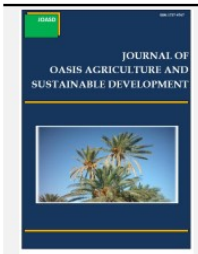

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Teucrium polium L.: Antimicrobial and Antioxidant Properties with an Emphasis on Oxidative Stress and Gastric Ulcer induced by ethanol in albinos rats

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Abstract

Teucrium species have been used in traditional medicine for treatment of different diseases. We aimed in the present study, at investigating the gastroprotective effect of *Teucrium polium* L methanolic extract (TPME) against ethanol-induced oxidative stress in rats. Adult male Wistar Wistar rats were used and divided into five groups of eight each: control, EtOH/HCl (80 % v/v, 4 g/kg b.w.), EtOH 80 % + two doses of TPME (100 or 200 mg/kg, b.w.) and EtOH/HCl + Famotidine (10 mg/kg, p.o.). Animals were orally (p.o.) pre-treated with TPME and then intoxicated with a single oral administration of EtOH/HCl (4 g/kg b.w.). Ulcerogenic parameters, oxidative stress indices, and histopathological examination of the stomach were assessed. The bioactive compounds of TPME were identified using high-performance liquid chromatography (HPLC). Results obtained indicated that pre-treatment with TPME significantly reduced the formation of ulcer at the two administered doses. Similarly, pre-treatments improved the antioxidant system, decreased acid output, lipid peroxidation, and improved the architecture of the gastric mucosa in ulcerated rats. HPLC results identified the presence of three phenolic acid derivates (p-coumaric, ferulic and syringic acids) and three flavonoids (myricetin, luteolin 7-O-glucoside and hyperoside). This study allowed us to conclude that TPME have a strong antioxidant and gastroprotective activities due to its richness on phenolic compounds.

1. INTRODUCTION

Peptic ulcer gastric disease is a worldwide gastrointestinal (GI) disorder that arises when the caustic effects of aggressive factors dominate over the defensive factors (McQuaid et al., 2018). These aggressive factors include physical stress, prominent tobacco consumption, alcohol or caffeine, certain types of medications, particularly the non-steroidal anti-inflammatory drugs and infection by *Helicobactor pylori* (Heibashy et al., 2014). In this context, Frank et al. (2005) reported that among these factors, high alcohol consumption is the greatest cause of gastric mucosal damage. Thus, the experimental model of ethanol-induced gastric ulcer was often employed to screen the anti-ulcer compounds (Liu et al., 2012). The most frequent synthetic drugs used to treat and prevent peptic ulcers include H2 receptor blockers (such as cimetidine, ranitidine, etc.) and proton pump inhibitors such as omeprazole, antacids, and cell protective factors. Because these synthetic drugs cause many negative impacts such as arrhythmia, impotence, hematopoietic alterations and hypersensitivity. Hence, use of natural anti-ulcer drug with less side effects is with great importance (Beiranvand et al., 2021). In fact, researchers are focused on exploring plants with potential healing effects on peptic ulcer disease. Among these plants, *Teucrium polium* L. (family Lamiaceae) is a wild-growing flowering plant, found abundantly in South-

Western Asia, Europe and North Africa. Traditionally, *T. polium* has been used for different pathological conditions such as gastrointestinal disorders, inflammations, diabetes and rheumatism (Bahramikia and Yazdanparast, 2012). Phytochemical investigations have shown that extract and individual components such as terpenoids, flavonoids, and essential oils isolated from different parts of *T. polium* have particular biological properties. The plant has different therapeutic effects including antioxidant, hypoglycemic, anticancer, anti-spasmodic, hypotensive, anti-nociceptive, anti-mutagenic, anti-inflammatory, hypolipidemic, cytotoxic, hepatic-protective, antiulcer, antibacterial, antifungal and memory enhancing effects (Alreshidi et al., 2020; Chabane et al., 2020; Rahmouni et al., 2021). Besides, *T. polium* is a common plant in folk medicine as a source of bioactive molecules acting on several human disorders. In this way, bibliographic data showed that phytochemical compounds isolated from *T. polium* aerial parts reached more than 130 molecules dominated by terpenoids (60%) (Bahramikia and Yazdanparast, 2012). The main identified compounds from T. polium roots, aerial parts, and inflorescence are apigenin, luteolin, rutin, cirsiliol, cirsimaritin, salvigenin, and eupatorin (Bahramikia and Yazdanparast, 2012; Alreshidi et al., 2020).

