Dual Protective Effects of *Taraxacum officinale* Ethanolic Extract against Cephalosporin-induced Anemia and HCl/ Ethanol-induced Gastric Ulcer in Wistar Rats

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ABSTRACT

Background: *Taraxacum officinale*, a traditional medicinal plant in Africa and notably Algeria, is widely used for its hematopoietic and gastrointestinal benefits. It is believed to stimulate red blood cell production and protect against gastric ulcers. This study aimed to evaluate the erythropoietic and antiulcer effects of its ethanolic extract in Wistar rats.

Methods: In the anemia model, 25 male rats were divided into four groups. Anemia was induced using cephalosporin (10 mg/kg) for 10 days, while treatment groups received ethanolic extract of *T. officinale* (EETO) (200 mg/kg, twice daily). Blood samples were analyzed for hematological and biochemical parameters. For the ulcer model, gastric lesions were induced with HCl/ethanol (1.50 µMol/60%) and treated with EETO (200 mg/kg) for 7 days. Gastric tissue was examined microscopically and malondialdehyde levels were measured.

Result: In anemic rats, *T. officinale* significantly increased red blood cells RBCs (52.10%), hemoglobin (38.56%), iron (50.95%) and several vitamins and minerals while reducing erythropoietin. In the ulcer model, the EETOreduced ulceration by 68% and oxidative stress markers. Histology confirmed mucosal protection. These findings support the therapeutic potential of *Taraxacum officinale* against anemia and gastric ulcers.

Key words: Anemia, *Taraxacum officinale*, Ulcer, Wistar rats.

INTRODUCTION

Nature is a rich source of remarkable plants with notable nutritional and medicinal benefits, such as *Buniumbul-bocastanum* (Bouhalla *et al.,* 2024; Bouhalla *et al.,* 2025) and dandelion (Ürüşan, 2023), which are encountered in Algeria.

Dandelion (*Taraxacum officinale*) has been valued since antiquity as a nutrient-rich remedy, traditionally used as a medicine for its diuretic, anti-inflammatory and hepato-biliary properties (Kisiel and Barszcz, 2000; Ahmad *et al.,* 2000).

Many medications, particularly antibiotics, can cause red blood cell lysis-a serious issue given RBCs' vital role in oxygenation. This raises significant safety concerns about frequent/long-term use of hemolytic drugs, despite their therapeutic necessity (Ighodaro *et al.,* 2020).

Anemia is an abnormality of the blood count. It is a health condition characterized by reductions in the number of red blood cells or hemoglobin, with the accompanying effect of a reduction in the ability of the blood to transport oxygen to the body's tissues. Abnormal low hemoglobin and circulating levels may result from decreased production of red blood cells, or increased lysis of red blood cells. Anemia is arguably the most common blood disorder, posing a critical health risk to at least a third of the world's population. It remains a major public health problem in many developing and underdeveloped countries. People of all ages, especially infants, young pregnant women and the elderly, are affected by this problem (Luka *et al.,* 2014).

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It is diagnosed by the blood count, an examination carried out on a blood test. It can be caused by destruction of red blood cells (hemolysis) or blood loss (hemorrhage) (De Benoist *et al.,* 2008).

Erythropoietin is the main hormone that regulates erythropoiesis (a complex, multi-step process of blood production). In addition to erythropoietin, the erythropoietic machinery requires a constant supply of certain minerals (iron, copper) and vitamins (A, B12, B6, C, E, folic acid, riboflavin and nicotinic acid). Insufficient supply of any of these propensity constituents can result in anemia (Owoyele *et al.,* 2011). Gastritis or gastric ulcer results in the destruction of gastric mucosa due to several inducers such as excessive acid secretion, pro-inflammatory cytokines and free radicals and Helicobacter pylori invasion (Boeing *et al.,* 2016). The presence of HCl with ethanol accelerates the process of gastric ulcerogenesis and impairs the protection of the mucous membranes against chemical agents like ethanol to exacerbate the lesions (Marotta *et al.,* 1999).

This work aims to highlight the anti-anemic effect as well as the anti-ulcer activity of *Taraxacum officinale* on Wistar rats.

MATERIALS AND METHODS

Collecting the plant

The plant material consists of the aerial part of *Taraxacum officinale* "Telfaf" at maturity. The harvest was carried out the Tazgait region of Mostaganem (Algeria) (Fig 1) in printer (March) 2020. The plant was washed with water to remove debris and soil particles.

