but without β-carotene. The coefficient of antioxidant activity (CAA) is determined by the following expression:

$$\text{AA\%} = 100 \times [(A_{E(0min)} - A_{E(120min)})/(A_{0(0min)} - A_{0(120min)})]$$

with $A_{0(0min)}$, $A_{0(120min)}$: absorbance of the control at $t = 0$ and $t = 120$ min, respectively;

$A_{E(0min)}$ et $A_{E(120\ min)}$: absorbance of the sample analyzed at $t = 0$ and $t = 120$ min, respectively.

**Animals and treatment.** Adult male Wistar rats (weighing 214.97 ± 16.12 g) were purchased from the Society of Pharmaceutical Industries of Tunisia (SIPHAT, Ben-Arours, TN). All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Tunis University and approved by the Animal Ethics Committee of National Institute of Health. The test was performed in compliance with the Commission Directive 2000/32/EC and the OECD Guideline 474. 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.

Animals were divided into six groups of eight animals each. Group 1 and 2 served as controls and received distilled water (10 mL kg$^{-1}$, b.w., p.o.) for 15 days. Groups 3 and 4 were pretreated with various doses of the SOFDE (100, 200 mg kg$^{-1}$, b.w., p.o.), group 5 received sulfasalazine (100 mg kg$^{-1}$, b.w., p.o.), while group 6 was pre-treated with the mixture (MIX: SOFDE, 50 mg kg$^{-1}$ b.w., p.o. + sulfasalazine, 50 mg kg$^{-1}$, b.w., p.o.). However, preliminary experiment indicated that the selected doses present the lowest that gives significant protective effects. Rats were fasted for 18 h before the last administration of SOFDE, MIX or reference molecules. After 60 min, each animal, except group 1, was received EtOH (4 g kg$^{-1}$, b.w.) by oral administration. Two hours later, rats were anaesthetized by intraperitoneal administration of sodium pentobarbital (40 mg kg$^{-1}$, b.w.) and sacrificed by decapitation,[38] blood was collected and plasma processed for electrolytes (free iron, calcium and magnesium), alkaline phosphatase (ALP) and C-reactive protein (CRP) determinations.

**Gastric and intestinal fluid accumulation.** The gastric and intestinal fluid was evaluated according to Dicarlo et al.[39] The fluid was collected and centrifuged at 3000 g during 5 min to eliminate insoluble materials. The supernatant was after measured using graduate tubes. After weighting the stomach and the small intestine, the difference between full and empty of two organs were determined.

**Evaluation of gastric and intestinal mucosal damage.** The stomach and small intestine of each animal was thrown out and opened along its greater curvature. The tissue was gently rinsed in NaCl 0.9%. The lesions in the gastric mucosa were macroscopically examined and the photographs of hemorrhagic erosions were taken by Canon EOS1100 D (ISO 6400) digital camera. Ulcer indexes were determined as the sum of the lengths of the whole gastric lesions (mm$^2$). Two independent, blinded observers performed the measurements of lesion lengths.

**Histopathological analysis.** Immediately after sacrifice, small pieces of stomach and duodenum were collected and washed with NaCl (0.9%). Tissue fragments were then fixed in a 10% neutral buffered formalin solution, embedded in paraffin and used for histopathological examination. 5 μm thick sections were cut, deparaffinized, hydrated and stained with

hematoxylin and eosin (HE). The gastric and small intestines sections were examined in blind fashion in all treatments.

**Plasma scavenging activities.** The plasma scavenging activity (PSA) in the different groups was measured using the DPPH radical according the method of Brand-Williams *et al.*[40] Briefly, 100 μL of plasma sample was added to 2 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol solution (100 mM). 1 mL of chloroform was added after incubation of the solution at 37 °C for 30 min and the mixture was centrifuged at 3000 *g* during 10 min. The absorbance of clear supernatant was then determined at 517 nm. DPPH solution was used as a control and the PSA, expressed as percentage, was calculated according to the following equation:

$$PSA\ (\%) = 100 \times (A_{517}\ (\text{control}) \times A_{517}\ (\text{sample})/A_{517}\ (\text{control})).$$

**Lipid peroxidation measurement.** Gastric and duodenal mucosa lipid peroxidation was evaluated by MDA measurement according to the double heating method.[41] Briefly, aliquots from stomach and duodenum mucosa homogenates were mixed with BHT–trichloroacetic acid (TCA) solution containing 1% BHT (w/v) dissolved in 20% TCA (w/v) and centrifuged at 1000 g for 5 min at 4 °C. Supernatant was blended with a solution containing (0.5 N HCl, 120 mM TBA buffered in 26 mM Tris) and then heated at 80 °C for 10 min. After cooling, the absorbance of the resulting chromophore was determined at 532 nm. MDA levels were calculated using an extinction coefficient for MDA–TBA complex of $1.56 \times 10^5$ $M^{-1}$ $cm^{-1}$.

**$H_2O_2$ determination.** The gastric and intestinal mucosa $H_2O_2$ level was determined according to Dingeon *et al.*[42] However, 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.

**Antioxidant enzyme activity assays.** SOD activity in the gastric and duodenal mucosa was determined using modified epinephrine assays.[43] At alkaline pH, superoxide anion induces 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 to 2 mL reaction mixture containing 10 μL of bovine catalase (CAT, 0.4 U $mL^{-1}$), 20 μL of epinephrine (5 mg $mL^{-1}$) and 62.5 mM of sodium carbonate/bicarbonate buffer (pH 10.2). Changes in absorbance were assessed at 480 nm.

The activity of catalase was recorded by measuring the initial rate of $H_2O_2$ disappearance at 240 nm.[44] The reaction mixture contained 33 mM $H_2O_2$ in 50 mM phosphate buffer (pH 7) and the CAT activity was calculated using the extinction coefficient of 40 $mM^{-1}$ $cm^{-1}$ for $H_2O_2$.

The activity of glutathione peroxidase was quantified following the procedure of Flohé and Gunzler.[45] Briefly, 1 mL of

**Table 1** Phytochemical composition and $IC_{50}$ values of the β-carotene bleaching inhibition and chelating effect of the *Salvia officinalis* flowers decoction extract (SOFDE) and butylated hydroxytoluene (BHT)[a]

| Parameters | Contents |
| --- | --- |
| Iron (μmol $L^{-1}$) | $3.29 \pm 0.08$ |
| Magnesium (mmol $L^{-1}$) | $4.25 \pm 0.02$ |
| Extraction yield (%) | $17.22 \pm 0.13$ |
| Calcium (mmol $L^{-1}$) | $6.64 \pm 0.04$ |
| Total tannins (mg TAE/g DM) | $60.41 \pm 3.87$ |
| Flavonols (mg RE/g DM) | $1.99 \pm 0.02$ |
| Total carotenoids (μg/100 mL) | $1.67 \pm 0.09$ |
| Total anthocyanins content (mg CG/g DM) | $3.79 \pm 0.29$ |
| β-Carotene bleaching inhibition $IC_{50}$ (μg $mL^{-1}$) | $56.77 \pm 2.34$ |
| Butylated hydroxytoluene $IC_{50}$ (μg $mL^{-1}$) | $20.83 \pm 0.71$ |

[a] Data are expressed as mean ± SEM ($n = 3$); SEM: standard error of the mean; DM: dry matter; TAE: tannic acid equivalent; CG: cyanidine glucosyl-3; RE: rutin equivalent.