Accordingly, the present study was designed to determine the phenolic composition of the methanolic extract of *T. polium*, and its antioxidant properties using 2 test systems. Furthermore, to evaluate the putative gastroprotective effect of the methanolic extract of *T. polium* (TPME) against oxidative stress induced by acute ethanol exposure in rat.

2. MATERIAL AND METHODS

2.1. Chemical and reagents

Folin–Ciocalteu reagent, sodium carbonate anhydrous (Na_2CO_3), gallic acid, sodium nitrite solution ($NaNO_2$), aluminum chloride hexahydrate solution ($AlCl_3$, $6H_2O$), vanillin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid, iron(III)chloride anhydrous ($FeCl_3$), and ascorbic acid were purchased from Fluka (Buchs, Switzerland). Butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), sodium hydroxide (NaOH), β-carotene, linoleic acid were purchased from Sigma-Aldrich (GmbH, Sternheim, Germany). Sulfuric acid (H_2SO_4) and Kalium-hexacyanoferrat (III); $K_3Fe(CN)_6$ were obtained from Merck (Darmstadt, Germany). Fluorescein sodium salt (FL), 2',7'-dichlorofluorescin-diacetate (DCFH-DA), 2',7'-dichlorofluorescin (DCFH), 2',7'-dichlorofluorescein (DCF), tert-butyl hydroperoxide (t-BuOOH), 6-hydroxy-2,5,7, 8-tetramethyl-2-carboxylic acid (Trolox), were all purchased from Sigma–Aldrich (Oakville, ON).

2.2. Plant material and preparation of crude extract

Leaves of *T. polium* were collected from the region of Korbous (Nabeul governorate, Tunisia DD: 36.81668 10.56865 DMS : 36° 49' 0" 10° 34' 7") in May 2023, during the flowering period. The harvested plant was identified at the Biotechnology center of the Technopark of Borj-

Fig. 1. Photographs of the studied plant: *Teucrium polium*

Cedria by Pr Abderrazek SMAOUI. Voucher specimens [HPM107] was deposited in the herbarium of the Laboratory of Aromatic and Medicinal Plants (LPAM). The leaves were cut into slices, oven–dried at 35°C and grounded. An aliquot of 100 g of dry powder was extracted with 1000 mL of methanol/water (80%) solution for 30 min under a magnetic stirring. The solution was filtered and centrifuged at 4500 g for 15 min and the supernatant was dried in a rotavap at 50°C and lyophilized and stored at 21°C, until use.

2.3. Determination of TPC and TFC

The level of total phenolics in *T. polium* extract was determined with the Folin–Ciocalteu reagent using the method described by Dewanto et al**.** (2002). To 125 µl of extract, 500 µl of reagent and 125 µl of distilled water were added. The mixture was shaken, added with 1250 µl of Na_2CO_3 (7%, w/v) and adjusted with distilled water to a final volume of 3 ml. After incubation for 90 min at 23°C in the dark, the absorbance **vs.** prepared blank was read at 760 nm in UV–visible spectrophotometer. TPC was expressed as mg gallic acid equivalents per gram of dry residue (mg GAE g^{-1} DR) using a calibration curve with gallic acid (0–400 µg ml^{-1}). All samples were analyzed in triplicate.

For total flavonoids content (TFC), an aliquot of the sample or (+)-catechin standard was added to test tubes containing 75 µL of a 5% $NaNO_2$ solution, and mixed for 6 min. Then, 0.15 mL of a freshly prepared 10% $AlCl_3$ solution was added. After 5 min at room temperature, 0.5 mL of 1 N NaOH was added. The final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture without the sample as a blank. The concentrations of flavonoid contents were calculated according to the equation that was obtained from the standard (+)-catechin graph, and were expressed as mg catechin equiv. g^{-1} DW (mg CE g^{-1} DR). All samples were analyzed in triplicate (Dewanto et al., 2002).