Extraction of polyphenols

The extraction of the plant was done by maceration according to the protocol of (You *et al.,* 2010) with a small modification. The plant material is crushed using a mortar, 200 g of the latter is added to 1000 ml of 70% ethanol. Allow the suspension to macerate at room temperature for 48 hours in the dark. After filtration using Whatman No. 4 paper, the filtrate was concentrated using a steam rotator at 45°C so that the ethanol was evaporated. The ethanolic extract of *Taraxacum officinale* will be collected in a dark glass bottle then stored at 4°C.

Determination of total polyphenols of extract of *Taraxacum officinale*

We used the Folin-ciocalteu method to determine the content of phenolic compounds according to (Li *et al.,* 2009). The concentration of total polyphenols is calculated from the calibration line established with gallic acid (0-200 µg/ml) and is expressed in mg of gallic acid equivalent per gram of extract.

Animals and accommodation conditions

40 male Wistar rats (150-180 g) from Pasteur Institute (Bouzareah, Algiers) were acclimatized for 2 weeks under standard conditions (12 h light/dark cycle, ventilated housing, ad libitum access to food/water). Animal care followed National Institute of Health (NIH-USA) guidelines.

Induction of anemia with cephalosporin in experimental rats

Anemia was induced in test animals with Cefuroxime, (10 mg/kg BW, 1 ml distilled water) was administered orally (2 × daily, 10 days) to induce anemia (modified Ighodaro *et al.,* 2020 protocol).

20 rats were randomly grouped into 4 groups (n=5/group):

- Group I: Untreated control.
- Group II: Cefuroxime only (10 mg/kg BW).
- Group III: EETO only (200 mg/kg BW).
- Group IV: Cefuroxime (10 mg/kg BW) + EETO.

Animals received oral doses of *T. officinale* ethanolic extract and Cephalosporin twice daily for 10 days with ad libitum access to food/water. After overnight fasting, blood was collected *via* retro-orbital eye puncture under light chloroform anesthesia prior to sacrifice. Samples were stored in dry and EDTA tubes for analysis. The experimental animals are as follows:

Group 1 (G1): Control group.
Group 2 (G2): Untreated anemic group (10 mg/kg cephalosporin).
Group 3 (G3): Non-anemic group treated with 200 mg/kg of EETO.



Fig 1: Geographical area of *Taraxacum officinale* harvest.

Group 4 (G4): Anemic group pre-treated with 200 mg/kg of EETO.

Estimation of hematological parameters (cell blood count)

Blood was collected in EDTA tubes for the determination of hematological parameters. A differential blood count was performed using a Coulter machine (ERMA PCE-210, Ermainc, Japan).

Estimation of serum iron and ferritin

The determination of serum iron and ferritin is carried out using a Cobas Integra 400 Plus automatic device according to (Fretellier *et al.,* 2014; Koivunen *et al.,* 2006) respectively.

Estimation of blood magnesium

The dosage of magnesium (mg) in serum is carried out using a colorimetric method using Chronolab kit.

Estimation of copper (Cu) and zinc (Zn) copper

(Cu) and zinc (Zn) determination in serum was carried out by atomic absorption spectrophotometry (SpectrAA 220 FS, Varian, Springvale, Australia) (Southon *et al.,* 1984).

Estimation of vitamins

- Estimation of vitamin C

The vitamin C content in blood serum was estimated according to the method of Omaye *et al.* (1979).

- Estimation of folate B9 and B12

The analytical performances of the *in vitro* quantitative determination techniques for folate B9 and vitamin B12 were evaluated on the Immulite 2000 analyzer by chemiluminescence using the method of (Colombier *et al.,* 2002).

Erythropoietin estimation

Erythropoietin was estimated using ELISA with Quantikine IVD ELISA kits (R and D Systems #DEP00).

Ulcer induction with HCl/ethanol in experimental rats

The study utilized 20 male rats divided into four groups (n=5 per group). Groups G1 and G2 received 1 ml physiological water orally, while G3 and G4 received 200 mg/kg EETO. Following a 24-hour fast on day 6, G2 and G4 were administered 1 ml HCl/ethanol solution (1.50 µMol/60%) on day 7 to induce gastric ulcers (Yang *et al.,* 2017). One hour post-induction, animals were anesthetized with chloroform and sacrificed. Blood samples were collected *via* retro-orbital puncture into heparinized tubes. Stomachs were excised, rinsed with saline and divided for histological analysis (10% formalin fixation) and lesion assessment (PBS preservation). Gastric mucosal injury was quantified as percentage of total surface area using microscopic evaluation (Morsy *et al.,* 2012). Experimental groups comprised: G1 (control), G2 (ulcer control), G3 (EETO-only) and G4 (EETO pretreatment + ulcer induction).

