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Chemical composition and protective role of *Pulicaria undulata* (L.) C.A. Mey. subsp. *undulata* against gastric ulcer induced by ethanol in rats

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Abstract

Pulicaria undulata subsp. *undulata* (Family; Asteraceae) is a medicinal plant used to treat inflammation. The objective of this study is to explore the protective effect of the ethanol extract of *P. undulata* subsp. *undulata* aerial parts against ethanol induced gastric ulcer in rats. The chemical composition of plant extract, the unsaponifiable matter and the fatty acid methyl esters were analyzed. The biological evaluation was carried out through measuring ulcer indices, oxidative stress markers, certain marker enzymes, inflammatory index and the histopathological assessment of the stomach in rats. The total unsaponifiable matter (94.29%) and the fatty acid methyl ester (82.96%) content were identified. Gastric ulcer recorded significant increase in gastric volume and lesion counts (p < 0.0001). Drastic changes in all biochemical parameters under investigation were observed. Protection with plant extract reversed the action of ethanol by variable degrees of improvement in comparison with the reference drug. The

presence of carbohydrates and proteins that acted as a mucilage lining the stomach inner wall give its protective action. In conclusion, *P. undulata* subsp. *undulata* succeeded to have anti-ulcerative protective effect. The measured biomarkers served as a good mirror to predict gastric ulcer and the presence of carbohydrates, protein and fibers present in the plant extract acted as a mucilage lining the inner intestinal wall and protect against ethanol induced gastric ulcer. Future study will be carried out to identify the biologically active compounds responsible for plant protection against the gastric ulcer.

Keyword: Biochemistry

1. Introduction

Stomach ulcers are the most common gastrointestinal and global disorders (Laloo et al., 2013). Mainly, the gastric ulcer occurs due to the imbalance between the destructive and offensive factors of the mucosal barrier (Chen et al., 2015). The destructive factors include stomach hydrochloric acid, mucosal hypoperfusion, free radicals, ethanol, *Helicobacter pylori* and excessive use of non-steroidal anti-inflammatory drugs (NSAIDs) (Vonkeman et al., 2007).

Ethanol is a harmful agent associated with severe pathologies. Ethanol affects the integrity of the gastric mucosa, increasing mucosal permeability and in certain cases causing bleeding (Guzmán-Gómez et al., 2018). The neutrophils in the site of injury elevate the concentrations of reactive oxygen species and other inflammatory mediators causing oxidative damage. Therefore, oxidative stress has been shown to play a critical role in the gastric mucosal damage (Pan et al., 2008).

Gastric ulcers prevention is a medical challenge (Jesus et al., 2013). Therefore, the medicinal plants are consider to be new promising alternative medications for the development drugs to control gastrointestinal illness (Antonisamy et al., 2014; Hajrezaie et al., 2015).

Pulicaria undulata (L.) C.A. Mey subsp. undulata (syn. *Pulicaria crispa* (Forssk.) Benth et Hook) is one of the most widespread desert plants. is an annual herb or sub-shrub with small yellow flowers (Eliebaa et al., 2018). It is one of the most widespread desert plants growing wild in Southern Egypt (Boulos, 2009). In Egypt, it is known as "Dethdath" and can be used to treat inflammation and as insect repellent (Maghraby et al., 2010). Mandaville (1990) cited the name *Pulicaria undulata* as the main name and *Pulicaria crispa* as a synonym, according to the international association for plant taxonomy (IAPT) nomenclature committee decision. *Pulicaria undulata* is used to treat inflammation (Ross et al., 1997) and as an herbal tea. In traditional medicine, its ingredients are used as perfumes, antihypoglycemic and antispasmodic drugs. Compounds like polyunsaturated fatty acids, sterols, minerals,

polysaccharides, terpenoids, proteins, and halogenated compounds are the main substances biosynthesized by desert plants with potential impact in pharmaceutical and food industries (Dendougui et al., 2000).