2.4. Identification of phenolic compounds using high performance liquid chromatography

Sample from *T. polium* was hydrolyzed according to the method of Proestos et al. (2008) which was slightly modified. The acidic hydrolysis was used to release the aglycones in order to simplify the identification process since the free forms of phenolic compounds are rarely present in plants and they occur as esters, glycosides or bound to the cell wall (Nuutila et al. 2002). 20 mL methanol containing butylated hydroxytoluene (BHT) (1 g/L) were added to 0.5 g of a dried sample. Then, 10 mL of 1M HCl were added. The mixture was stirred and sonicated for 15 min and refluxed in a water bath at 90°C for 2 h. The obtained mixture was injected into an HPLC. The phenolic compound analysis was carried out using an Agilent Technologies 1100 series liquid chromatography (RP–HPLC) coupled with an UV– vis multi-wave length detector. The separation was carried out on a 250, 4.6-mm, 4-lm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulfuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program was as follows: 15%A/85%B 0–12 min, 40%A/60% B 12–14 min, 60% A/40% B 14–18 min, 80% A/20% B 18–20 min, 90% A/10% B 20–24 min, 100% A 24–28 min. The injection volume was 20 µL, and peaks were monitored at 280 nm. Samples were filtered through a 0.45 mm membrane filter before injection. Phenolic compounds were identified according to their retention times and spectral characteristics of their peaks against those of standards, as well as by spiking the sample with standards.

For quantitative analysis, the limits of detection and quantification were calculated from the parameters of the calibration curves obtained by injection of known concentrations of different standard compounds namely: catechin hydrate (y=3.63 +1.8; R2= 1.00); p-coumaric acid (y=34.14 +5.88; R2= 0.99); sinapic acid (y=41.61 x-2.38; R2= 1.00); and trans-hydroxycinammic acid (y=49.68 +24.601; R2= 1.00). The results were expressed in mg per g of dry residue.

2.5. DPPH free radical assay

DPPH scavenging potential of the *T. polium* sample was determined using a colorimetric method based on the proportionality between degradation of DPPH solution color and the antioxidant capacity (Hanato et al., 1988). Briefly, 250µL of methanolic solution of stable radical DPPH (0.2 mM) was added to 1 mL of increasing concentrations (add the range of concentration and how are they prepared) of extract. After shaking and 30 min of incubation at room temperature in the dark, optical density (OD) was measured at 517 nm and inhibition percentages (IP %) were calculated according to the following formula:

$$IP\%=((Ac-As)/Ac)*100$$

Where; Ac is the OD of control and As is the OD of the extract sample. Results were expressed in half-maximal inhibitory concentration (IC_{50}, µg/mL).

2.6. Ferric reducing power assay (FRAP)

This antioxidant activity was focused on the reduction of the trivalent iron produced by the $FeCl_3$ (Oyaizu, 1986). The intensity of the blue-green color was measured at 700 nm. Results were expressed as EC_{50} value (µg/ml) which is the effective concentration giving an absorbance of 0.5 and was obtained from linear regression analysis.

2.7. *In vivo* Experimental study

Healthy adult male Wistar rats (weighing 220–250 g; housed 5 per cage) were purchased from Pasteur Institute of Tunisia. They were provided with standard food (standard pellet diet- Badr Utique-TN) and water *ad libitum* and maintained in animal house at controlled temperature (22 ± 2 °C) with a 12 h light–dark cycle.Rats were divided into 5 groups of 8 animals each and treated as described in Table 1. Then, rats were fasted for 24h before the last administration of TPME or reference molecules. After 60 min, each animal, except group 1, received EtOH/HCl (4g/kg, b.w.) by oral administration. One hour later, rats were euthanized for sample collection.

2.7.1. Evaluation of gastric mucosal damage

The stomach of each animal was removed and opened along its greater curvature. The lesions in the gastric mucosa were macroscopically examined. Ulcer indexes were determined as the sum of the lengths of the whole gastric lesions (in mm^2).

2.7.2. Gastric volume juice determination

Gastric juice was collected and centrifuged at 3000 g during 5 min to remove insoluble materials. The supernatant was after measured using graduate tubes.

2.8. Lipid peroxidation measurement and antioxidant enzyme activity assays

Gastric tissue homogenate was freshly prepared in phosphate buffer 100 mM (pH 7) containing a mixture of mammalian protease inhibitors and then centrifuged at 3000g for 10min (4°C).