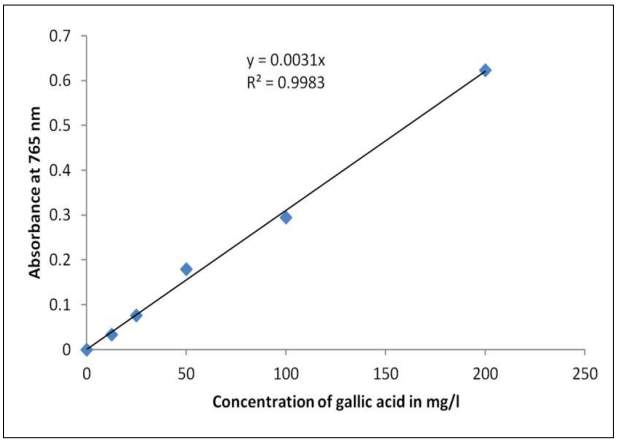


Fig 2: Calibration curve of gallic acid of total polyphenols from ethanolic extract of *Taraxacum officinale*.

RESULTS AND DISCUSSION

Antibiotics are defined as substances in small quantities produced from microorganisms (Bertrand, 2005). These compounds which alter the normal functioning of bacteria can inhibit their growth (bacteriostatic antibiotic) or destroy them (bactericidal antibiotic) (Hefnawy *et al.,* 1999). Cephalosporins form a large group of semi-synthetic antibiotics of lactam core derivatives; they are used in clinical medicine. They are linked to the fundamental structure and the mechanism of bactericidal action of penicillins. They are used for the treatment of infections caused by Gram-negative and Gram-positive bacteria. Cephalosporins are among the oldest and most frequently prescribed natural antimicrobial agents (AL-Ghannam, 2008).

Chronic cephalosporin use can induce hemolytic anemia (Garratty, 2012). This study evaluated *Taraxacum officinale* as a natural anti-anemic adjunct to cefuroxime therapy.

Content of total polyphenols of ethanolic extract of *Taraxacum officinale* (EETO)

The results of total polyphenol content of EETO in Fig 2, indicating that the extract has a proportion of total polyphenols, which is 88.33 mg EAG/g.

Plants of the genus *Taraxacum*, known as dandelion, have long been used in traditional medicine due to the presence of chicoric acid (dicaffeyl tartaric acid), which belongs to phenylpropanoids and one of the main constituents of *T. officinale* (Ivanov, 2014).

When the volume percentage of ethanol was less than 50%, content of total phenols, chicoric and caffeic acid and derived acids increased with increases in ethanol concentrations (Tsai *et al.,* 2012).

Phenolic acids, plant-derived secondary metabolites, are potent antioxidants with significant health benefits (Ivanova *et al.,* 2005). Our study found 88.33 mg/100 g polyphenols, comparable to Yang *et al.* (2017) (99.9± 0.7 mg/100 g) but higher than Domitrovic *et al.* (2010) (3.11± 0.14 mg GAE/g), likely due to solvent polarity effects (Xi *et al.,* 2009).

Effect of ethanolic extract of *Taraxacum officinale* on Hb, RBC and PCV in anemic rats induced by cephalosporins

According to Table 1, compared to controls, the anemic group (G2) showed significant reductions ($p<0.05$) in RBCs (43.11%), Hb (17.19%) and hematocrit (29.79%). EETO pretreatment (200 mg/kg) effectively restored these parameters, increasing Hb (52.10%), RBCs (38.56%) and hematocrit (40.29%).

According to the WHO, a hemoglobin threshold (Hb<11 g/dL) was chosen for the determination of anemia (Fehr *et al.,* 2009).

Effect of ethanolic extract of *Taraxacum officinale* on minerals

Table 2 shows the effect of EETO on iron, ferritin, magnesium, zinc and copper in experimental rats. Iron shows the same variations as ferritin in all groups. Iron and ferritin values are expressed as the average of five rats. The results also indicate that administration of cephalosporin at 10 mg/kg (bw) to rats in the positive control group led to a significant decrease ($P<0.05$) in iron, ferritin, magnesium, copper and zinc with the levels of 49.67%, 48.12%, 43.03%, 47.17% and 45.20% respectively compared to the control group. On the other hand, pre-treatment of rats with the EETO significantly restored iron, ferritin and magnesium, copper and zinc ($P<0.05$) to 50.95%, 47.40%, 45.78%, 53.81% and 44.96% respectively.

