Antioxidant and Anti-Ulcer Activities of *Teucrium Polium* Aqueous Extract

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Abstract : *Teucrium polium* is an important medicinal plant belonging to the family of Lamiaceae. It is used in traditional medicine for the treatment of inflammations, rheumatism, diabetes and ulcers. The aim of this study was to assess the *in vitro* antioxidant effects and the *in vivo* anti-ulcer activity of the aqueous extract of aerial part from *Teucrium Polium* (TPAE). The antioxidant effect of the TPAE extract was evaluated by ABTS and phenanthroline assays. Acute toxicity of PSSE was carried out based on OECD guidelines 425. The anti-ulcer effect of the extract was evaluated using the ethanol induced ulcer in rat. The estimation of polyphenols and flavonoids showed that the aqueous extract contains a high amount of polyphenols and flavonoids: **151.19 ± 1.22 µg gallic**

acid equivalents/mg of dry weight and 38.12 ± 2.01 µg quercetin equivalent/mg dry weight, respectively. TPAE extract had an ABTS scavenging and metal chelating effects. Oral administration of the TPAE extract produced a signifcant ($p < 0.0001$) anti-ulcer effect with a dose of 400 mg/kg compared to the negative control. Our findings highlight the medicinal use of TPAE in traditional medicine and as an additional source of natural and safe anti-ulcer agents

Keywords: Teucrium Polium, *polyphenols, flavonoids, antioxidants, anti-ulcer effect.*

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1. Introduction

The human body produces reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical and hydrogenperoxide by many enzymatic systems through oxygen consumption. In normal amounts, these ROS are beneficial as growth regulators and signal transducers (Holmström and Finkel., 2014). However, during oxidative stress, large amounts of these ROS can be produced and may be dangerous because of their ability to attack numerous molecules, including proteins and lipids. In fact, it has been reported that ROS contributes largely to cellular aging, mutagenesis, and coronary heart disease through sever always including membrane destabilization, DNA breakage and generally by oxidizing low-density lipoproteins (LDL) (Fang et al., 2002). Antioxidant agents can aid in the scavenging of different reactive oxygen species as well as the prevention of many diseases (Loucif et al., 2022c). The cell can reduce the impact of ROS either by an endogenous system or by an exogenous system using antioxidants (Cheesman K, Slater, 1993). Antioxidants are compounds that can delay or inhibit the oxidation of lipids and other molecules and by doing so inhibit the initiation and propagation of oxidative chain reactions (Loucif et al., 2022a). there is a growing interest in the search for antioxidants in plants (Loucif et al., 2022b). Previous findings clearly show that the consumption of plant-derived polyphenolic compounds and natural antioxidant supplements may be used to protect the body against various diseases, including ulcer, cancer, cardiovascular, and neurodegenerative diseases. Natural antioxidants help the endogenous antioxidant system to reverse oxidative damage or protect oxidative stress-induced deterioration (Sen et al., 2013). Phenolic compounds and flavonoids have been found to have therapeutic applications against different diseases caused by oxidative stress, and several researchers demonstrated the correlation between polyphenolic compounds and the antioxidant activity of plant extracts (Loucif et al., 2021).

Teucrium polium belonging to the Lamiaceae is endemic in Algeria. It is used in traditional medicine for the treatment of inflammations, rheumatism, diabetes and ulcers (Tariq et al., 1989). The aim of the present study was to assess the *in vitro* antioxidant effects and the *in vivo* anti-ulcer activity of the aqueous extract of aerial part from *Teucrium Polium*. The antioxidant effect of the extract was evaluated.

2. Material and Methods

2.1. Plant material

The plant *Teucrium Polium* (aerial parts) was collected in the Setif region in the North-Eastern of Algeria, during the flowering period

2.1.1. Preparation of the extracts

100g of *Teucrium Polium* powder was mixed with 1 L of boiled distilled water (100 °C), and after 20 minutes, it was removed from the heat. The mixture was filtered using Wattman filter paper n°1 and then dried at 45 °C to obtain *Teucrium Polium* aqueous extract (TPAE), which was dissolved in water for further analysis (Ferreira et al., 2006).

2.2. Animals

In this investigation, adult female Wistar rats (150–180 g) and albino mice (25–30 g) were used. These animals were purchased from the 'Institut Pasteur d'Algérie', Algiers. Rats and mice were housed in cages under standard conditions of 12:12 h light/dark cycle and 25 ± 1 °C for seven days before the experiments. They were given free access to water and standard diet (ad libitum), and kept under standard conditions mentioned in the Animals By-Laws N° 425–2008. Experimental assays were approved by the Committee of the 'Association Algerienne des Sciences en Experimentation Animale' (http://aasea.asso.dz/ articles/) under law No. 88-08/1988, associated with veterinary medical activities and animal health protection (N° JORA: 004/1988).