Lipid peroxidation was evaluated as described by Ohkawa et al. (1979). Briefly, samples were incubated with sodium phosphate buffer at 37°C for one hour and then precipitated with trichloroacetic acid. 1% of thiobarbituric acid was added and the mixture was placed in boiling water for fifteen minutes. Lipid peroxidation was calculated in terms of nmol/mg protein using molar extinction coefficient of 156000 M^{-1} cm^{-1} at 532 nm.

Catalase activity was evaluated as described by Aebi (1984). Briefly, samples were mixed with H_2O_2 and phosphate buffer (pH= 7). The absorbance was recorded at 240 nm and Catalase activity was expressed in µmol of H_2O_2 consumed/minute/mg of protein.

Superoxide dismutase (SOD) activity determination was performed according to Rtibi et al (2015). Briefly, 10 µl of bovine catalase (0,4 U/µl) was added to our samples and the whole was then mixed with 20 µl epinephrine (5mg/ml) and 62,5 mM sodium carbonate-sodium bicarbonate buffer. The absorbance was read at 480 nm and expressed by Unit of SOD/min/mg protein.

GPx was determined as described by Rtibi et al. (2015), 0.4 ml of phosphate buffer is added to 0.05 ml of sample. 0.53 ml of distilled water is added before stirring the mixture and adding 0.01 ml of GSH and 0.01 ml of 1-chloro-2,4-dinitrobenzene (CDNB). Once the reaction is triggered, the optical density is measured at a

Table 1. Experimental Groups and Treatments

Group	I (control)	II (ulcerated group)	III (positif control)	IV (treated group)	V (treated group)
Treatment	Treated by bi-distilled water (5mL/kg, b.w., p.o)	-Treated with saline solution (10mL/kg) -After 1h 1mL/rat of acidified ethanol solution (60mLEtOH+1.2mL HCl+38.8mL H_2O)	Treated with famotidine (10mg/kg, b.w. p.o) during 7 days	Treated with 100mg/kg, b.w p.o of TPME	Treated with 200mg/kg, b.w p.o of TPME

wavelength λ= 340 nm every 30 seconds for 2 minutes. GPx was expressed by 10^{-3} mM/min/mg of Protein.

2.9. Statistical analysis

Statistical comparison between groups was evaluated by ANOVA followed by Sidak's test for all *post-hoc* multiple comparisons. A *p*-value of 0.05 or less was considered as statistically significant.

3. RESULTS

3.1. Phytochemical analysis

The total amounts of phenolic and flavonoid contentained in leavesof *T. polium* are summarized in Table 2. The values were 275.89 ± 15.75 mg GAE/g DM and 128,66 ± 9,84 mg CE/g DM respectively, for total polyphenol (TPC) and flavonoids content (TFC).

RP-HPLC coupled with a UV-Vis multi wavelength detector was employed to separate and to quantify phenolic compounds. Fig. 2 shows the chromatogram relative to the *T. polium m*ethanolic extract. Five phenolic compounds were successfully identified including 3 phenolic acids (*p*-coumaric acid (15.02mg/gDR±1.55), ferulic acid (1.23 mg/gDR±0.03), and syringic acid (2.11 mg/gDR±0.03)) and 2 flavonoids (luteoline 7-*O*-glucoside (1.21 mg/gDR±0.05) and hyperoside (1.05 mg/gDR ±0.01). Furthermore, phytochemical investigation allowed us to depict *p*-coumaric acid as major phenolic compound.

3.2. In vitro antioxidant activity.

In the current study, the antioxidant ability of *T. polium m*ethanolic extract was screened through *in vitro* methods, namely DPPH and FRAP tests in order to evaluate their scavenging ability toward DPPH radical, as well as the ability to reduce Fe^{3+} to Fe^{2+} respectively. As shown in Table 2, *T. polium* exhibited capacity to neutralize the DPPH radical with an IC_{50} value equal to 180±15.9µg/mL. Concerning the ferric reducing activity power (FRAP), the results showed that $EC_{50\%}$=230 ± 10.75 µg/mL.