**Table 2** Liquid chromatography-high resolution electrospray ionization mass spectrometry (LC-HRESIMS) analysis of *Salvia officinalis* flowers decoction extract

| Peak no. | Identification[a] | Formula | $[M]^-$ H $m/z$[b] | Retention time (min) | Concentration (ppm) |
| --- | --- | --- | --- | --- | --- |
| 1 | Quinic acid | $C_7H_{12}O_6$ | 191.00 | 2.142 | 181.883 |
| 2 | Gallic acid | $C_7H_6O_5$ | 169.00 | 3.986 | 158.708 |
| 9 | 1,3-Di-*O*-caffeoylquinic acid | ý$C_{25}H_{24}O_{12}$ | 515.00 | 16.761 | 6.433 |
| 3 | Protocatchuic acid | $C_7H_6O_4$ | 153.00 | 6.825 | 155.249 |
| 11 | *p*-Coumaric acid | $C_9H_8O_3$ | 163.00 | 20.642 | 279.886 |
| 16 | Luteolin-7-*O*-glucoside | $C_{21}H_{20}O_{11}$ | 447.00 | 59.969 | 48.608 |
| 20 | Naringin | $C_{27}H_{32}O_{14}$ | 579.00 | 25.560 | 16.987 |
| 21 | Apegenin-7-*O*-glucoside | $C_{21}H_{20}O_{10}$ | 431.00 | 67.712 | 14.217 |
| 23 | Salviolinic acid | $C_{36}H_{30}O_{16}$ | 717.00 | 28.004 | 268.318 |
| 24 | *Trans* cinnamic | $C_9H_8O_2$ | 147.00 | 31.617 | 733.142 |
| 25 | Quercetin | $C_{21}H_{20}O_{11}$ | 301.00 | 31.740 | 40.272 |
| 26 | Kampherol | $C_{15}H_{10}O_6$ | 285.00 | 31.840 | 1.708 |
| 31 | Cirsilineol | $C_{18}H_{16}O_7$ | 343.00 | 38.998 | 5.271 |

[a] The compounds are suggested according to the dictionary of natural products and the characteristic fragmentation pattern. [b] The formulae were deduced from the quasi molecular ion peak $[M + H]^+$.

reaction mixture containing 0.2 mL of gastric or intestinal mucosa supernatant, 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 was stopped by the addition of 0.5 mL TCA (5%, w/v). After centrifugation at 1500$g$ for 5 min, aliquot (0.2 mL) from supernatant was combined with 0.5 mL of phosphate buffer 0.1 M pH 7.4 and 0.5 mL DTNB (10 mM) and absorbance was read at 412 nm. The GPx activity was expressed as nM of GSH consumed/min/mg protein.

**Non-enzymatic antioxidants levels.** The total concentrations of thiol (–SH) groups in the gastric and intestinal mucosa were

determined by Ellman's method.[46] Aliquots of gastric or duodenal mucosa were mixed with 800 μL of 0.25 M phosphate buffer (pH 8.2) and 100 μL of 20 mM EDTA, and the optical density was measured at 412 nm (A1). Subsequently, we added 100 μL of 10 mM DTNB and the reaction mixture was incubated at 37 °C during 15 minutes and a new value (A2) was determined. The thiol groups concentration was calculated by the difference between A2 and A1 using a molar extinction coefficient of $13.6 \times 10^3$ M$^{-1}$ cm$^{-1}$. The results are expressed in nM of thiol groups per mg of protein.
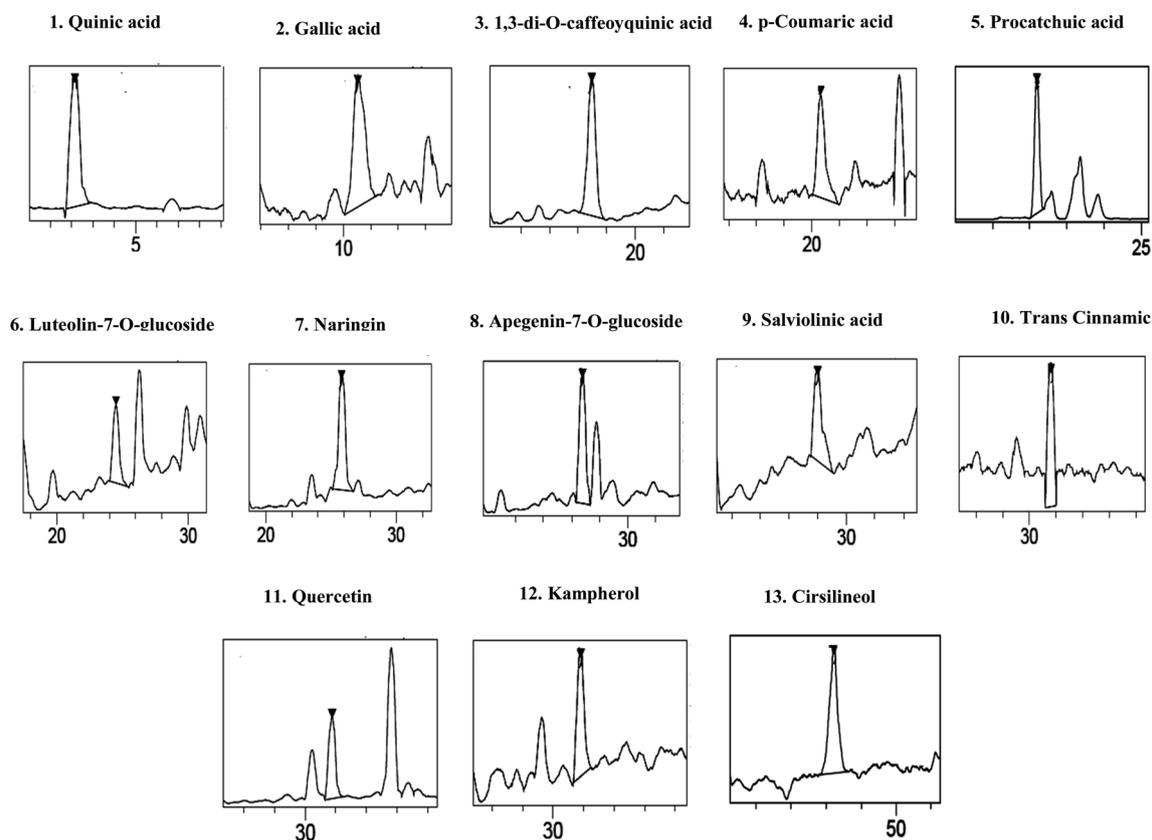


**Fig. 1** Chromatographic profile and characterization of phenolic compounds of *Salvia officinalis* L. flowers decoction extract (SOFDE) (assignments of peaks are given in Table 2).

**Table 3** Evaluation of gastric and small bowel fluid accumulation. Animals were pre-treated with two doses of SOFDE (100 and 200 mg kg$^{-1}$, b.w., p.o.), mixture (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.) and sulfasalazine (100 mg kg$^{-1}$, b.w., p.o.) or vehicle (NaCl 0.9%). One hour after, animals received EtOH (4 g kg$^{-1}$, b.w., p.o.) by gavage for 2 h. Assays were carried out in triplicate[a]

| Groups | Weight of stomach content (g) | Protection (%) | Weight of intestinal content (g) | Protection (%) |
|---|---|---|---|---|
| Control | $0.51 \pm 0.18$ | | $0.34 \pm 0.37$ | |
| EtOH | $5.39 \pm 1.36$* | 0 | $2.13 \pm 0.90$* | 0 |
| EtOH + SOFDE-100 | $4.57 \pm 1.22$# | 15.21 | $0.98 \pm 0.72$# | 53.99 |
| EtOH + SOFDE-200 | $4.08 \pm 0.87$# | 24.30 | $0.75 \pm 0.30$# | 64.79 |
| EtOH + SULF | $3.85 \pm 1.43$# | 41.56 | $0.56 \pm 0.12$# | 73.71 |
| EtOH + MIX | $2.99 \pm 1.03$# | 59.37 | $0.39 \pm 0.09$# | 81.69 |

$^a$ *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to EtOH group.