2.3. Phenolics content determination

2. 3. 1. Determination of total phenolic content (TPC)

The total phenolic content of TPAE was estimated spectrophotometrically using the Folin–Ciocalteu method (Singleton et al., 1965). A volume of 20 µl of the extract was added to 100 µl of Folin– Ciolcalteu reagent (10%). Then, a volume of 75 µl of sodium carbonate solution (7, 5%) was added. The obtained mixture was incubated for 2 h in darkness at ambient temperature, and the absorbance was determined at 765 nm. Gallic acid was used as a reference to establish the calibration curve from which the concentration of polyphenols was calculated and the results were expressed in micrograms equivalent of gallic acid per milligram of extract (µg GA/mg of extract).

2. 3. 2. Determination of total flavonoids content (TFC)

The quantification of the total flavonoid content was performed by the trichloro-aluminum method (Topçu et al., 2007) with some modifications. Briefly, 130 µl of methanol were added to 50 µl of a sample (1mg extract/1ml water). Subsequently, 10 µl of 1M potassium acetate and 10 µl of 10% aluminum nitrate were added. The mixture obtained was incubated at room temperature for 40 minutes, and the bsorbance was read at 415 nm. Quercetin at different

concentrations was used to realize the calibration curve to estimate the concentration of flavonoids found in the aqueous extract and the results have been given in micrograms equivalent of quercetin per milligram of extract (µg EQ/mg of extract).

2. 4. Antioxidant activities

2. 4. 1. Phenanthroline Assay

This test was carried out according to the method described by Szydłowska-Czerniak et al. (2008) A volume of 10 µl of a sample at different concentrations was placed into a 96 well roundbottomed plate. Then, 50 µl of FeCl3 (0.2%), 30 µl of phenanthroline (0.5%), and finally, 110 µl of methanol was added. The microplate was incubated 20 minutes at 30°C in a dark, and the absorbance of the solution was measured at 510 nm. BHT and BHA were used as antioxidant standards.

2.4.2. ABTS Radical Cation Decolorization Assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Re et al. (1999). After the preparation of the oxidation solution of ABTS, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.700±0.020 at 734 nm with water. Then, 160µl of ABTS solution was added to 40 µl of a sample at different concentrations. After 10 min, the absorbance was measured at 734 nm. Water was used as a control, together with BHA and BHT were utilized as antioxidant references for comparison of the activity. The results were given as the IC_{50} (µg/ml), which was calculated utilizing the following equation: $ABTS^{\bullet+}$ Scavenging effect (%) = [(AControlASample)/AControl] ×100.

2.5. Acute oral toxicity in mice

In order to assess the biosafety of the TPAE, the acute toxic class method described by OECD guidelines 423 (OECD, 2001) was used. The animals were randomly divided in experimental groups containing 6 animals each (3 males and 3 females). Prior TPAE administration, all animals were fasted overnight with free access to clean tap water. The treated and control groups were weighed and then received by gavage a single dose of 2000 mg/kg or 5000 mg/kg and distilled water, respectively. After dosing, all animals were individually observed for mortality, toxicological and other symptoms (morphological, physiological, and behavioral changes), with special attention given during the first 4 h, and periodically during the first 24 h, and then daily for the following 14 days.

2.6. Gastroprotective effect evaluation in rats

The method followed is that one described by Gharzouli et *al.*, (2002). It consists in verifying the protective action of TPAE at 200 and 400 mg/kg against the ulcer caused in animals by administration of pure ethanol. A total of four batches of eight rats were set up for the test, one batch for each dose (200 and 400 mg/kg) of TPAE, one batch for the negative control (CMC 1.5

%) and one batch for ranitidine (40 mg/kg) which is used as a standard (positive control). The rats fasted for 24 hours with free access to the glycosylated water, they were placed separately in individual cages before experimentation. An hour after removing the water, and at time T=0, the rats received intragastrical 1 ml/200 g of sample (crude extract at different dose or ranitidine). The control group received only CMC (1.5%). One hour later, each rat received intragastrical 0.5 ml/200 g of pure ethanol. Half an hour after administration of the ethanol, the rats were sacrificed. The stomach of each rat was removed, opened with great curvature using a chisel, washed with a physiological solution and then spread well and fixed on a tablet to better observe the ulcers formed. The stomachs were photographed for better vision. The total area of the lesions and the total area of the stomach were measured using Image J 1.52o software (Wayne Rasband, National Institutes of Health, USA). The percent of the ulceration was calculated for each group treated according to this formula: % Ulceration = [UAs/ UAc] ×100. Where; UAc: ulcer area of the negative control. UAs: ulcer area of the Sample. Histological analyzes of the glandular gastric mucosa were performed to determine the severity of the ulcers. Antioxidant activity was determined using following biochemical parameters: estimation of lipid peroxidation (MDA), reduced glutathione (GSH), catalase (CAT), Superoxide dismutase (SOD) and total proteins.