3.3. Effect of *T. polium* against Ethanol/HCl-Induced Gastric Ulcers

The administration of *T. polium* extract at 100 and 200 mg/kg for 7 days did not result in any sign of toxicity and mortality of the rats, since during that period of observation, there was no abnormal behavior among the animals in terms of salivation, diarrhea, hyperexcitability, respiratory suffering, and mortality, compared with the control group. Based on that, it was concluded that the 2 doses of *T. polium* extract were safe to be administered to the animals, in order to test and evaluate their gastroprotective

Table 2. Total phenolic (TPC) and flavonoid contents (TFC) from plant methanolic extract, DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP) tests.

Specie	TPC (mg GAE/ g DM)	TFC (mg CE/ g DM)	DPPH ($IC_{50\%}$µg/mL)	FRAP ($EC_{50\%}$µg/mL)
T. polium	275.89 ± 15.75	128,66 ± 9,84	180±15,9	230 ± 10,75

Values are the means of three replicates and standard deviation.

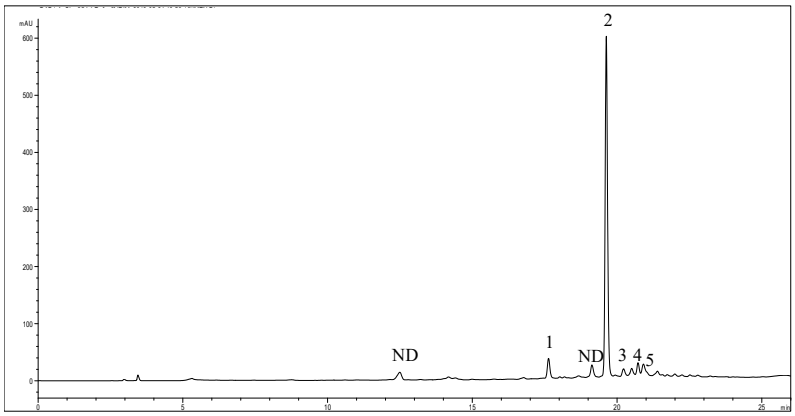

Fig. 2. RP-HPLC chromatograms of *T. polium* methanolic extract. Signal was monitored at 280 nm. The peak numbers correspond to: 1: syringic acid; 2: *p*-coumaric acid; 3: ferulic acid; 4: luteolin 7-O-glucoside ; 5 : hyperoside.

effects.

The administration of HCl/EtOH cause damage to the gastric mucosa of rats (Group 2) as evidenced by the ulcer index (UI=2.59±0.65). This damage results in the presence of several bleeding points and furrows. Indeed, Famotidine treatment significantly reduced this ulcer index to 0.69 ±0.25. Nevertheless, *T. polium* extract treatment minimized the pathologic changes induced by EtOH/HCl, reducing inflammatory cell infiltration and submucosal edema in a dose-dependent manner. In particular, the administration of TPME at 100 mg/kg significantly ameliorated the gastric tissue, allowing it to maintain its normal structure (UI=0.81±0.32). The protective effect of TPME at 200 mg/kg was similar to that afforded by the pharmaceutical drug famotidine (UI=0.72±0,43).

The same tendency is obtained for the percentage of protection (PP) of the gastric mucosa which is significantly improved following the administration of TPME at a dose of 200 mg kg^{-1} (79.72%).

Variaitions in the volume of gastric juice (GV) are reported in Table 3. It indicated that the ulceration caused by the EtOH/HCl mixture led to an increase in the GV to 4.21±0.51 mL accompanied by an increase in acidity (pH = 2.5±0.85), in comparison to control group. Besides, Group 3 which received Famotidine reported a reduced GV of 2.72±0.40ml ($p<0.05$) and increased pH to 3.6±0.83mL. Concerning groups 4 and 5, the administration of the TPME (100 and 200 mg kg^{-1}) restored the GV and pH parameters. Notably, no significant difference between used doses of TPME that have similar effects as Famotidine.

3.4. Effect of *T. polium* extract on lipid peroxidation

Results showed a significant increase in malondialdehyde (MDA) level in HCl/EtOH group in comparison with control group (FAM) (5.08 ± 0.25 vs 4.31 ± 0.33 nmol/mg proteins, (Fig. 3). Treatment of ulcerated rats with two doses of *T. polium* extract (100 and 200 mg/kg) induced significant decrease of malondialdehyde level in comparison with HCl/EtOH group (4.55 ±0.42 and 4.2± 0.21 nmol/mg proteins respectively for the doses at 100 and 200 mg/kg).