The goal of this study was to investigate the wild plant collected from Northern Sinai; *P. undulata* subsp. *undulata* for its chemical composition and evaluate the effect of its aerial parts ethanol extract as protective agent against stomach ulcers in rats model. The evaluation focused on stomach ulcer indices; gastric volume, lesions count, and pH value. Oxidative stress markers as glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) were also estimated. Inflammatory index as interlukin-10 (IL-10), intracellular adhesion molecule-1 (ICAM-1) and tumor necrosis factor alpha (TNF-α) were evaluated. The work was extended to measure certain marker enzymes as succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), gluose-6-phosphatase (G-6-Pase), acid phosphatase (AP) and $5'$- nucleotidase ($5'$NT) which are consider as mitochondrial, cytoplasm, microsomal, lysosomal and plasma membrane marker, respectively. Histological analysis of the stomach architecture was also taken into consideration.

2. Materials and methods

2.1. Chemicals

All chemicals used in the present study were of high analytical grade, products of Sigma (USA), Merck (Germany), BDH (England), Riedel de Hàen (Germany) and Fluka (Switzerland).

2.2. Apparatus

An atomic absorption spectrometer, PyeUnicam SP 1900 Spectrophotometer (Pye Unican Ltd., Cambridge, England), was used for element detection of Hg^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Li^+, Se^{2+} and Co^{2+}. Flame emission spectrometry was used for Na^+, Ca^{2+}, Mg^{2+}, P^{3+}, and K^+ assay. Both the unsaponifiable and the saponifiable fractions of *Pulicaria undulata* were studied to identify their contents using GLC analysis. The unsaponifiable matter and fatty acids were performed on Agilent Technologies (6890N)−Network-GC system equipped by a flame ionization detector. GLC analysis of the unsaponifiable matter was performed using capillary column (HP-5 phenyl methyl siloxane) and oven temperature at 80 °C/8 minutes from 80 to 350 °C. Nitrogen gas was used as a carrier gas. The operating conditions for fatty acid methyl esters analysis were capillary column HP-5% 5-phenyl methyl siloxane (30 m × 320 µm × 0.25 µm), column maximum temperature was 325 °C. Detector and injection temperature was 250 °C. Oven temperature was from 50-350 °C with a rate 50 °C/10 minutes. An automatic Kjeldahl-Foss apparatus, model 16210 (Foss America Inc., Fishkill, NY), and a Markham distillation

apparatus (Markham, Ontario, CA) for determination of total protein content were used.

2.3. Plant material

The aerial parts of *Pulicaria undulata* (L.) C.A. Mey subsp. *undulata* (syn. *Pulicaria crispa* (Forssk.) Benth et Hook) were collected in April 2016, from El-Hasana Region, 80 km south of El-Arish, Northern Sinai, Egypt. It was identified by the plant taxonomist Dr Mohamed El-Gebalyand according to Boulos (2009). A voucher specimen was deposited in the herbarium of the National Research Centre, Dokki, Giza, Egypt (CAIRC).

2.4. Preparation of ethanolic extract

The air-dried powdered aerial parts of *Pulicaria undulata* (800 g) was exhaustively extracted with ethanol (90%) using a Soxhlet apparatus (Maghraby et al., 2010). The ethanolic extract was distilled in *vacuu*m at 45 °C to yield 54.6 g (representing 6.83% of the air-dried aerial parts).

2.5. Phytochemical analysis

The percentage of total protein was measured by determining the nitrogen content by the Kjeldahl's method (AOAC, 1985) using a Markham distillation apparatus. The total protein content was calculated in mg/g of the dried matter in the sample (N $\times$ 6.25).

The total carbohydrates as well as soluble sugars were determined as glucose by the phenol-sulfuric acid method (Cohen et al., 1989). The crude fiber content of the sample digested with 5% HCl were determined (AOAC, 1985). Micro- and macroelements were determined in the digested solution by acid mixture (Dean and Rains, 1975). Investigation of the lipoidal matter (yield of 8.32% on dry weight basis) was carried out by saponification of *n*-hexane extract by refluxing with alcoholic KOH (0.5 M) for 2 h (Johnson and Davenport, 1971). The unsaponifiable matters were extracted with ether, evaporated and analyzed by GLC. The free fatty acids obtained from saponification were subjected to methylation (MeOH, 4%$-$5% dry H_2SO_4) for 2 hours, extracted with ether, evaporated and analyzed by GLC (Iverson; Sheppard, 1975).