2.6.1. Histopathological preparations

The histological sections were made at the pathology anatomy laboratory (CHU of Sétif). A portion from glandular part of stomach from each experimental group was fixed in formalin (10%). They were cut into small pieces. These samples are dehydrated by passage through three successive ethanol baths of 30 min (70, 90 and 100°C). Then they are thinned in two baths of 20 minutes of toluene and included in the paraffin (two baths of 2 hours each). The operation is automated using an automaton (TISSUE-TEK). The final inclusion is then carried out in metal molds. The paraffin blocks obtained are then cut with a microtome and the 5 µm thick sections were spread on slides with a 2% gelatin gel and then dried in an oven set at a temperature of 35-42°C., rehydrated and dried, stained with hematoxylin-eosin. The tissue sections were examined by a pathologist using microscopic analysis. The slides were later photographed.

2.6.2. Evaluation of *in vivo* antioxidant activity of gastric homogenate

a. Preparation of homogenate

The glandular portion from each stomach was cut, weighted and homogenized in 50 mM Tris HCl buffer (pH 7.4) using Dounce homogenizer in ice-cold condition to obtain 10 % (w/v) homogenate. Then the homogenate was centrifuged at 4000 g at - 4 °C for 15 min and the supernatant was collected and stored at -20 °C for the following biochemical parameters estimation: lipid peroxidation (MDA), reduced glutathione (GSH), catalase (CAT), Superoxide dismutase (SOD) and total proteins.

b. Estimation of gastric total proteins content

Gastric total proteins were determined by the method of Gornall et *al.*, (1949) using the Biuret kit total protein reagent (potassium iodide, potassium sodium tartrate, copper sulphate and sodium hydroxide). Proteins give a blue-violet color with copper sulphate in alkaline medium. In brief, 1 ml of Biuret reagent was mixed with 25 µl of the tissue homogenate or standard (bovine serum albumin), then the mixture was incubated at room temperature for 10 min. The absorbance was then read at 540 nm. Total protein amount was calculated according to the following formula:

Total protein (mg/ml) = $(A_{sample}/A_{standard}) \times n$. Where n is standard concentration.

c. Estimation of catalase activity

Catalase (CAT) activity was measured by the method of Clairborne (1985) with slight modification. The principle of this assay is based on the hydrogen peroxide (H2O2) breakdown in the presence of catalase to the water (H_2O_2) and oxygen (O_2). A solution of 19 mM H_2O_2 (2.9 ml) in 50 mM phosphate buffer pH 7.4 was put into a quartz cuvette, 50 µl of tissue homogenate was added. The rate of decomposition of H_2O_2 in presence of CAT was monitored spectrophotometrically at 240 nm immediately and at every 15 seconds for 1 min; the enzymatic activity was expressed as nmole H_2O_2/min/mg.

d. Assessment of reduced glutathione

Reduced glutathione (GSH) was measured by the method of Ellman (1959). The assay is based on the oxidation of GSH by 5, 5' -dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent). DTNB and GSH react to generate 2-nitro-5-thiobenzoic acid (TNB) which has an intense yellow color and maximum absorbance at 412 nm. For this assay, 50 µl of the tissue homogenate was diluted in 10 ml of phosphate buffer (0.1 M, pH 8). To 3 ml of the mixture of dilution, 20 µl of DTNB (0.01 M) were added and after 5 min of incubation, the yellow color developed was read at 412 nm. The concentration of GSH was calculated using the molecular absorption coefficient (ε TNB: $13.6 .10^3$ $M^{-1}.cm^{-1}$), (Razinger et *al.*,2008). The results were expressed as mmol/mg of tissue.

e. Determination of superoxide dismutase activity

The method used to determine superoxide dismutase (SOD) activity was based on the capacity of SOD to inhibit pyrogallol autoxidation, according to Marklund and Marklund (1974) and Gao et al., (1998). Supernatant aliquots (5 µl) were added to 1 ml of buffer solution (50 mM Tris HCl, pH 8.2) and 10 µl pyrogallol (20 mM). An increase in absorbance was measured at 420 nm every 30 s for 1 min against a blank. Inhibition % = $(A_{control}-A_{sample}/A_{control}) \times 100$. The amount of enzyme that produces 50% inhibition of pyrogallol autoxidation, relative to the control, was defined as one unit of SOD activity.

f. Lipid peroxidation estimation

Stomach tissue lipid peroxidation (LPO) was assessed by measuring malondialdehyde (MDA) formation following the method of Ohkawa et al., (1979). The principle of this method consists of the reaction of MDA with thiobarbituric acid (TBA) in acid conditions and a higher temperature (100°C) to form a pink MDA-(TBA)2 complex. Briefly, 0.5 ml of TCA (20 % w/v) was added to 0.5 ml of tissue homogenate, then 1 ml of TBA (0.67 % w/v) was added. The mixture was incubated at 100 °C for 15 min, cooled immediately in ice and mixed with 4 ml of n-butanol and centrifuged at 3000 rpm for 15 min. The absorbance of the clear pink supernatant was measured spectrophotometrically at 532 nm against a blank. The concentration of MDA was calculated using the molecular absorption coefficient (ε MDA-TBA: 156 $mM^{-1}.cm^{-1}$), (Razinger et *al.*, 2008). The results were expressed as nmole of MDA/ g of tissue (nmole MDA/g of gastric tissue).