Although the stored iron is deposited in these cells in the form of ferritin (Cattan, 2004). When there is a lack of iron, the iron stored in the form of ferritin can be released. evidence. In general, the macrophage (villus apex macrophage and others), through the process of erythro-phagocytosis, can continuously supply iron to the plasma. Indeed, during this process (Loréal *et al.,* 2012).

Zinc has anti-inflammatory properties and a deficiency can cause immune disorders (Derai, 2014).Copper deficiency has recently emerged as a cause of anemia (Esposito *et al.,* 2003).

Anemia could lead to abnormal blood–zinc status in the body (Jeng and Chen, 2022).

Effect of ethanolic extract of *Taraxacum officinale* on vitamins

Table 3 shows the effects of the EETO on the vitamins contained in rats. Values are expressed as the average of five rats. According to our results, we note that the concentration of vitamins C, B9 and B12 in rats in the group pre-treated with the ethanolic extract of the plant increases significantly ($P<0.05$) by 85.58%, 89.22% and 56% respectively compared to untreated anemic rats. The concentration of vitamins C, B9 and B12 were too low in the positive control group with 85.82%. 86.91% and 35.62% respectively compared to the control group. A moderate improvement was observed in rats in the group pre-treated with the EETO at 200 mg/kg (bw).

Vitamin C is capable of mobilizing iron stores in the reticuloendothelial system, it also potentiates the enzymatic reaction of iron incorporation for the synthesis of heme (Giancaspro *et al.,* 2000).

Table 1: Effect of ethanolic extract of *Taraxacum officinale* on Hb, RBC and hematocrit in anemic rats induced by cephalosporins.

	G1	G2	G3	G4
Hb g/dL	16.32±0.44	10.21±0.44	12.96±0.11	16.62±0.37
GR × 10^6/µl	7.19±0.59	4.09±0.8	5.60±1.44	8.54±0.42
Hemato %	44.7±3.03	31.38±2.67	37.94±2.78	52.56±2.1
GB × 10^3/µl	4.96±0.11	6.72±0.47	4.08±0.19	4.96±0.25
Plqts × 10^3/µl	383.98±6.74	135.6±24.35	358.8±33.88	384.04±4.94
Lymphocytes × 10^3/µl	3.46±0.08	1.14±0.13	2.04±0.59	3.46±0.279
TCMH pg	28.66±0.84	23.2±1.48	25.54±1.24	31.22±3.08
Neutrop × 10^3/µl	4.36±0.23	0.74±0.18	1.74±0.82	4.24±0.37

Table 2: Effect of ethanolic extract of *Taraxacum officinale* on minerals.

Groups	G1	G2	G3	G4
Fer (µmol/L)	15.3±1.2	7.7±1.09	12.9±0.89	15.7±1.3
Ft (ng/mL)	29.3±1.2	15.2±0.83	22.4±1.34	28.9±1.67
Mg (mmol/l)	0.79±0.16	0.45±0.03	0.68±0.11	0.83±0.05
Zn (mg/dl)	78.54±0.8	41.52±2.02	46.26±5.18	89.9±8.88
Cu (µmol/l)	18.14±0.19	9.94±0.82	16.1±1.22	18.06±0.21

Table 3: Effect of ethanolic extract of *Taraxacum officinale* on vitamins.

Parameters	G1	G2	G3	G4
Vit C (mg/l)	71.26±3.5	10.1±3.72	65.2±4.12	70.09±226
Vit B9 (nmol/l)	19.26±3.5	2.52±1.6	8.2±4.2	23.38s±3.5
Vit B12 (pg/ml)	200.4±22.1	129±27	196.6±28	293.2±130

A combined deficiency of cobalamin (B12) and iron can have opposing effects on red blood cell volume and give rise to anemia (Mahmood *et al.,* 2014).