The level of GSH was performed by colorimetric method using the method of Sedlak and Lindsay.[47] In fact, 5 mL of supernatant was mixed with 4 mL of cold distilled water and 1 mL of TCA (50%). The tubes were vortexed for 10 minutes and centrifuged at 1200 g for 15 minutes. 2 mL supernatant was mixed with 4 mL of 0.4 M Tris buffer (pH 8.9). 0.1 mL of DTNB (0.01 M) were added to the reaction medium. The absorbance was recorded rapidly at 412 nm against the blank containing only the buffer.

**Protein determination.** Protein concentration was determined according to Hartree, which is a slight change of the Lowry method. Serum albumin was used as a standard.[48]

**Iron measurement, calcium and magnesium determination.** Free iron, calcium and magnesium concentrations were performed using commercially available diagnostic kits (Biomaghreb, Ariana, TN, ISO 9001 certificate).

**Quantitative determination of C-reactive protein (CRP) and ALP activity.** Alkaline phosphatase activity and C-reactive protein content were assessed using commercially available diagnostic kits (Biomaghreb, Ariana, TN, ISO 9001 certificate).

**Statistical analysis.** The data were analyzed by one-way analysis of variance (ANOVA) and expressed as means ± standard error of the mean (S.E.M.). All analyzes were performed using the SAS (Statistics Analysis System). All statistical tests were two-tailed, and a $P$ value of 0.05 or less was considered significant.

## Results

### Phytochemical composition of *Salvia officinalis* flowers decoction extract (SOFDE) and *in vitro* antioxidant capacity

**Colorimetric and chromatographic analysis of SOFDE.** We firstly showed that calcium is the most abundant elements in sage flowers extract while iron comes in last place (6.64 ± 0.04 mmol L$^{-1}$ and 3.29 ± 0.08 μmol L$^{-1}$, respectively). The SOFDE (extraction yield = 17.22 ± 0.13%) also contains a high content of total tannins (60.41 ± 3.87 TAE/g DM), flavonols (1.99 ± 0.02 mg RE/g DM) and anthocyanins (3.79 ± 0.29 mg CG/g DM), but a moderate concentration of total carotenoids (1.67 ± 0.09 μg/100 mL) (Table 1).

The HPLCPDAESI-MS/MSLC-HRESIMS analysis of SOFDE allowed to the identification of 13 phenolic compounds. Six phenolic acids which include quinic acid, protocatechuic acid, gallic acid, 1,3-di-O-caffeoyquinic acid, *p*-coumaric acid and salviolinic acid (Table 2). In addition, the chromatographic elution profile of flavonoids (Fig. 1) showed seven flavonoids compounds such as naringin, quercetin, kampherol, apegenin-7-o-glucoside, *trans* cinnamic, luteolin-7-O-glucoside and cirsilineol.

**In vitro antioxidant capacity of SOFDE.** Concerning the antioxidant capacity, we showed in Table 1 that the inhibition of β-carotene bleaching effect of SOFDE and Butylated hydroxyl toluene (BHT) increased significantly in a dose-dependent manner. The inhibit rice concentration 50 of SOFDE (IC$_{50}$ = 56.77 ± 2.34 μg mL$^{-1}$) appear significantly higher than BHT (IC$_{50}$ = 20.83 ± 0.71 μg mL$^{-1}$) used as reference molecule.

**Evaluation of gastric and small bowel fluid accumulation.** As shown in Tables 3 and 4, gastric and duodenal ulceration was accompanied by a significant increase in fluid accumulation as well as a decrease in mucus weights. The administration of SOFDE and MIX has been significantly restored, all of these parameters in a dose-dependent manner. We found the most relevant correction was recorded in the group that was received a mixture dose of SOFDE and sulfasalazine. In addition, sulfasalazine was also protected against disruption of the secretory profile.

**Qualitative and quantitative macroscopic evaluation.** Macroscopic examination of the glandular part of the stomach and small intestine was performed at the opening of the gastrointestinal segments. EtOH-treatment induced hemorrhagic lesions on the glandular part of the stomach and along the duodenum (Fig. 2). However, SOFDE, MIX and sulfasalazine treatments significantly protected gastric and duodenal mucosa from alcohol-induced injury (Table 3).

**Histopathological evaluation of gastric and duodenal lesions.** Histological observation of ethanol-induced gastric and intestinal lesions in EtOH group revealed a comparative extensive congestion, surface coating alteration, edema, necrotic lesions, epithelial and vascular cells alteration. We also observed a haemorrhage, hyperaemia as well as inflammatory
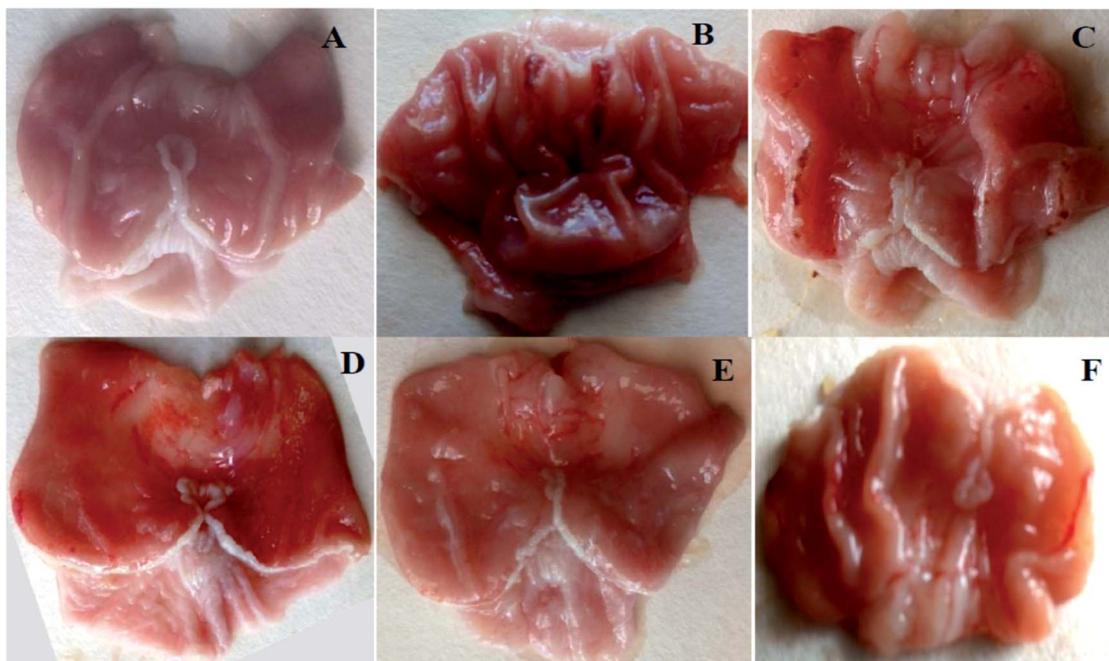
**Table 4** Effects of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced acute macroscopic gastric and small bowel injury. Animals were pre-treated with two doses of SOFDE (100 and 200 mg kg$^{-1}$, b.w., p.o.), mixture (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.) and SULF (100 mg kg$^{-1}$, b.w., p.o.) or vehicle (NaCl 0.9%). One hour after, animals received EtOH (4 g kg$^{-1}$, b.w., p.o.) by gavage for 2 h. Assays were carried out in triplicate[a]