2.7. Statistical analysis

Statistical tests were carried using Graph Pad Prism (Version 7.00). *In-vitro* results were expressed as mean ± standard deviation (SD) and in vivo results were expressed as mean ± standard error of means (SEM). Results were analyzed for significance using one-way analysis of variance (ANOVA) followed by Dunnett's test, at 5% probability level.

3. Results

3.1. Total polyphenols, and flavonoids contents

The results presented in Table 1, showed that the TPAE contained a high total polyphenols and flavonoids (151.19 ± 1.22 µg gallic acid equivalents/mg of dry weight) and (38.12 ± 2.01 µg quercetin equivalent/mg dry weight), respectively.

Table 1: Total polyphenols and flavonoids content of TPAE extract.

Extract	Total phenolic content (a)	Total flavonoid content (b)
TPAE	151.19 ± 1.22	38.12 ± 2.01

TPAE: *Teucrium Polium* aqueous extract, (a): µg GAE/mg and (b): µg QE/mg.

3.2. Antioxidant activities

3.2.1. Phenanthroline Activity

The antioxidative activity was observed in TPAE using phenanthroline test as shown in Table 2. This assay showed that the TPAE had a strong antioxidant activity with an $A_{0.5}$ of

200.85±3.17µg/mL.

3.2.2. ABTS Radical Cation Decolorization Activity

ABTS scavenging assay showed that the TPAE exhibited a good effect with an IC_{50} of 29.76 ± 1.06 µg/mL (Table 2). This suggests a significant antioxidant activity of TPAE.

Table 2: Antioxidant activities of TPAE

Extract/ standard	ABTS scavenging activity	Antioxidant activity by phenanthroline assay
	IC_{50} (µg/mL)	$A_{0.5}$ (µg/mL)
TPAE	29.76 ± 1.06****	200.85±3.17****
BHA	1.81±0.10	7.13±1.01
BHT	1.29+0.30	12.14+0.79

**** p <0.0001 compared to corresponding standards. TPAE: *Teucrium Polium* aqueous extract, BHA: butylatedhydroxyanisole, BHT: butylatedhydroxytoluene and ABTS: 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid).

3.3. Acute oral toxicity in mice

At the end of the 14 days of observation, no mortality or morbidity were recorded for either sexe at two doses of 2000 mg/kg and 5000 mg/kg. Moreover the animals did not show any toxic effects, behavioral or morphological changes

3.4. Gastroprotective effect

3.4.1. Evaluation of the degree of ulceration by the calculation of the surfaces

Intragastric administration of absolute ethanol to the untreated group of rats (the vehicle (1.5% CMC) as negative control) produced large bandlike hemorrhagic erosions in the glandular stomach (75.26±4.00%). Pretreatment with TPAE at the tested doses (200 and 400 mg/kg) offered different degrees of protection to the mucosa against all such damages caused by ethanol (20.99±1.65% and 1.07±0.05%, respectively; P≤0.001 and P≤0.0001, respectively). Compared with the 1.5% CMC vehicle-treated animals. The tested doses of TPAE extracts dose-dependently reduced significantly the gastric ulcer. The highest dose of TPAE exhibited a better reduction of ulcer gastric than the positive control ranitidine (4.02±0.12%; P≤0.5) (Table 3).

Table 3: Effects of TPAE on gastric ulcer in rats.

Sample	Negative control (CMC)	TPAE (200mg/kg)	TPAE (400mg/kg)	Positive control (ranitidine)
Inhibition of ulcer (%)	75.26±4.00	20.99±1.65^{**c}	1.07±0.05^{****b}	4.02±0.12****

PAE: *Teucrium Polium* aqueous extract. Bars represent means±SEM (n=8). ***; P≤0.001, ****; P≤0.0001 vehicle as negative control. ^{b*}; P≤0.05, ^{c**}; P≤0.01, *vs* ranitidine as positive control.

3.4.2. Macroscopic examination of TPAE effect on ethanol-induced gastric mucosa damage in rats

The assay revealed an effect of ethanol on gastric tissues in the absence and in the presence of TPAE at different doses, and the results are shown in figure 1. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa in the vehicle as negative control animals (ulcer control group) (figure 1. A). The positive control group (ranitidine 40 mg/kg) showed milder injuries to the gastric mucosa (figure 1. B) compared to the ulcer negative control rats. However, oral administration of TPAE (figure 1. C) effectively reversed the ethanol-induced gastric injury in a dose-dependent manner, with significant reduction of the gastric ulcer area. The protective properties of the TPAE extract at its highest dosage; 400 mg/kg (figure 1. C3) appeared a best protective activity than to the positive group (figure 1 B).