2.6. Animals and ethics

Male Wistar albino rats (100$-$120 g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water

and diet. Animal experiments were conducted according to established animal welfare guidelines. Anesthetic procedures, handling with animals and their termination were complied with the ethical guidelines of the Medical Ethical Committee of the National Research Centre, Giza, Egypt (Approval no. 18192) and performed for being sure that the animals do not suffer at any stage of the experiment.

2.7. Acute toxicity study

Sixty male Wistar albino rats (100−120 g) were divided into four groups to estimate the acute toxicity at different plant concentrations (250, 500, 1000 mg/kg b.wt). Animals were observed for 15 days. No dead rats were observed along the experiment period reviling the extract safety. Therefore, we selected the dose of 500 mg/kg b.wt for the *in vivo* study.

2.8. Doses and route of administration

Absolute ethanol was orally administrated at a dose of 0.5 mL/100 g body weight on 24 hours empty stomach (Mard et al., 2008). Plant extract was orally given at a dose of 500 mg/kg b.wt/day for one week according to the acute toxicity test. Ranitidine as a reference antiulcer drug was orally administrated at a dose of 100 mg/kg b.wt/day for one week (Mard et al., 2008).

2.9. Experimental groups

Forty eight male rats were equally divided into six groups. Group1 was normal healthy control rats. Groups 2, 3 were normal healthy rats administrated with plant extract or ranitidine drug, respectively. Group 4 received the ethanol dose on 24 hrs empty stomach, and sacrificed after one hour. Groups 5−6 were normal healthy rats administered with plant extract or ranitidine daily for 7 days prior an oral dose of absolute ethanol on 24 hrs empty stomach and sacrificed one hour later.

2.10. Sample preparations

Stomach tissue was homogenized in normal physiological saline solution (0.9% NaCl) (1:5 w/v). The homogenate was centrifuged at 4 °C for 15 min at 3000 rpm and the supernatant was stored at −80 °C for further estimation of the marker enzymes and antioxidant parameters.

2.11. Estimation of gastric lesion counts

Stomach was removed, opened from the long curvature, washed with normal saline, expand and fixed on the dissection plate and lesion numbers were counted by magnifying lens (Szelenyi and Thiemer, 1978).

2.12. pH level

Stomach was removed; gastric content was collected and centrifuged at 3000 rpm for 15 min. The supernatant volume was examined for its pH value using pH strips.

2.13. Biochemical determinations

All animal groups were subjected to determinate gastric ulcer indices; lesion counts, total acidity, and gastric volume. Oxidative stress markers; malondialdehyde (MDA) (Buege and Aust, 1978), superoxide dismutase (SOD) (Nishikimi et al., 1972) and glutathione (GSH) (Moron et al., 1979) were estimated in gastric tissue. Certain marker enzymes; succinate dehydrogenase (SDH) (mitochondrial marker) (Rice and Shelton (1957), lactate dehydrogenase (LDH) (cytoplasm marker) (Babson and Babson, 1973), gluose-6-phosphatase (G-6-Pase) (microsomal marker) (Swanson, 1955), acid phosphatase (AP) (lysosomal marker) (De Duve and Wattiaux, 1956) and $5'$- nucleo-tidase ($5'$NT) (plasma membrane marker) (Bodansky and Schwartz, 1963) were also carried out in gastric tissue. Inflammatory index were estimated by ELISA technique; interleukin-10 (IL-10) (Abcam, ab100765, Cambridge, USA), intracellular adhesion molecule-1 (ICAM-1) (Abcam, ab100763, Cambridge, USA), and tumor necrosis factor alpha (TNF-α) (Abcam, ab100785, Cambridge, USA) were also evaluated in gastric tissue. Gastric total protein was also estimated (Bradford, 1976).

2.14. Histopathological analysis

Stomach tissue slices were fixed in 10% paraformaldehyde and embedded in paraffin wax blocks. Sections of 5 μm thick were stained with hematoxylin & eosin (H&E) and Masson's trichrom, then examined under light microscope for determination of pathological changes (Hirsch et al., 1997).