| Groups | Mucus weight (g) | Protection (%) | Ulcer index (mm$^2$) | Protection (%) | Small bowel injury index (mm$^2$) | Protection (%) |
|---|---|---|---|---|---|---|
| Control | 4.53 ± 0.57 | | 0 | | | |
| EtOH | 1.74 ± 0.56* | 0 | 73.60 ± 4.05* | 0 | 20.82 ± 3.09* | |
| EtOH + SOLAE-100 | 2.62 ± 0.98# | 50.57 | 51.48 ± 3.83# | 30.05 | 13.34 ± 4.01# | 35.93 |
| EtOH + SOLAE-200 | 2.95 ± 0.46# | 69.54 | 39.80 ± 1.50# | 45.92 | 10.26 ± 1.37# | 50.72 |
| EtOH + SULF | 2.82 ± 0.26# | 62.07 | 29.75 ± 0.26# | 59.58 | 7.41 ± 0.36# | 64.41 |
| EtOH + MIX | 3.02 ± 0.19# | 73.56 | 10.13 ± 2.36# | 86.24 | 6.94 ± 0.43# | 66.67 |

[a] *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to EtOH group.
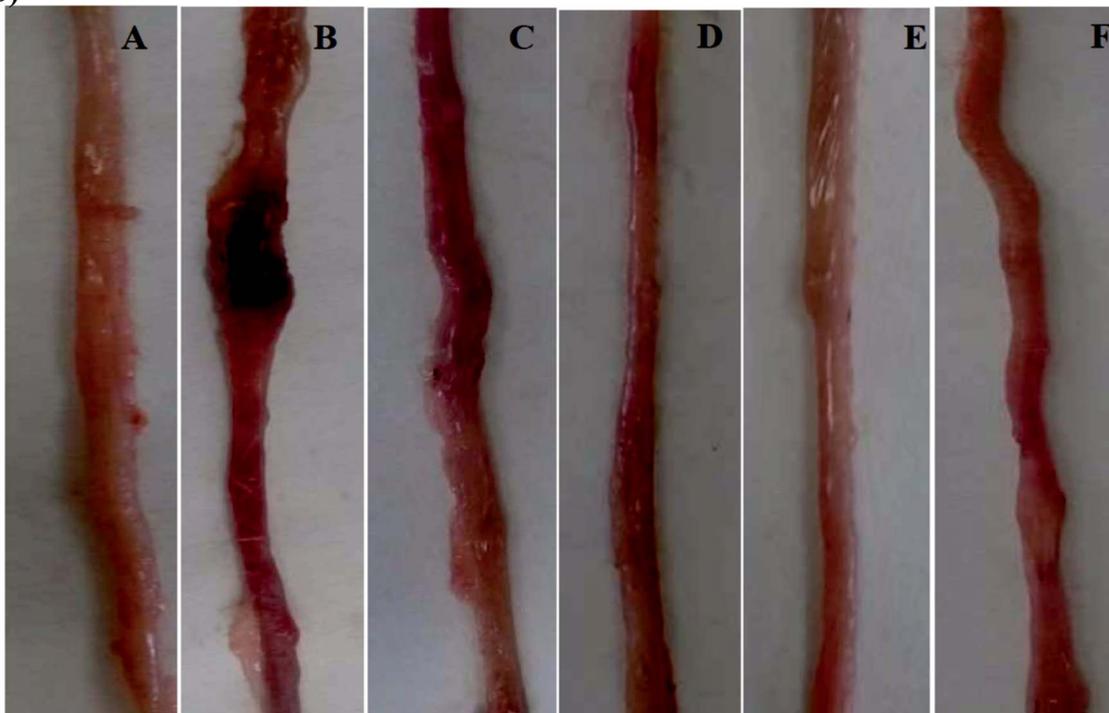
**(a)**