Effect of ethanolic extract of *Taraxacum officinale* on erythropoietin (EPO) concentration)

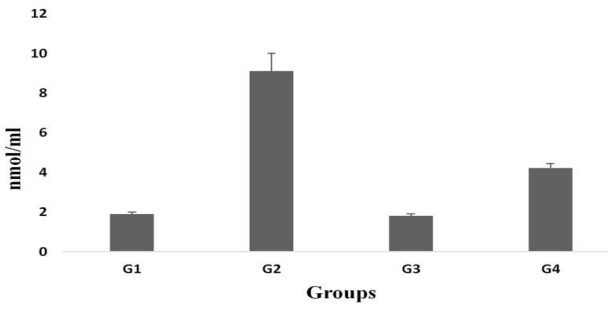
Table 4 shows the effects of the EETO on the erythropoietin content of rats. Daily administration of cephalosporin significantly increased the erythropoietin concentration of the positive control group. This percentage is 30.4% higher than that of the control group. On the other hand, the pre-treatment of rats in the fourth group recorded an improvement in the concentration of erythropoietin to 36.98% compared to the positive control rats.

Ferrucci *et al.* (2005) suggest that inflammatory anemia evolves from a pre-anemic stage, characterized by a compensatory increase in erythropoietin that maintains a normal hemoglobin level.

So according to these results, *Taraxacum officinale* extract is able to improve blood constituents in most erythropoietic markers (Hb, GR, Hematocrit, Hb, Iron, Ft, Mg, Cu, Zn, vitamin C, B9, B12 and erythropoietin) in animals in the group treated with 200 mg/kg of the plant extract. Previous studies show that the plant had an inhibitory effect on vascular permeability (Jeon *et al.,* 2008), that it has antioxidant activities because it stimulates catalase and glutathione reductase (You *et al.,* 2010) and subsequently the red blood cells become more resistant to hemolysis. Rodriguez-Fragoso *et al.* (2008) show that *Taraxacum officinale* can be used to improve blood circulation.

Table 4: Effect of ethanolic extract of *Taraxacum officinale* on erythropoietin concentration.

Groups	G1	G2	G3	G4
EPO (UI/L)	13±0,75	19,52±1,19	14,12±0,23	12,3±0,48



G1: Control; G2: 1 ml physiological water (7 days) then 10 ml of HCl/ethanol (1 day); G3: 200 mg/kg of EETO (7 days); G4: 200 mg/kg of EETO (7 days) then 10 ml of HCl/ethanol (1 day). The values represent the mean (m) of 5 determinations ± SEM (n =5).

Fig 3: Malondialdehyde (MDA) content (nmol/ml) in rats treated or/not with the EETO and/or HCl/ethanol.

Effect of ethanolic extract of *Taraxacum officinale* on malon-dialdehyde (MDA)

Fig 3 shows EETO's effect on lipid peroxidation. HCl/ethanol significantly increased MDA (8.92 vs 1.7 nmol/ml in controls, $p<0.05$). EETO pretreatment reduced MDA by -6 nmol/ml (71.76% decrease vs control group).

Our study shows that *Taraxacum officinale* extract decreased MDA levels. This trend was also observed by (Yang *et al.,* 2017). This trend was also observed by Yang *et al.* (2017). Turkistani, (2019) obtained similar results with the aqueous extract of *Taraxacum officinale*.

Macroscopic study of the stomach

Percentage inhibition of gastric mucosal injury (%INH)

Microscopic examination allowed us to note the effectiveness and gastro-protective action of the EETO. It should be noted that the administration of a dose of 200 mg/kg of EETO at a dose of 200 mg/kg of EETO, in gastric ulcerated rats (G4), significantly inhibited ($P<0.05$) the ulceration rate of 68%, against the group of ulcerated rats (0%) G2. On the other hand, daily administration of EETO has an inhibition value of 98.2% similar to that of the control (Fig 4).

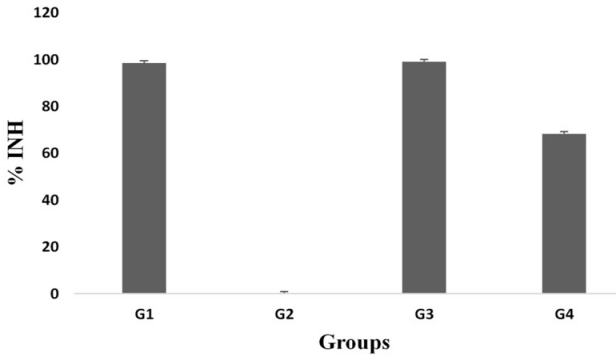
The same results are observed by (Turkistani, 2019), who showed that the aqueous extract of *T. officinale* containing these phytochemicals would be strongly linked to anti-ulcer benefits.