2.15. Statistical analysis and calculations

All data were expressed as mean $\pm$ SD of eight rats in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program accompanied with lease significance difference between groups (LSD) at $p \leq 0.05$.

$$\text{Percentage of change} = \frac{\text{Mean of control} - \text{Mean of treated group}}{\text{Mean of control}} \times 100$$

$$\text{Percentage of improvement} = \frac{\text{Mean of treated group} - \text{Mean of ulcerative group}}{\text{Mean of control}} \times 100$$

3. Results

3.1. Investigation of phytoconstituents of *P. undulata* protein, fibers, total hydrolysable carbohydrates and soluble sugar content

Considerable percentage of total protein content (9.55% on dry weight basis) was detected. The content of crude fibers was 24.56 %. Total hydrolysable carbohydrates were 24.29% on dry weight basis, while soluble sugar content being 15.98%.

3.2. Micro- and macro elements

Macroelements (%) was calculated referring to the air-dried powdered plant (data not shown), where P^{3+}, K^+, Mg^{2+}, Ca^{2+} and Na^+ amounted 0.19, 1.73, 8.01, 11.22 and 9.50%, respectively. Microelements were Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+} and Se^{2+} which amounted 960, 84, 17, 3.03 and 3.54 ppm, respectively. Hg^{2+}, Pb^{2+} and Cd^{2+} were less than 0.1 ppm.

3.3. Sterols, terpenes, and hydrocarbons content

The percentage of unsaponifiable matter (USM) content amounted to 69.72% and the total fatty acids amounted to 22.43%, on dry weight basis. GLC analysis of the USM content (Table 1) revealed stigmasterol and β-sitosterol as the main sterols and *n*-heneicosane (21.88 %) the highest hydrocarbon content. In the present study, the extract containing high amount of saturated aliphatic hydrocarbons (88.90%). β-Amyrin and squalene were also detected.

3.4. Fatty acid methyl esters

Twenty-one fatty acid methyl esters were identified, representing 82.96% of the total saponifiable matter (Table 2). GLC analysis of fatty acids methyl esters revealed that the most abundant and unsaturated fatty acid was 11,14,17-eicosatrienoic acid ($C_{20:3}$) followed by *cis*-13-docosenoic acid ($C_{22:1}$), whereas palmitic acid ($C_{16:0}$) was the main saturated fatty acid. From Table 2, it can be concluded that 11,14,17-eicosatrienoic acid, palmitic acid, and *cis*-13-docosenoic acid represent the major components (14.43, 10.64, and 8.55%, respectively). . Saturated fatty acids represent 46.94% of the total fatty acid content, whereas monounsaturated, diunsaturated, triunsaturated, tetraunsaturated fatty acids represent 13.82, 1.57, 18.95, 1.68% of the total fatty acid content, respectively. The percentage of saturated fatty acids (46.94%) is higher than that of polyunsaturated ones (36.02%).

3.5. Gastric ulcer index

Insignificant changes in gastric volume and pH level were observed control rat administrated with *P. undulata* subsp. *undulata* aerial parts ethanol extract and

Table 1. GLC analysis of the unsaponified fraction of *Pulicaria undulata*.

Peak No.	Rt [min]	Compounds identified	Area (%)
1	11.03	*n*-Pentadecane	0.91
2	12.25	*n*-Hexadecane	1.28
3	13.63	*n*-Heptadecane	0.26
4	14.85	*n*-Octadecane	0.58
5	16.52	*n*-Nonadecane	3.11
6	16.99	*n*-Eicosane	6.92
7	17.30	*n*-Heneicosane	21.88
8	19.55	*n*-Docosane	19.34
9	20.38	*n*-Tricosane	2.24
10	21.64	*n*-Tetracosane	5.76
11	22.66	*n*-Pentacosane	7.08
12	23.57	*n*-Hexacosane	2.63
13	24.52	*n*-Heptacosane	3.26
14	25.36	*n*-Octacosane	0.92
15	25.48	*β*-Amyrin	0.56
16	25.98	Squalene	0.28
17	26.24	*n*-Nonacosane	0.26
18	27.04	*n*-Triacontane	0.58
19	28.61	Cholesterol	0.34
20	29.45	Campesterol	0.41
21	30.39	Stigmasterol	1.38
22	31.22	*β*-Sitosterol	1.20
Total hydrocarbons			88.90
Total terpenes			0.84
Total sterols			3.33
Total identified compounds			93.07