**(b)**



Fig. 2 Gastric (a) and duodenal (b) morphology showing the protective effects of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced ulcer. Animals were treated with two doses of SOFDE (100 and 200 mg kg$^{-1}$, p.o.), MIX (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.), SULF (100 mg kg$^{-1}$, p.o.) or vehicle (H$_2$O). (A) H$_2$O + NaCl; (B) H$_2$O + EtOH; (C, D) SOFDE (100 and 200 mg kg$^{-1}$, p.o., respectively) + EtOH; (E) MIX (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF 50 mg kg$^{-1}$, b.w., p.o.) + EtOH; (F) sulfasalazine (100 mg kg$^{-1}$, b.w., p.o.) + EtOH.
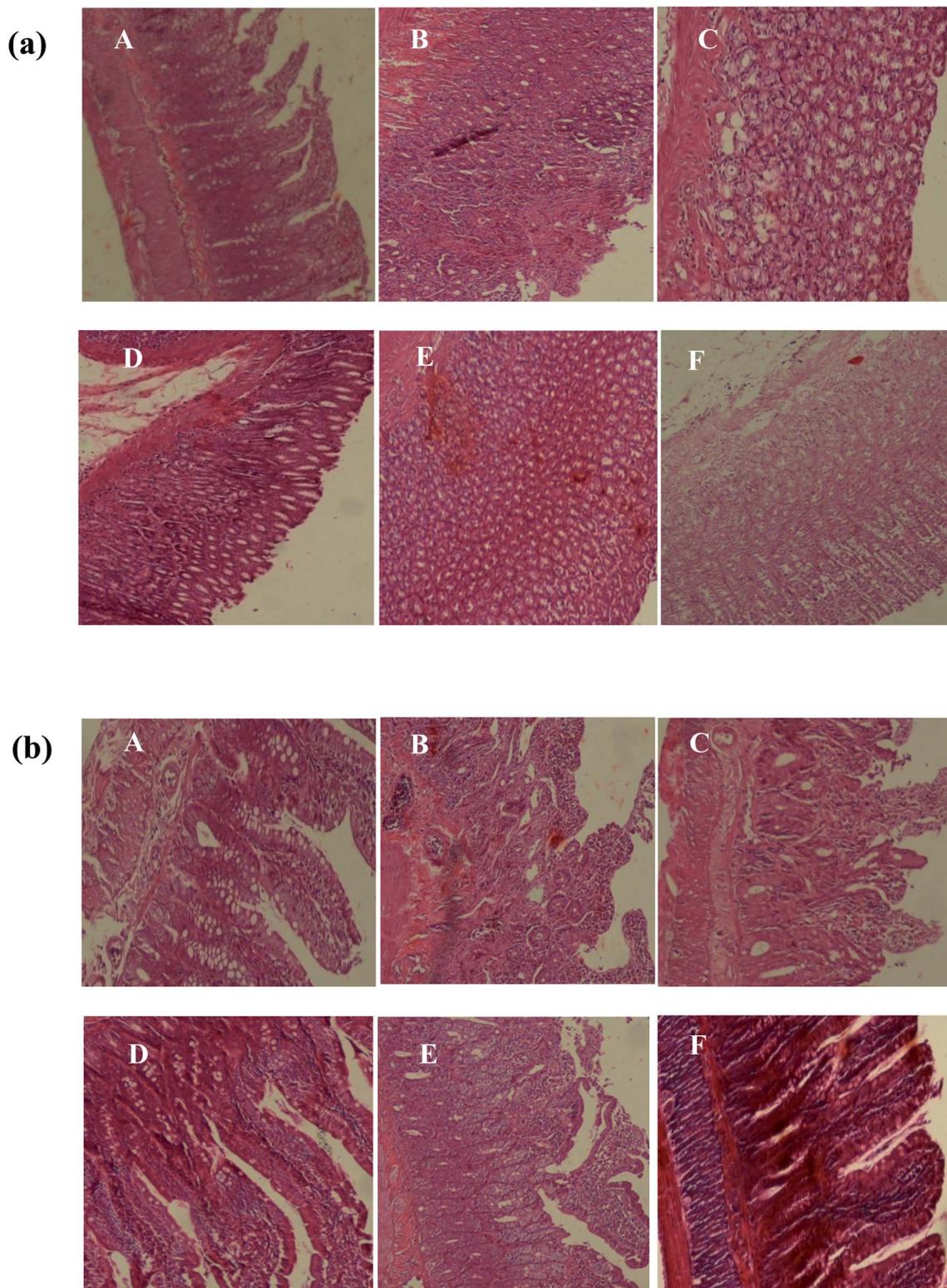
Fig. 3   Gastric (a) and duodenal (b) histology showing the protective effects of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced ulcer. Animals were treated with two doses of SOFDE (100 and 200 mg kg$^{-1}$, p.o.), MIX (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.), SULF (100 mg kg$^{-1}$, b.w., p.o.) or vehicle (H$_2$O). (A) H$_2$O + NaCl; (B) H$_2$O + EtOH; (C, D) SFDE (100 and 200 mg kg$^{-1}$, p.o., respectively) + EtOH; (E) MIX (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF 50 mg kg$^{-1}$, b.w., p.o.) + EtOH; (F) sulfasalazine (100 mg kg$^{-1}$, b.w., p.o.) + EtOH.
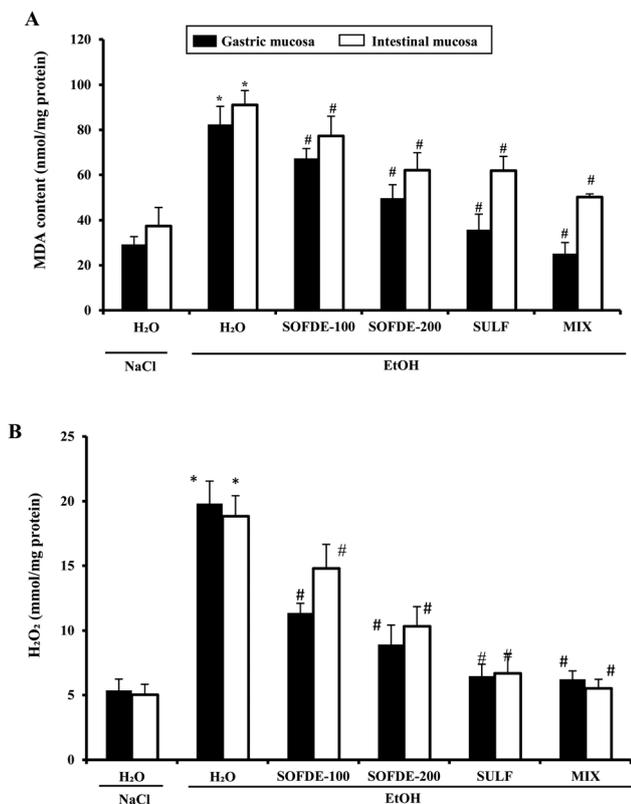
Fig. 4 Effect of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced changes in stomach and intestinal mucosa MDA (A) and $H_2O_2$ (B) levels. Animals were pre-treated with two doses of SOFDE (100 and 200 mg kg$^{-1}$, b.w., p.o.), SULF (100 mg kg$^{-1}$, b.w., p.o.) and MIX (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.) or vehicle (NaCl 0.9%). One hour after, animals received EtOH (4 g kg$^{-1}$, b.w., p.o.) by gavage for 2 h. Assays were carried out in triplicate. *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to EtOH group.

cell infiltration in the stomach and small bowell mucosa as well as submucosa (Fig. 3).

Pretreatment with SOFDE showed a dose-dependent protection of the gastric and intestinal mucosa as revealed by the reduction of lesions, mucosal and submucosal edema as well as leucocytes infiltration. Importantly, we showed that the group received the mixture registered the most important protection. A similar protective effect had also observed in sulfasalazine pretreated rats.

**Effect of SOFDE, sulfasalazine and mixture on EtOH-induced gastroduodenal lipoperoxidation and hydrogen peroxide increase.** To investigate the implication of oxidative stress in the antiulcerogenic effect of SOFDE, we firstly assessed the MDA and hydrogen peroxide levels. EtOH administration significantly increased MDA levels in gastric and duodenal mucosa. Alcohol-induced lipoperoxidation was significantly reversed by SOFDE, MIX or sulfasalazine pre-treatment in a dose-dependent manner (Fig. 4).

We also showed the effect of EtOH and SOFDE on intracellular mediator such as hydrogen peroxide level in gastric and intestinal mucosa (Fig. 4). In addition, alcohol group had

a significant increase in hydrogen peroxide level in gastric and intestinal tissues when compared to negative control group. SOFDE and sulfasalazine treatment significantly and dose-dependently reduced the EtOH-induced this intracellular mediator deregulation. More importantly, our result found that MDA and $H_2O_2$ levels were reversed by MIX pre-treatment more significantly than sulfasalazine and SOFDE each alone.

**Effects on plasma scavenging activity.** EtOH administration significantly decreased the plasma scavenging activity when compared to control group (Fig. 5). By contrast, PSA percentage was significantly and dose-dependently increased after SOFDE pre-treatment. A similar effect was also observed for sulfasalazine, used as reference molecules, but less important than the group treated with the mixture.

**Effect of SOFDE, SULF and MIX on EtOH-induced antioxidant enzyme activities depletion.** On other hand, we examined the effect of SOFDE, SULF and EtOH treatment on antioxidant enzyme activities (Fig. 6). As expected, gastric and duodenum injuries were accompanied by a significant decrease of superoxide dismutase (A), catalase (B) and glutathione peroxidase (C) activities. Sage decoction extract treatment significantly corrected the enzyme activities decrease caused by alcohol administration in a dose-related manner. The MIX exerted a more important effect than SOFDE and sulfasalazine each alone.

**Effect of SOFDE, SUL and MIX on EtOH-induced non-enzymatic antioxidants levels deregulation.** We also investigated the gastric and duodenal non-enzymatic antioxidants levels (Fig. 7). We showed that alcohol intoxication significantly reduced thiol groups (A) as well as reduced glutathione (B) contents. However, SOFDE exhibited significant and dose-dependent regulation of all those parameters. We noticed that the MIX exerts a more important effect than sulfasalazine alone.

**Effect of SOFDE, SUL and MIX on free iron, magnesium and calcium levels.** We further looked at the EtOH and SOFDE on intracellular mediators such as calcium, free iron and magnesium levels (Table 5). As expected, alcohol group showed a significant increase of those parameters in gastric and duodenal mucosa when compared to negative control group. SOFDE and SULF each alone significantly protected against EtOH-induced intracellular mediators disturbances while the mixture exerted a more pronounced effect.

**Effect of SOFDE, SUL and MIX on EtOH-induced inflammation.** Serum CRP and ALP activities significantly increased in the EtOH treated group when compared to control. Importantly, we found a protective effect against the inflammatory markers increase were observed in the SOFDE and SULF groups. We also showed a powerful anti-inflammatory activity of SOFDE against harmful effects of EtOH (Table 6). More importantly, we showed that the MIX presented a more important anti-inflammatory capacity when compared to SOFDE and SULF treatment each alone.