3.5. Effect of *T. polium* extract on enzymatic antioxidant levels: CAT, SOD and GPx activities

Table 3. Effect of oral pre-treatment with *T. polium* extract on gastric ulcer parameters in rats (n = 8/group).

	Group 1 (Normal Control)	Group 2 HCl/EtOH	Group 3 FAM	Group 4 TPME (100mg kg^{-1})	Group 5 TPME (100mg kg^{-1})
UI	-	2,59±0,65 ^{a}	0,69±0,25 ^{b}	0,81±0,32 ^{c}	0,72±0,43 ^{b}
PP	-	-	86,24 ^{a}	59,56 ^{c}	79,72 ^{b}
GV	6.26^{d}	4,21±0,51^{a}	2,72±0,40 ^{b}	2,21±0,22 ^{c}	1,98±0,77 ^{c}
GpH	5.09^{d}	2,5±0,85 ^{c}	3,6±0,83 ^{b}	4,8±0,64 ^{a}	4,7±0,49 ^{a}

UI: Ulcer index (mm); GV: gastric volume ml; GpH: gastric pH; PP: percentage of protection. Group 1: Ulcer control (pre-treated with 10 mL/kg of NaCl 0.9% followed by EtOH/HCl); Group 2: Positive control (pre-treated with famotidine 20 mg/kg, followed by EtOH/HCl). Groups 3 and 4: pre-treated with TPME at 100 and 200 mg/kg, respectively, followed by EtOH/HCl; Values are the means of three replicates and standard deviation. Values within the same line with different superscripts (a, b, c) are significantly different at $p < 0.05$.

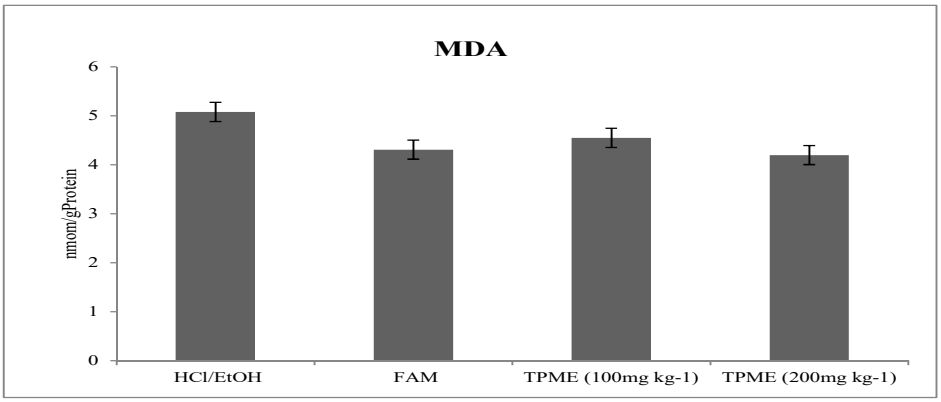

Fig. 3. Effect of *Teucrium polium* methanolic extract (TPME) and famotidine (FAM) ethanol (EtOH)-induced changes in stomach mucosa MDA levels in rats. Animals were pre-treated with 2 doses of TPME (100and 200 mg/kg, b.w., p.o.).

As shown in Table 4, EtOH/HCl treatment increased the gastric levels of antioxidant enzymes, when compared to the control group ($p < 0.05$). Hence, the results suggest that TPME acts as a ROS scavenger.

Gastric SOD,CAT and GPx activities significantly decreased; in ulcerated group (128.4±15.7; 1.57±0.28 and 209.3±36.3 respectively) in comparison to the control group treated with famotidine. Whereas, treatment with both doses of TPME significantly increased the gastric SOD, and increased the gastric levels of CAT and GPx compared with the ulcerated group and values are closed to those of control group treated with famotidine.

Table 4. Effect of oral pre-treatment with *T. polium* extract (100 and 200 mg/Kg) on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities. Values are the means ± SD of three independent assays. Values with different superscripts (a-d) are significantly different at $p < 0.05$ as compared to control group.