ranitidine drug. Ulcerogenic rats showed significant increase in gastric volume by 627.27% ($p < 0.0001$) and significant decrease by 21.50% ($p < 0.01$) in pH level referring to the control group. Ulcerogenic rats protected with plant extract and ranitidine drug showed significant decrease in gastric volume by 25.00 and 17.50% ($p < 0.01$), respectively and an insignificant decrease in pH value by 15.00 and 10.00%, respectively compared to the ulcerative group. According to these observations, we noticed that rats protected with plant extract and ranitidine exhibited improvement by 181.81 and 127.27% in gastric volume and by 11.77 and 7.84% in pH level, respectively (Fig. 1).

Ulcerogenic rats showed seven lesions/stomach. Ulcerogenic rats protected with plant extract and the selected drug showed significant decrease ($p < 0.001$) in lesion

Table 2. Gas-liquid chromatographic analysis of fatty acids of *Pulicaria undulata* methyl esters.

Peak no.	Rt [min]	Compounds identified	Relative area (%)
1	8.29	Caprylic ($C_{8:0}$)	1.23
2	9.52	Capric acid (10:0)	7.97
3	10.78	Lauric acid (12:0)	1.49
4	21.22	Myristic acid (14:0)	3.83
5	21.82	Myristoleic acid (14:1)	0.85
6	23.07	Pentadecanoic acid (15:0)	2.38
7	29.44	Palmitic acid (16:0)	10.64
8	30.08	Margaric acid (17:0)	1.87
9	31.38	Palmitoleic acid (16:1)	1.60
10	34.01	Stearic acid (18:0)	3.25
11	35.15	Oleic acid (18:1)	0.48
12	36.59	Linoleic acid (18:2)	1.57
13	37.34	Linolenic acid (18:3)	4.52
14	42.09	Arachidic acid (20:0)	1.87
15	42.55	11,14,17-Eicosatrienoic acid (20:3)	14.43
16	43.38	Arachidonic acid (20:4)	1.68
17	45.78	Behenic acid (22:0)	5.05
18	46.76	*cis*-13-Docosenoic acid (22:1)	8.55
19	48.52	Tricosanoic acid (23:0)	3.04
20	50.20	Tetracosanoic acid (24:0)	5.56
21	55.54	Nervonic acid (24:1)	2.34

No: Number; R_t (min): Retention time in minutes.

counts by 57.14 and 42.85%, respectively as compared with the stomach ulcer rats, while ulcerogenic rats treated with plant extract and ranitidine recorded significant decrease by 71.42 and 75.14%, respectively as compared with the ulcerative group (Fig. 1).