## Discussion

In the present work, we investigated the phytochemical properties as well as the individual or synergistic protective actions
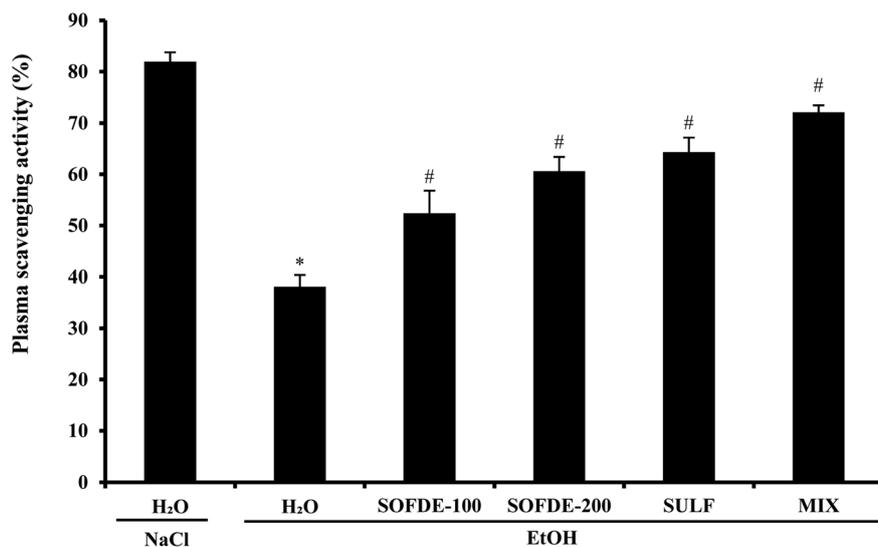
**Fig. 5** Effect of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced disturbances in plasma scavenging activity (PSA). Animals were pre-treated with two doses of SOFDE (100 and 200 mg kg$^{-1}$, b.w., p.o.), SULF (100 mg kg$^{-1}$, b.w., p.o.) and MIX (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.) or vehicle (NaCl 0.9%). One hour after, animals received EtOH (4 g kg$^{-1}$, b.w., p.o.) by gavage for 2 h. Assays were carried out in triplicate. *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to EtOH group.



**Fig. 6** Effect of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced changes in stomach and intestinal mucosa antioxidant enzyme activities: SOD (A), CAT (B) and GPx (C). Animals were pre-treated with two doses of SOFDE (100 and 200 mg kg$^{-1}$, b.w., p.o.), SULF (100 mg kg$^{-1}$, b.w., p.o.) and MIX (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.) or vehicle (NaCl 0.9%). One hour after, animals received EtOH (4 g kg$^{-1}$, b.w., p.o.) by gavage for 2 h. Assays were carried out in triplicate. *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to EtOH group.
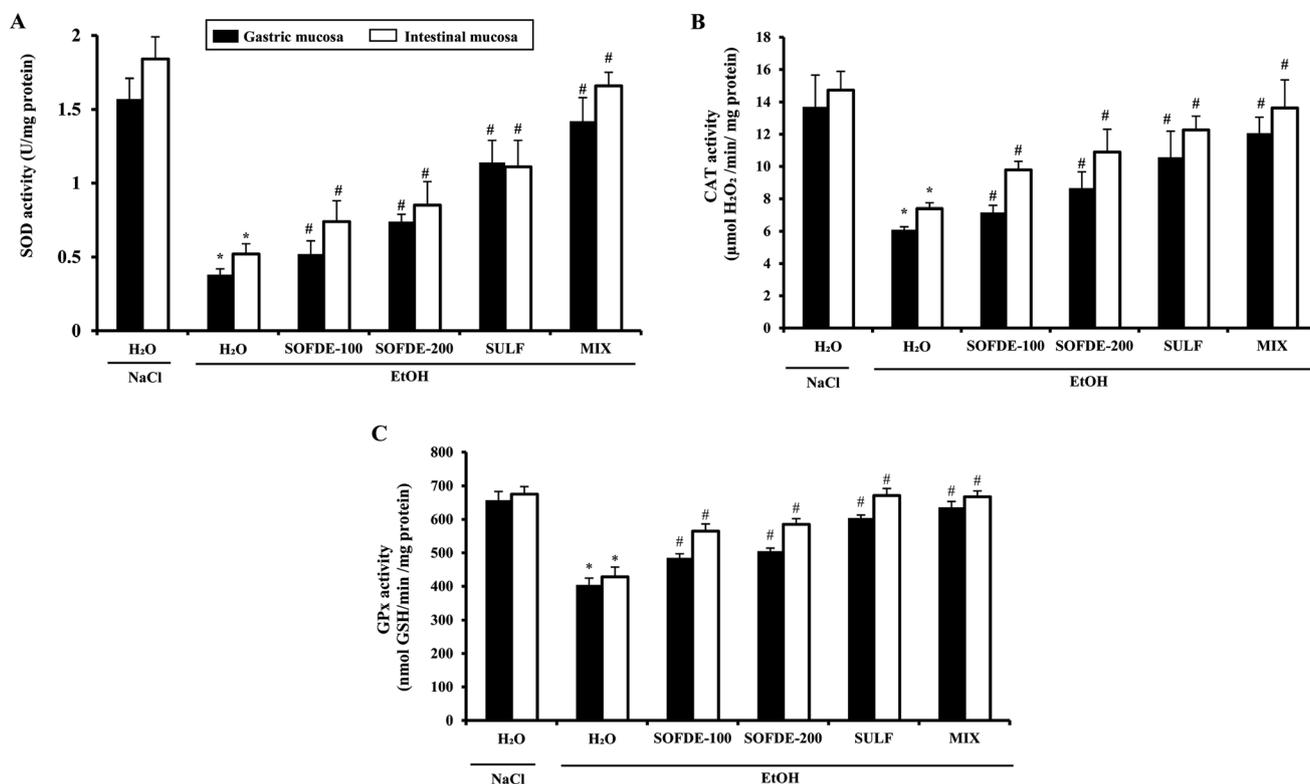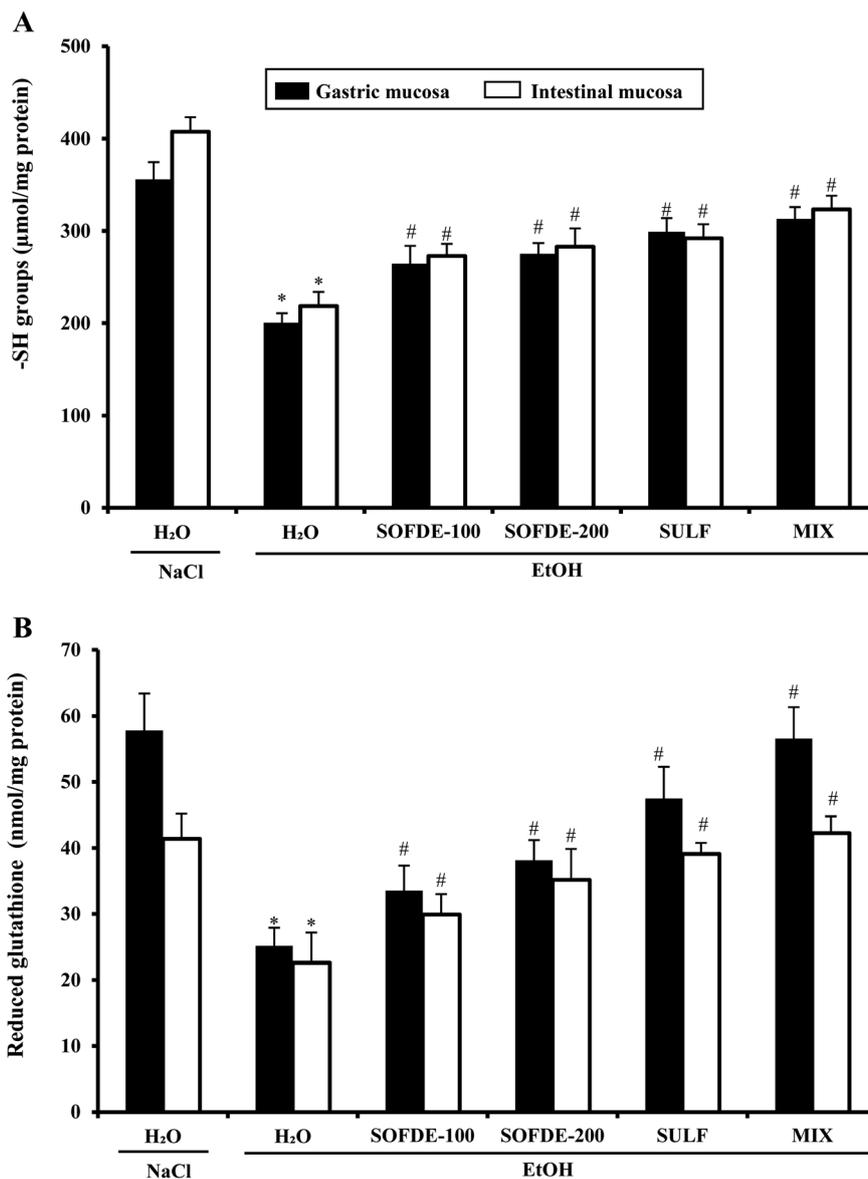
Fig. 7 Effect of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced changes in stomach and intestinal mucosa sulfhydryl groups (A) and reduced glutathione (B). Animals were pre-treated with two doses of SOFDE (100 and 200 mg $kg^{-1}$, b.w., p.o.), SULF (100 mg $kg^{-1}$, b.w., p.o.) and MIX (SOFDE, 50 mg $kg^{-1}$, b.w., p.o. + SULF, 50 mg $kg^{-1}$, b.w., p.o.) or vehicle (NaCl 0.9%). One hour after, animals received EtOH (4 g $kg^{-1}$, b.w., p.o.) by gavage for 2 h. Assays were carried out in triplicate. *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to EtOH group.