	Group 1 Normal control	Group 2 HCl/EtOH	Group 3 FAM	Group 4 TPME (100mg kg^{-1})	Group 5 TPME (100mg kg^{-1})
SOD (U/mgProtein)	202, ± 12,5 ^{a}	128,4 ± 15,7 ^{b}	229,5 ± 20,3 ^{a}	214,8 ± 16,3 ^{a}	232,7 ± 20,5 ^{a}
CAT (Mol H_2O_2/min/mgProtein)	3,21 ± 0,22 ^{b}	1,57 ± 0,28 ^{c}	3,96 ± 0,48 ^{a}	3,45 ± 0,95 ^{b}	3,59 ± 0,82 ^{b}
GPx (10^{-3} mM/min/mgProtein)	311,6 ± 11,4 ^{b}	209,3 ± 36,3 ^{d}	369,5 ± 46,2 ^{a}	329,1 ± 17,8 ^{c}	349,6 ± 18,8 ^{b}

4. DISCUSSION

Gastric ulcer is a frequent gastrointestinal tract disorder that affects about 10% of the world population (Beiranvand and Bahramikia, 2020). A gastric ulcer occurs due to an imbalance between the protective and aggressive factors. Gastric ulcers are usually caused by *Helicobacter pylori* bacteria or non-steroidal anti-inflammatory drugs (NSAIDs), and other factors such as alcohol drinking, smoking and dietary habits (Gugliandolo et al., 2021). These can break down the stomach's defence against the acid it produces to digest food (Gugliandolo et al., 2021). Ethanol is mostly used to induce gastric ulcers in animal models. Ethanol ingestion causes gastric cell necrosis and vascular injury, and consequently, ulceration (Rtibi et al., 2015).

Many studies using ethanol-induced gastric ulcer models have been extensively performed in animals to investigate the protective effect of plants (Birdane et al., 2007).

The current investigation evaluated the gastro-protective effect of TPME against EtOH/HCl-induced gastric ulcer. Gastric ulcer was induced by oral administration of EtOH/HCl. In accordance with previous studies there are no sign of toxicity and mortality of the rats, compared with the control group (Chabane et al., 2020; Mehrabani et al., 2009). Based on that, it was concluded that the 2 doses of TPME were safe to be administered to the animals, in order to test and evaluate their gastroprotective effects. Gastric lesions produced by EtOH/HCl (Group 2) resulted in inflammation of the mucosa and the formation of several ulcers (UI=2,59±0,65) with a decrease in gastric juice volume from 6.26 to 4.21mL and pH from 5.09 to 2.5 compared with control group. In turn, the oral pre-treatment with 100 and 200mg/Kg of TPME ameliorated the gastric parameters and this is by restoration of mucosa gastric pH and volume. Moreover, EtOH-induced gastric ulceration has been previously shown to be attenuated by many plants extracts. These medicinal plants show in their chemical composition a variety of bioactive molecules, which protect the stomach mucosa through the induction of gastroprotective mechanisms or acting as natural antioxidants (Mehrabani et al., 2009).

A study conducted by Mehrabani et al. (2009) reported the same tendency for the *T. polium* from Iran reduced the ulcer indices by >50% after one week, >80% after 2 weeks, and >90% after 4 weeks. Another work of Chabane et al (2020) demonstrated after oral administration in

mice at doses of 1000 and 2000 mg/kg b.wt, of *T. polium* methanolic extract (from Algeria) no signs of liver and kidney toxicity were detected by analysis of biochemical parameters and by histological examination.

In addition and according to several works, the healing effect of *T. polium* may be due to antioxidant activity, *T. polium* was shown to have strong *in vitro* antioxidant properties (Ilhami et al., 2003; Mehrabani et al., 2009). In the present study, *T. polium* extract showed its richness in total polyphenols and flavonoids. On the other hand, antioxidant activities of *T. polium* have been widely studied (Sharififar et al., 2009; Tepe et al., 2011; Stankovic et al., 2012; De Marino et al., 2012), using the DPPH radical-scavenging and FRAP assays. In our study, we found that TPME presents a high scavenging capacity and a good ability to reduce iron. A recent study by Noumi et al (2020) reported that methanolic extract of *T. polium* from Saudi Arabia showed promising antioxidant activities ($IC_{50\%}$= 0.087 mg/mL for DPPH test and $EC_{50\%}$= 0.292 mg/mL for the FRAP test). This antioxidant effect of TPME has been linked to many of its bioactive molecules. Teucrium contains quite high values of phenolic compounds, which are one of the most significant categories of natural antioxidants (Sharififar et al., 2008) such flavonoids which were reported to have anti-ulcer and gastroprotective properties (Zayachkivska et al., 2005). Besides, one of the main components of TPME, which is responsible for most of its antioxidant effect, is the *p*-coumaric acid which is present in an interesting amount in our case. This compound exerts its antioxidant activity via augmenting the endogenous antioxidant enzymes and scavenging a variety of reactive species.