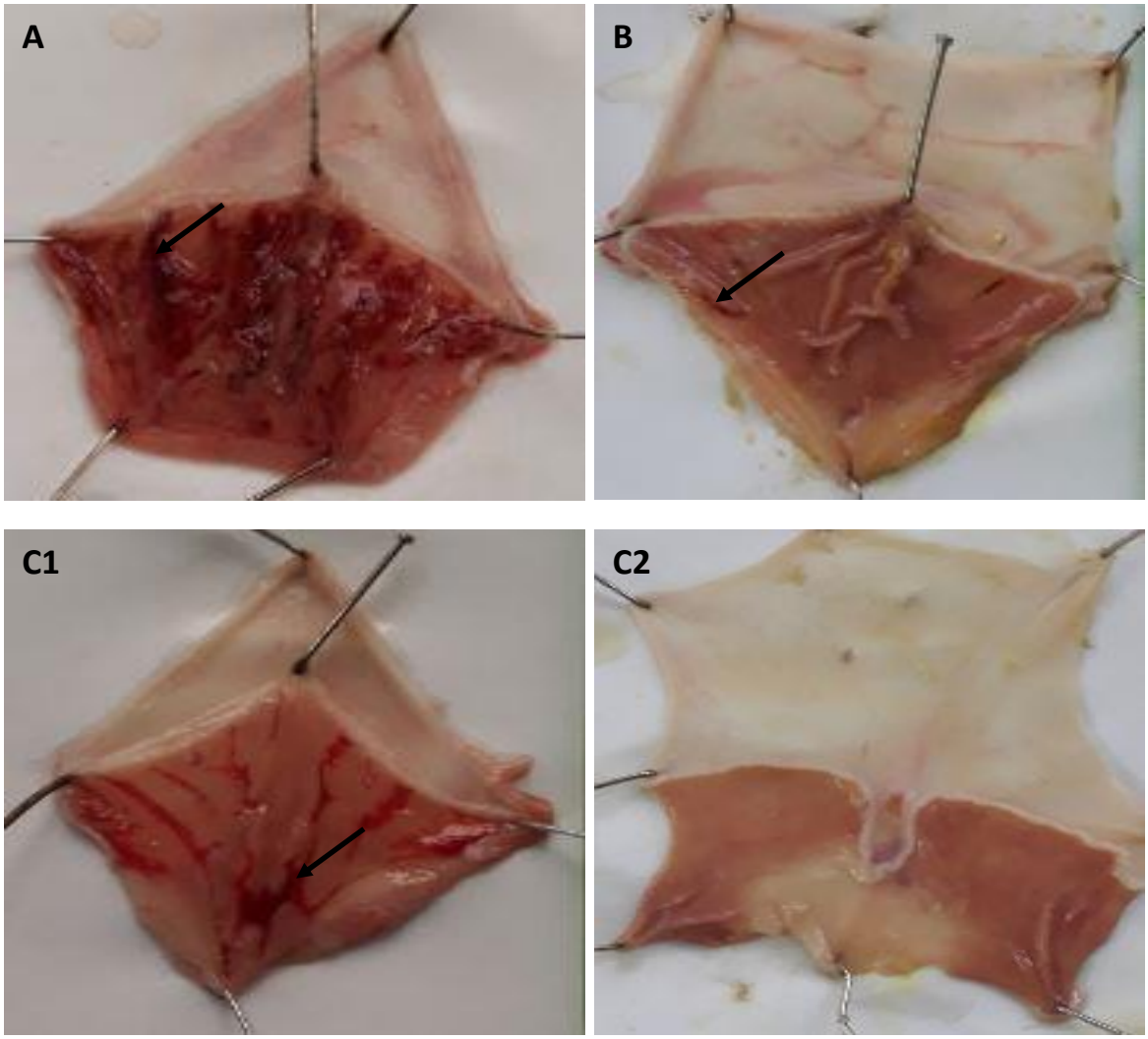

Figure 1: Effects of TPAE on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats. (A) The group pre-treated with CMC vehicle as negative control. (B) The group pre-treated with ranitidine (40 mg/kg) as positive control. (C1 and C2):

The group pre-treated with TPAE (200 and 400 mg/kg, respectively). PAE: *Teucrium Polium* aqueous extract. Black arrow: elongated bands of hemorrhagic lesions.

3.4.3. Histopathological examination of TPAE effect on ethanol-induced gastric mucosa damage in rats

Histological study of ethanol-induced gastric mucosal damage in rats in the absence and in the presence of ranitidine, TPAE at different doses are shown in figure 2. Stomach of vehicle plus ethanol-treated rat in ulcer control group (Figure 2. A) showing necrosis in the superficial mucosal epithelium (red arrow) with oedema, inflammatory cell infiltration (blue arrow) and congestion of blood vessels (green arrow) in submucosa. Stomach of ranitidine plus ethanol-treated rat in positive control group (Figure 2 B) showing intact histological structure of the mucosa and submucosa compared to that in ulcer control rats (Figure 2. A). Rat pretreated with TPAE (Figure 2. C) have relatively better protection in a dose-dependent manner. TPAE (200 mg/kg) reduced the oedema, inflammatory cell infiltration and congestion of blood vessels compared to the negative control. A complete absence of edema and leucocytes infiltration (blue arrow) was observed in rat pretreated with TPAE (400mg/kg).