of *Salvia officinalis* flowers decoction extract and sulfasalazine on EtOH-induced peptic ulcer.

The phytochemical study firstly showed that SOFDE presents an important antioxidant activity assessed by to the bleaching inhibition potency of β-carotene ($IC_{50} = 56.77 \pm 2.34$ μg $mL^{-1}$). A similar result was obtained by Martins *et al.*[49] who observed a good β-carotene bleaching inhibition ($IC_{50} = 50.87 \pm 3.73$ μg $mL^{-1}$). The antioxidant activity of SOFDE could be, in part, attributed to its high phenolic compounds levels. In this context, our data also suggest that SOFDE presents a high concentration of flavonols, total tannins and a moderate concentration of total anthocyanins and carotenoids. These levels fully corroborated those of Akhondzadeh *et al.*[22] The

antioxidant capacity is mainly attributed to the hight level of phenolic acids and flavonoids in this fraction such as quinic, protocatchuic, 1,3-di-O-caffeoyquinic, *p*-coumaric and salviolinic acids, and naringin, quercetin, kampherol, apigenin-7-*o*-glucoside, luteolin-7-*o*-glucoside and cirsilineol. It has been previously found that the majority of these bioactive molecules has been identified in aqueous leaf extracts and has been shown for a high antioxidant capacity against DPPH radical.[32]

*In vivo*, we firstly revealed that acute alcohol administration distorted the gastric and duodenal mucosa and submucosa, which are accompanied by surface coating, epithelial cells alterations, as well as edema and leukocyte infiltration. Indeed, prostaglandin deficiency within the digestive mucosa is defined

**Table 5**  Subacute effect of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced changes in stomach and intestinal mucosa and plasma free iron, magnesium and calcium levels in rats. Animals were pre-treated with two doses of SOFDE (100 and 200 mg kg⁻¹, b.w., p.o.), mixture (SOFDE, 50 mg kg⁻¹, b.w., p.o. + SULF, 50 mg kg⁻¹, b.w., p.o.) and SULF (100 mg kg⁻¹, b.w., p.o.) or vehicle (NaCl 0.9%), and were challenged with a single oral administration of EtOH (4 g kg⁻¹, b.w., p.o.) or NaCl (0.9%) for two hours. Assays were carried out in triplicate[a]

| Groups | Free iron | | | Calcium | | | Magnesium | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Gastric mucosa (µmol per mg per protein) | Intestinal mucosa (µmol per mg per protein) | Plasma (µmol L⁻¹) | Gastric mucosa (mmol per mg per protein) | Intestinal mucosa (mmol per mg per protein) | Plasma (mmol L⁻¹) | Gastric mucosa (µmol per mg per protein) | Intestinal mucosa (µmol per mg per protein) | Plasma (µmol L⁻¹) |
| Control | 22.02 ± 1.92 | 7.37 ± 1.05 | 1.28 ± 0.06 | 0.66 ± 0.08 | 0.73 ± 0.08 | 0.501 ± 0.04 | 194.32 ± 20.78 | 209.27 ± 19.69 | 24.30 ± 2.60 |
| EtOH | 63.09 ± 4.07* | 20.18 ± 1.78* | 2.26 ± 0.21* | 2.57 ± 0.32* | 3.11 ± 0.85* | 0.99 ± 0.08* | 364.63 ± 17.87* | 334.46 ± 17.12* | 98.66 ± 4.20* |
| EtOH + SOFDE-100 | 50.01 ± 3.90# | 17.37 ± 1.40# | 2.02 ± 0.13# | 2.48 ± 0.78# | 2.70 ± 0.58# | 0.86 ± 0,07# | 297.09 ± 19.74# | 289.61 ± 18.21# | 84.57 ± 4.09# |
| EtOH + SOFDE-200 | 44.22 ± 1.41# | 15.62 ± 1.33# | 1.69 ± 0.09# | 1.87 ± 0.54# | 2.26 ± 0.30# | 0.71 ± 0.05# | 266.26 ± 19.01# | 263.46 ± 20.21# | 63.70 ± 3.44# |
| EtOH + SULF | 34.13 ± 3.87# | 12.99 ± 2.56# | 1.42 ± 0.15# | 2.08 ± 0.79# | 2.08 ± 0.78# | 0.77 ± 0.06# | 231.69 ± 22.61# | 244.77 ± 21.22# | 53.22 ± 5.33# |
| EtOH + MIX | 30.45 ± 3.39# | 8.42 ± 1.69# | 1.31 ± 0.08# | 1.44 ± 0.41# | 1.31 ± 0.35# | 0.55 ± 0.07# | 200.86 ± 21.10# | 216.74 ± 17.12# | 49.51 ± 3.67# |

[a] *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to EtOH group.

as the major pathogenic mechanism of the ethanol-induced digestive system diseases. The deficit in endogenous prostaglandins plays an essential role in the pathogenic process by making the mucosa more vulnerable to the aggression, without direct implication in the digestive lesions.[50] Our data are in line with previous report using EtOH as ulceration inducer.[51] Ethanol causes injures in the vascular endothelial cells of the gastric and intestine mucosa and induces microcirculatory disturbance and hypoxia, related to massive production of free radicals.[52]