Microscopic analysis revealed:

- The control group (G1) presented a normal gastric structure (mucosa, muscularis, serosa) (Fig 5a) and which is similar with the group (G3) (Fig 5c).
- HCl/ethanol treatment caused increased gastric thickness with edema and polynuclear neutrophils (Fig 5b).
- EETO pretreatment (G4, 200 mg/kg) showed significant lesion reduction, prevented thickness increase and reduced dema/polynuclear neutrophils (Fig 5d).

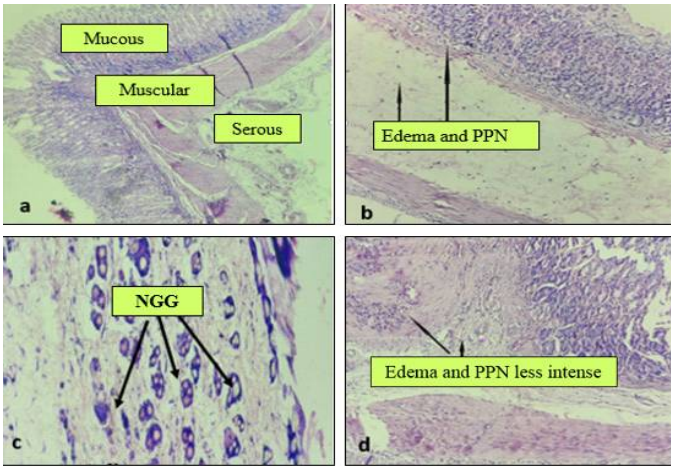
Among the various factors that contribute to gastritis and gastric ulcer is the consumption of alcohol with high acid content in the stomach. HCl/ethanol is widely used to induce chronic gastritis in animal models of this disease since their similarity in symptoms to those of humans (Santin *et al.,* 2013). Ethanol is one of the agents that promotes gastritis by increasing oxidative stress and inflammation, leading to necrosis and tissue depletion of gastric mucus and reducing gastric blood flow (Breviglieri *et al.,* 2017; Zanatta *et al.* al., 2009). Ethanol reduces gastric blood flow, which is attributed to hemorrhage and necrosis of stomach tissues (Zanatta *et al.,* 2009). Solubilizing ethanol also increases gastric mucosal loss (Marotta *et al.,* 1999). It induces hemorrhage and necrosis with an associated increase in lipid peroxidation rates. The presence of HCl with ethanol accelerates the process of gastric ulcerogenesis (Marotta *et al.,* 1999).

In the present study, the models were established by administering a dose of HCl/ethanol for one day to induce chronic gastritis, which was expressed by reddish



G1: Control; G2: 1 ml physiological water (7 days) then 10 ml of HCl/ Ethanol (1 day); G3: 200 mg/kg of EETO (7 days); G4: 200 mg/kg of EETO (7 days) then 10 ml of HCl/Ethanol (1 day). The values represent the mean(m) of 5 determinations ±SEM (n =5).

Fig 4: Percentage inhibition of gastric mucosal lesion (%) in rats treated or not with EETO and/or HCl/ethanol.



NGG: Normal gastric glands. PPN: Polymorphonuclear neutrophils. (a): G1: Control; (b): G2: 1ml physiological water (7 days) then 10 ml of HCl/Ethanol (1 day); (c): G3: 200 mg/kg of EETO (7 days); (d): G4: 200 mg/kg of EETO (7 days) then 10 ml of HCl/Ethanol (1 day).

Fig 5: Histological sections of stomach of group.

discoloration of the inner layer, as well as hemorrhage and edema due to increased oxidative stress and inflammation. EETO reduce gastritis compared to the positive control, it has a positive and preventive effect to treat gastritis and ulcer, with better protection against ulcer, because the maintenance of the gastric mucosa plays an important role in gastric protection (Suo *et al.,* 2016). Mahmood *et al.* (2010) claimed that phenols as well as flavonoids in *Taraxacum officinale* have anti-ulcerogenic activity.

CONCLUSION

According to the results of this study, it can be said that the consumption of *Taraxacum officinale* during a treatment causing anemia such as cephalosporin or another medication, can maintain healthy homeostasis, particularly regarding red blood cells, hematocrit and white blood cells. It is capable of reducing lipid peroxidation, which is one of the main manifestations of oxidative damage to organs and reducing gastric lesion surfaces. It is important to use this plant and introduce it into the diet as a preventative measure, or in case of erythropoietic or gastric problems and further work would be necessary to produce a phytomedicine based on *Taraxacum officinale*.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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