In fact, drug candidates that can provide high efficacy and low toxicity are needed value for the prevention and treatment of gastric ulcers (Zhou et al., 2020). The findings showed that the gastroprotective effect of Teucrium is attributed to its antioxidant activity. TPME enhances the synthesis of endogenous antioxidant enzymes and decreases lipid peroxidation in other models encompassing oxidative stress. Obviously, in ulcerated rats, a decrease in the activity of SOD, CAT, and GPx enzymes was observed, compared with normal rats, while TPME (100 and 200mg/Kg) and famotidine significantly reversed the ethanol-induced changes in these enzymes levels.

Human exposure to oxidative stress induces, with varied degrees of importance, protein oxidation, lipids peroxidation and nitrite release, causing accumulation of reactive oxygen species which are closely related with gastric ulcer (Ben Mansour et al., 2022). ROS cause lipid peroxidation manifested by MDA elevation as observed in the current study, and result in reduced membrane integrity of surface-epithelial cells, thereby causing gastric ulcers (Beiranvand and Bahramikia, 2020). Lipid peroxidation level is an indicator of the generation of ROS in the tissue. However, SOD converts the reactive superoxide radical to H_2O_2 in the gastric mucosa. CAT, as an important antioxidant enzyme, converts H_2O_2 to water and oxygen. It has been shown that under ethanol-induced gastric mucosal damage, the CAT activity and GPx content in gastric tissue reduces. This reduction leads to an increase in ROS accumulation and, consequently, an increase in lipid peroxidation (Antonisamy et al., 2014).

The gastroprotective effect of TPME, in a model of ethanol-induced gastric ulcer, suggests its possible useful use in the treatment or prevention of gastric ulcer.

In fact, *T. polium*'s gastroprotective effects are likely due to a multifaceted approach, addressing key factors involved in gastric ulcer development and healing. First, *T. polium* contains various compounds, including flavonoids and terpenoids, that exhibit strong antioxidant properties (Bahramikia et al. 2022), these antioxidants help neutralize free radicals, which play a significant role in the development and exacerbation of gastric ulcers. By reducing oxidative stress, *T. polium* helps protect the gastric mucosa from damage. Second, *T. polium* has been shown to possess anti-inflammatory properties, which help reduce inflammation in the gastric mucosa (Al-Naemi et al., 2024). This reduction in inflammation contributes to the healing and prevention of ulcers. Moreover, some studies suggest that *T. polium* may influence prostaglandin synthesis. Prostaglandins play a crucial role in maintaining the integrity of the gastric mucosa. So, by modulating prostaglandin levels, *T. polium* may contribute to its gastroprotective effects (Mehrabani et al., 2009).

5. CONCLUSION

This study demonstrates that TPME ameliorates EtOH/HCl-induced gastric mucosal injury. This gastroprotective effect of TPME is mediated by augmenting the antioxidant and cytoprotective defenses and by alleviating oxidative stress and thus, could have a therapeutic potential in EtOH/HCl -induced gastric ulcers. Further studies are envisaged for the isolation of its

bioactive compounds and highlight their mechanism of action.

ABBREVIATIONS

DPPH:2,2-Diphenyl-1-picryl hydrazyl; ROS: Reactive Oxygen Species; TPC: Total Phenolic Content; GI: gastrointestinal; EtOH: ethanol; HCl: hydrogen chloride; TFC: total flavonoid contents; OP: optical density; FRAP: Ferric reducing power assay; SOD: Superoxide dismutase; CAT: catalase; GPx: glutathion peroxidase; TPME: *Teucrium polium* methanolic extract.

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AUTHOR CONTRIBUTION

Rim Ben Mansour. Experimentation, data curation, writing the original manuscript; Imen Ghozzi. Contribution to investigation, data curation (*in vivo* assays); Riadh Ksouri. Contribution to conceptualization and to resources. Raja Serairi-Beji. contribution to data curation, supervision, writing paper.

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