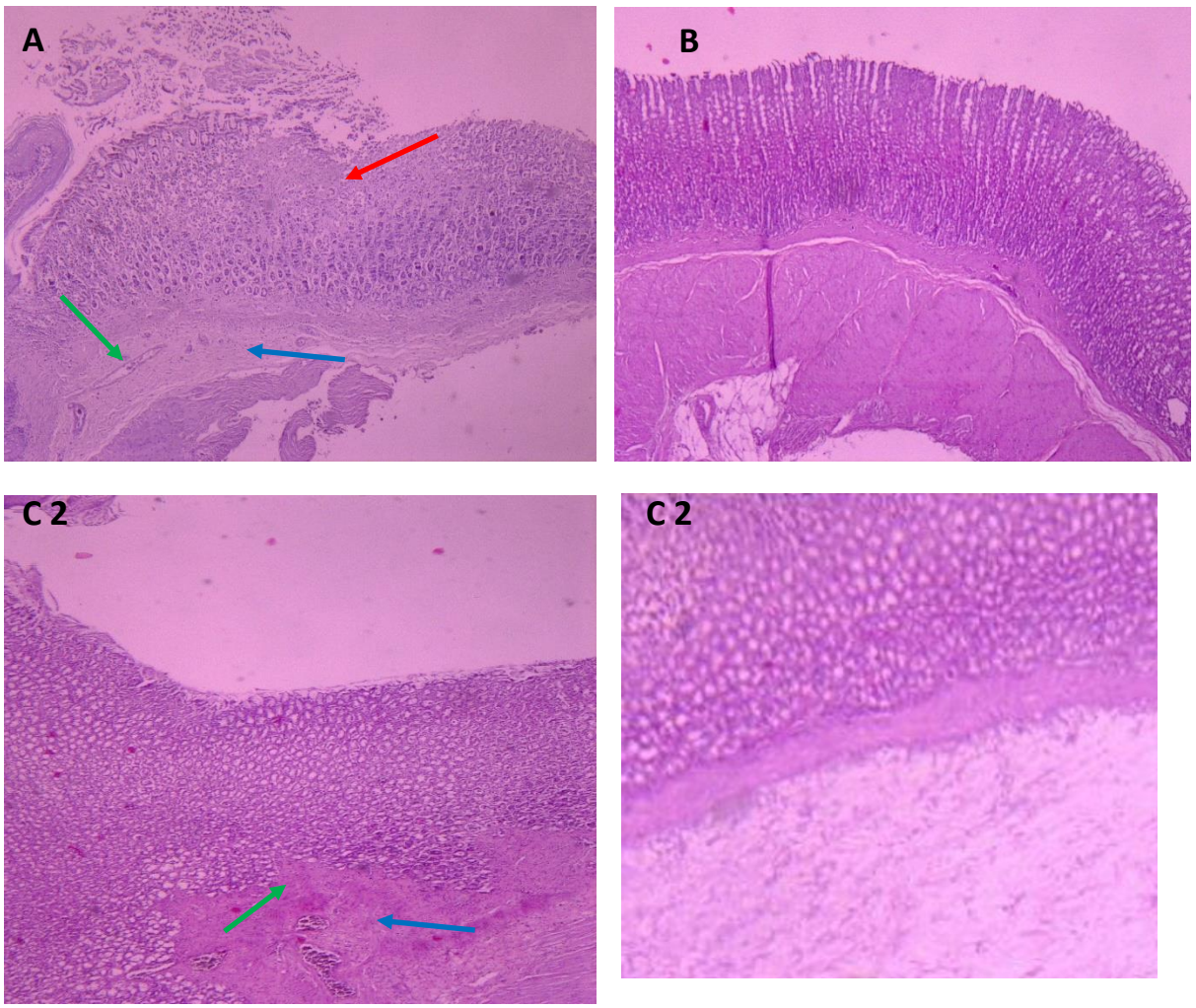

Figure 2: Histological examination for the protective effect of TPAE against ethanol-induced gastric damage in rat stomach tissue (magnification 100x). (A) The group pre-treated with CMC vehicle as negative control. (B) The group pre-treated with ranitidine (40 mg/kg) as positive control. (C1 and C2): The group pre-treated with TPAE (200 and 400 mg/kg, respectively). PAE: *Teucrium Polium* aqueous extract. Red arrow: surface epithelium damage and hemorrhagic necrosis penetrating deeply into gastric mucosa. Blue arrow: edema of submucosa and inflammatory cell infiltration. Green arrow: congestion of blood vessels.