Importantly, our data showed a protective effect of subacute treatment with SOFDE (15 days) against gastric and intestinal fluid accumulation, weight of mucus and lesions induced by EtOH administration. Our extract also contributed in the reduction of macroscopic and histopathological observed damages. The therapeutic effect of MIX is more pronounced than SULF (100 mg kg⁻¹, b.w., p.o.) used as reference molecule as well as the high dose of SOFDE (200 mg kg⁻¹, b.w., p.o.). This beneficial effect can be explained by the fact that this drug has a single course of action[53] while sage decoction extract acts through several different mechanisms.[54] When those two mechanisms are combined in the gastrointestinal tract, synergism will occur through several biochemical targets and pathways. However, for phenolic compounds many mechanisms might be involved such as intracellular mediators by chelation of metal ions ($Fe^{2+}$, $Cu^{2+}$), membrane stabilization,[55] inhibition of pepsinogen production[56] and increased mucus production, characterized by a film formed by the polymerization of glycoproteins which makes it possible to trap the bicarbonates and delay the penetration of endoluminous $H^+$ ions. This situation establish a pH gradient ranging from less than 3 at the level of the luminal face of this layer, to more than 7 on the mucosal surface.[57] However, the mixture of natural active substances and drugs has been used in previous work to treat cancer diseases[58] as well as its antibacterial activity.[59]

In the present study, we also showed a high concentration of total tannins (60.41 ± 3.87 mg TAE/g DM). These molecules could prevent ulcer development throughout vasoconstricting effects or to their proteins precipitation in the ulceration site, producing an impermeable coating over the lining that restrains gut secretions and defends the underlying mucosa from lesions.[60] We also found a high level of flavonls (kaempferol and quercetin), that present anti-ulcer and gastroprotective properties.[61]

We also showed that EtOH intoxication induced a depletion of plasma scavenging activity (PSA), as well as an increase of hydrogen peroxide and MDA level, which is defined as an indicator of the ROS generation in tissues and plasma. In addition, we observed a decrease of thiol group and GSH levels, as well as depletion antioxidant enzyme activities such SOD, CAT and GPx. However, its well known that acute administration of EtOH leads oxidative imbalance through several pathways such as the generation of reactive oxygen species.[62] Indeed, SOD converts the reactive superoxide radical to $H_2O_2$, which was decreased in the gastric and intestine mucosa. When this intracellular mediator was not scavenged by CAT, it could leads to lipid peroxidation after generation of hydroxyl radical.[63] More importantly, we

**Table 6** Subacute effect of *Salvia officinalis* flowers decoction extract (SOFDE), mixture (MIX) and sulfasalazine (SULF) on EtOH-induced changes in plasma CRP and ALP levels in rats. Animals were pretreated with two doses of SOFDE (100 and 200 mg kg$^{-1}$, b.w., p.o.), mixture (SOFDE, 50 mg kg$^{-1}$, b.w., p.o. + SULF, 50 mg kg$^{-1}$, b.w., p.o.) and SULF (100 mg kg$^{-1}$, b.w., p.o.) or vehicle (NaCl 0.9%), and were challenged with a single oral administration of EtOH (4 g kg$^{-1}$, b.w., p.o.) or NaCl (0.9%) for two hours. Assays were carried out in triplicate[a]

| Groups | C-Reactive protein (CRP) ($\mu g\ dL^{-1}$) | Alkaline phosphatase (ALP) plasma ($U\ L^{-1}$) at 37 °C |
|---|---|---|
| Control | 0.34 ± 0.01 | 68.29 ± 3.72 |
| EtOH | 1.48 ± 0.37* | 190.44 ± 5.04* |
| EtOH + SLAE-100 | 0.92 ± 0.21# | 161.33 ± 1.67# |
| EtOH + SLAE-200 | 0.79 ± 0.12# | 105.42 ± 4.30# |
| EtOH + SULF | 0.69 ± 0.08# | 83.19 ± 5.86# |
| EtOH + MIX | 0.46 ± 0.01# | 71.27 ± 8.84# |

[a] *: $p < 0.05$ compared to control group and #: $p < 0.05$ compared to EtOH group.

showed that SOFDE and sulfasalazine administration each alone or in combination (MIX) abolished acute EtOH-induced oxidative stress in the gastric and the duodenal mucosa. These finding are similar with previous study which has reported that the sage decoction extract contains a good amount of total polyphenols, flavonoïds, condensed tannins and a high level of rosmarinic and salviolinic acids and apegenin-7-*O*-glucoside.[49] These molecules are the primal source of the antioxidant ability of this plant and are including in the scavenging of free radicals as hydroxyl radical (OH˙) which is the major cause of lipid peroxidation.[64] Furthermore, those sulfhydryl groups are in part involved in gastric cytoprotection[65] as well as in the maintain of mucosal barrier integrity and free radicals scavenging.

Importantly, we showed an increase of intracellular mediators such as calcium, free iron and magnesium in plasma, gastric and duodenal mucosa in response to oxidative stress induced by ethanol administration. These data are in line with several previous studies.[66,67] However, we can now suggest that SOFDE exerts a beneficial effect by chelating free iron and scavenging $H_2O_2$ and regulation of the calcium and magnesium homeostasis. Our results also supposes that pretreatment with SOFDE protects against overcharge of cells of the gastric and intestinal mucosa by free iron and $H_2O_2$ induced by ethanol sub-acute administration. Moreover, these later are involved in the generation of hydroxyl radical (OH˙),[68] which plays the major role in oxidative damage by affecting the molecular structures.[69] In this respect, Jan *et al.*[70] conclude that living organisms create a complex endogenous and exogenous antioxidant defense system to restrict the production of this damaging radical.

Finally, we have shown in the present work that EtOH intoxication induced inflammation as assessed by a significant increase in plasma CRP and ALP when compared to control group ($P < 0.05$). On the other hand, the pre-treatment with SOFDE, sulfasalazine and MIX significantly decreased the studied biomarkers. These data are in line with those of Mosli *et al.*[71] Indeed, several studies have shown that EtOH is associated with an inflammatory state *via* the expression of pro-inflammatory cytokines.[51] However,

oxidative stress is well known to be related to inflammatory gastric and bowel disease.[72] Moreover, quinic, salviolinic, procatchuic and *p*-coumaric acids which are identified with abandoned amount in SOFDE possess an important anti-inflammatory activity.[29,73]

## Conclusion

In conclusion, our data clearly demonstrated strong synergic protective effects between *Salvia officinalis* decoction extract and sulfasalazine against ethanol-induced gastric and small bowel injuries. This gastro-duodenal protection might be due in part to its antioxidant and anti-inflammatory properties as well as its opposite effects on intracellular mediators such as hydrogen peroxide, free iron and calcium. Therefore, the mixture between bioactive compounds from plant and standards drugs is considered as an alternative to protect against gastro-intestinal disorders and to avoid unpredictable side effects of commercial drugs.

## Ethical consideration

All procedures on animals in this study were approved with the National Institute of Health recommendations for the use and care of animals.

## Abbreviations

| | |
|---|---|
| CAT | CAT |
| EtOH | Ethanol |
| GPx | Glutathione peroxidase |
| GSH | Reduced glutathione |
| $H_2O_2$ | Hydrogen peroxide |
| MDA | Malondialdehyde |
| MIX | Mixture |
| ROS | Reactive oxygen species |
| –SH | Sulfhydryl groups |
| SOD | Superoxide dismutase |
| SOFDE | *Salvia officinalis* flowers decoction extract |
| SULF | Sulfasalazine |

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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