3.4.4. *In vivo* antioxidant activity of gastric homogenate

3.4.4.1. Estimation of gastric total proteins content

Treatment with TPAE (400 mg/kg) resulted in an increase of total gastric protein level compared to the vehicle (P≤0.0001). TPAE (400 mg/kg) showed no significant difference in gastric proteins contents compared to ranitidine (P>0.05), (Table 4).

3.4.4.2. Estimation of catalase activity

Treatment of rats with the TPAE good effects on CAT activity. TPAE at 400 mg/kg doses increased the CAT activity (P≤0.01) compared to the vehicle. TPAE (400 mg/kg) showed no significant difference in catalase activity compared to the positive control; ranitidine (P>0.05), (Table 4).

3.4.4. 3. Assessment of reduced glutathione

Table 4 showed that, TPAE extract (400 mg/kg) increased the GSH levels to 6.58±0.29 nmol/g tissue (P≤0.0001) when compared to the vehicle (1.22±0.19 nmol/g tissue). TPAE extract (400 mg/kg) showed a similar GSH level compared toranitidine as positive control (P>0.05).

3.4.4. 4. Determination of superoxide dismutase activity

Oral treatment with TPAE (200 and 400 mg/kg) extracts inhibited the pyrogallol autoxidation, when compared to the vehicle (P≤0.01 to P≤0.001). The inhibition capacity of TPAE (400 mg/kg) was not significantly different compared to ranitidine as positive control (P>0.05), (Table 4).

3.4.4.5. Lipid peroxidation estimation

Lipid peroxidation (LPO) and hence MDA gastric content was markedly elevated following ethanol administration in normal rats (Table 4). TPAE dose-dependently attenuated the damage induced by ethanol (P≤0.001to P≤0.0001). At tested doses, TPAE reduced the amount of MDA to values (35.75±1.98 to 18.86±0.97 nmol/g tissue) which were lower than that in vehicle (82.8±1.24 nmol/g tissue).

Table 4: Effectsof TPAE on total protein level, CAT activity, GSH level, SOD activity and MDA level of stomach tissue in ethanol-induced gastric mucosal lesions in rats.

Antioxidant parameters	Extract doses		Positive control	Negative control
	200mg/kg	400mg/kg		
Total proteins (mg/ml)	0.21±0.03 $^{ns\ b}$	0.36±0.01$^{****\ a}$	0.35±0.02 ****	0.03±0.01
Catalase activity (nmol/min/mg)	1.89±0.14 $^{ns\ b}$	2.45 ± 0.11$^{**\ a}$	2.43±0.09 **	1.2±0.08
GSH level (nmol/gtissue)	3.32±0.39$^{*\ b}$	6.58±0.29$^{****\ a}$	6.82±0.49 ****	1.22±0.19
SOD activity (%)	37.79±3.22$^{**\ b}$	52.19±1.14$^{***\ a}$	50.45±6.63 ***	18.18±2.13
Gastric MDA level (nmol/g tissue)	35.75±1.98$^{***\ b}$	18.86±0.97 $^{****\ b}$	22.05±3.53 ****	82.8±1.24

PAE: *Teucrium Polium* aqueous extract. Bars represent means±SEM (n=8). ^{ns}; no significant difference (P>0.05), *; P≤0.05, **; P≤0.01, ***; P≤0.001, ****; P≤0.0001 *vs*vehicle as negative control. ^{a}ns; no significant difference (P>0.05),^{b}; P≤0.05 *vs* ranitidineas positive control.

4. Discussion

Free radicals are thought to contribute to several disorders in the body. Hydroxyl radical (OH•) is extremely reactive, more toxic than other radical species and can attack biologic molecules such as DNA, proteins and lipids. Thus, the scavenging ability of hydroxyl radicals is widely accepted as a way to evaluate the potential of antioxidants (Loucif et al., 2021), the later are a group of compounds that inhibit oxidation and reduce free radicals directly or indirectly. Oxidative stress may bealleviated *in vivo* by exogenous administration of antioxidants. Some synthetic antioxidants showed potential adverse effects on the body. Thus, research attention is turning to find more safe, effective, and natural antioxidants to resist oxidative stress (Benteldioune et al., 2019). There is a growing interest in natural antioxidants such as polyphenols, present in medicinal and dietary plants that could help prevent oxidative damage. The TPAE was assessed for its possible antioxidative activities by employing two complementary tests, phenanthroline (Phen) and ABTS methods. The Phen assay is based on the capacity of antioxidants (reductants) to reduce Fe^{3+} to Fe^{2+} (Tongpoothorn et al., 2012). TPAE showed high metal chelating capacity using the Phen test. Phenolic compounds have been reported to be chelators of free metal ions (Loucif et al., 2020b). To assay the ABTS radical scavenging of TPAE, cationic ABTS radical

decolorization was carried out. The ABTS radical is relatively stable but readily reduced by antioxidants. The scavenging activity against cationic ABTS radical indicates the ability of the extract to act as electron donors or hydrogen donors in free radical reactions (Loucif et al., 2022a). TPAE exhibited a strong scavenging capacity on ABTS. These antioxidant effects of plant extract are very probably attributed to its high phenolic compounds and flavonoids (Loucif et al., 2020a). In fact, antioxidant activities are partially linked to the presence of phenolic compounds (Loucif, 2022). Also, the literature showed that a good correlation was found between antioxidant activity and the content of polyphenols and flavonoids (Li et al., 2008).

This study determined the toxicity profile in mice after acute oral administration of the TPAE, and its effects of TPAE after acute oral administration in mice were observed. Our findings of present study showed that there are no visible signs of toxicity (behavioral, neurological or physical changes) or mortality were observed at two test doses (2000 mg/kg and 5000 mg/kg) within the 14 days of observation. It is concluded that the minimum lethal dose of the plant extract tested in this study was over 5000 mg/kg. Overall, the findings of this study indicate that the plant tested is non-toxic. According to Loomis and Hayes classification (1996), A chemical substance with an LD_{50} within the range of 5000–15000 mg/kg is considered as practically non-toxic.

Alcohol consumption can produce acute hemorrhagic gastric erosions, and excessive ingestion can result in gastritis characterized by mucosal oedema, sub-epithelial hemorrhages, cellular exfoliation, and inflammatory cell infiltration (Chen et *al.*, 2015). Ethanol is well known as a potent necrotizing agent that destroys the defensive factors of the mucosa, leading to the depletion of gastric wall mucus (Wallace, 2001). It is also reported that acute exposure of the gastric mucosa of rats to ethanol can result in gastric lesions similar to those occurring in gastric ulcer in humans; hence, ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity (Boligon et *al.*, 2014). In the present study, absolute ethanol administration induced severe hemorrhagic lesions. These finding were supported by the histopathological changes, where severe disruption to the surface epithelium, necrotic lesions penetrating deeply into mucosa, extensive oedema of the submucosal layer, inflammatory cells infiltration and and congestion of blood vessels occurred. Oral administration of TPAE effectively reversed the ethanol-induced gastric injury in a dose-dependent manner, with significant reduction of the gastric ulcer area compared to the negative control. In addition, TPAE ameliorated the aforementioned gastric histopathological changes induced by ethanol. Previous studies confirmed that Polyphenol was responsible for the effect of plants against ethanol-induced gastric ulcer (Loucif, 2022). Moreover, is growing evidence to suggest that ethanol-induced gastric mucosal injury is closely related to the increased ROS level (Pan et *al.*, 2008), and increased ROS provoke oxidative injury, cell death, and epithelial damage (Palle et *al.*, 2018). Previous findings have revealed also that ROS augment gastric acid secretion by histamine release and reduces mucus production by decreasing prostaglandin synthesis (Jesus et

al., 2013; Palle et *al.*, 2018). On the other hand, the body per se has enzymatic and non-enzymatic defenses, including GSH, SOD and CAT against ROS-induced lipid peroxidation (Mates et *al.*, 1999). GSH and SOD are known to scavenge superoxide, hydrogen peroxide, hydroxyl and lipid peroxyl radicals, and thus attenuate the tissue damage. CAT as a preventive antioxidant triggers the rapid conversion of peroxyl radical into biologically safe substances, like water (Wong et *al.*, 2013). MDA, an index of lipid peroxidation, can usually be quantified to identify lipid peroxidation (Pillai et *al.*, 2005). Thus, to address the role of oxidative stress in our model of ulceration, we assessed several oxidant-antioxidant parameters in the gastric tissues of rats. The experimental results showed that ethanol markedly increased MDA level, an effect that was accompanied by a decrease in GSH, CAT and SOD activities, supporting in this way the role of oxidative stress in the pathogenesis of ethanol-induced gastric ulcer. On the contrary, the treatment with TPAE resulted in a significant increase in the activities of SOD and the levels of GSH, as well as a decrease in MDA formation, indicating their antioxidant activity. This may explain at least in part their gastroprotective effect. The last effect is usually attributed to the chemical constituents found in this plant. In fact, compounds like those ones found in TPAE (phenolics and flavonoids) have been reported to possess antioxidant actions and to protect the stomach from ulcerogeny (Zakaria et *al.*, 2016, Shin et *al.*, 2021).

Conclusion

Our work exhibited that TPAE extract contain good level of phenolics. TPAE extract shows remarkable anti-oxidant potential compared to the standards used, and the extract exhibited a good anti-ulcer effect compared to ranitidine. These findings explain the pharmacological properties of *Teucrium Polium* and provided a scientific base for its use in traditional medicine. More investigation is required to identify and isolate the components responsible for these activities in *Teucrium Polium* extract.

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Declaration of Competing Interest

Authors declare no conflict of interest

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