3.6. Oxidative stress markers and protein content

Ulcerogenic rats showed significant decrease in SOD and GSH level by 60.25 and 45.01% ($p < 0.001$), respectively as compared with the normal healthy rats. Ulcerogenic rats protected with plant extract and ranitidine showed significant increase in SOD activity by 38.23 and 64.37% ($p < 0.001$) and insignificant increase in GSH level by 18.05 and 1.03%, respectively as referred to the stomach ulcerative group. Therefore, rats protected with plant extract and ranitidine exhibited improvement by 15.19 and 25.58% in SOD activity and 9.92, 1.02% in GSH level, respectively (Fig. 2).
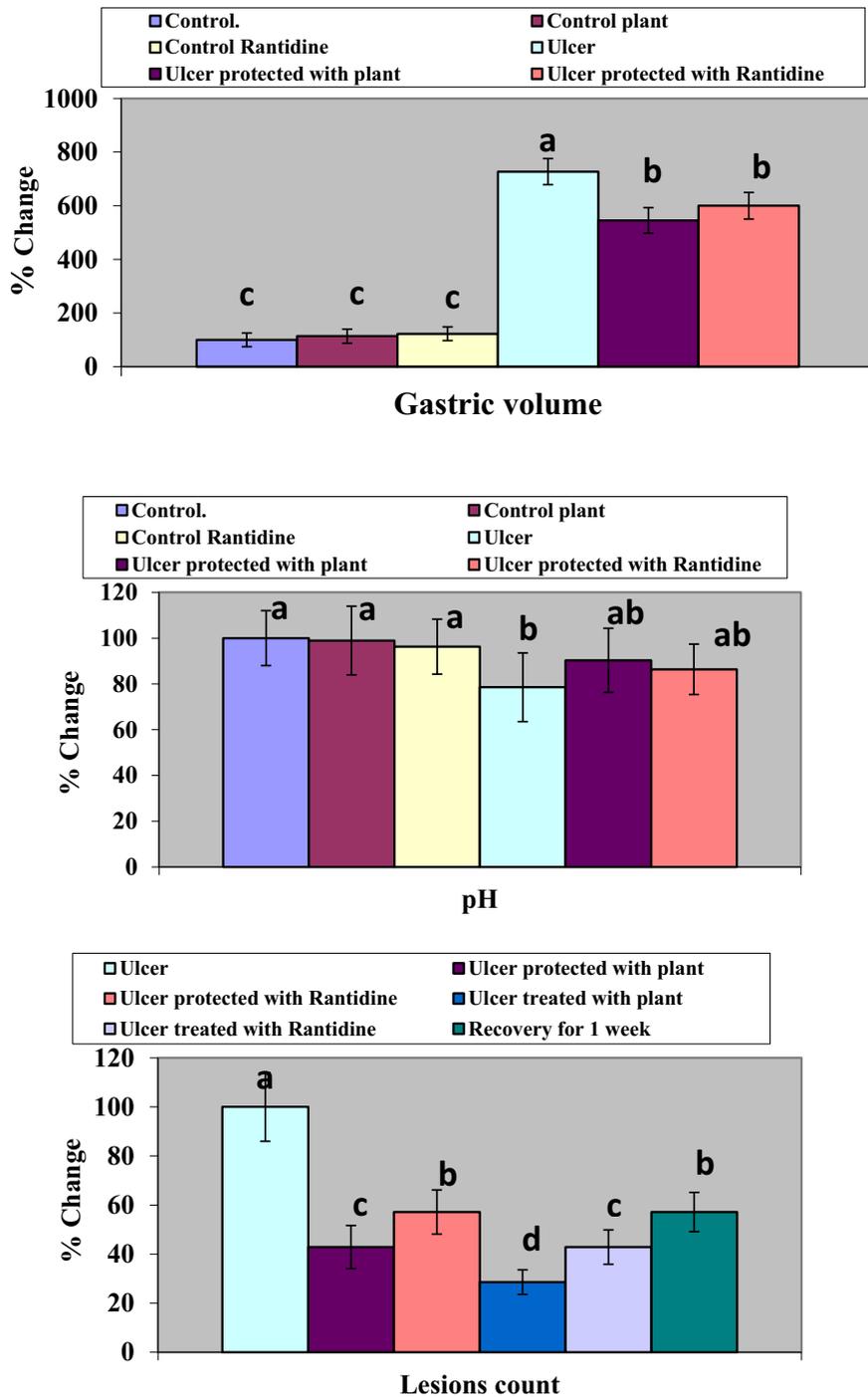
Fig. 1. Percentage changes of gastric volume, pH, lesion counts versus control group. Groups having the same letters are non-significantly different, while those having different letters are significantly different at p ≤ 0.05.

With respect to MDA level, ulcerogenic rats showed significant increase in MDA level by 873.80% ($p < 0.0001$) compared to the control group. Protection with plant extract and ranitidine showed significant decline in MDA level by 52.07 and 44.74% ($p < 0.001$), respectively as compared with the stomach ulcerative rats. Hence, we noticed that rats protected with plant extract and ranitidine exhibited improvement in MDA level by 507.14 and 435.71%, respectively (Fig. 2).
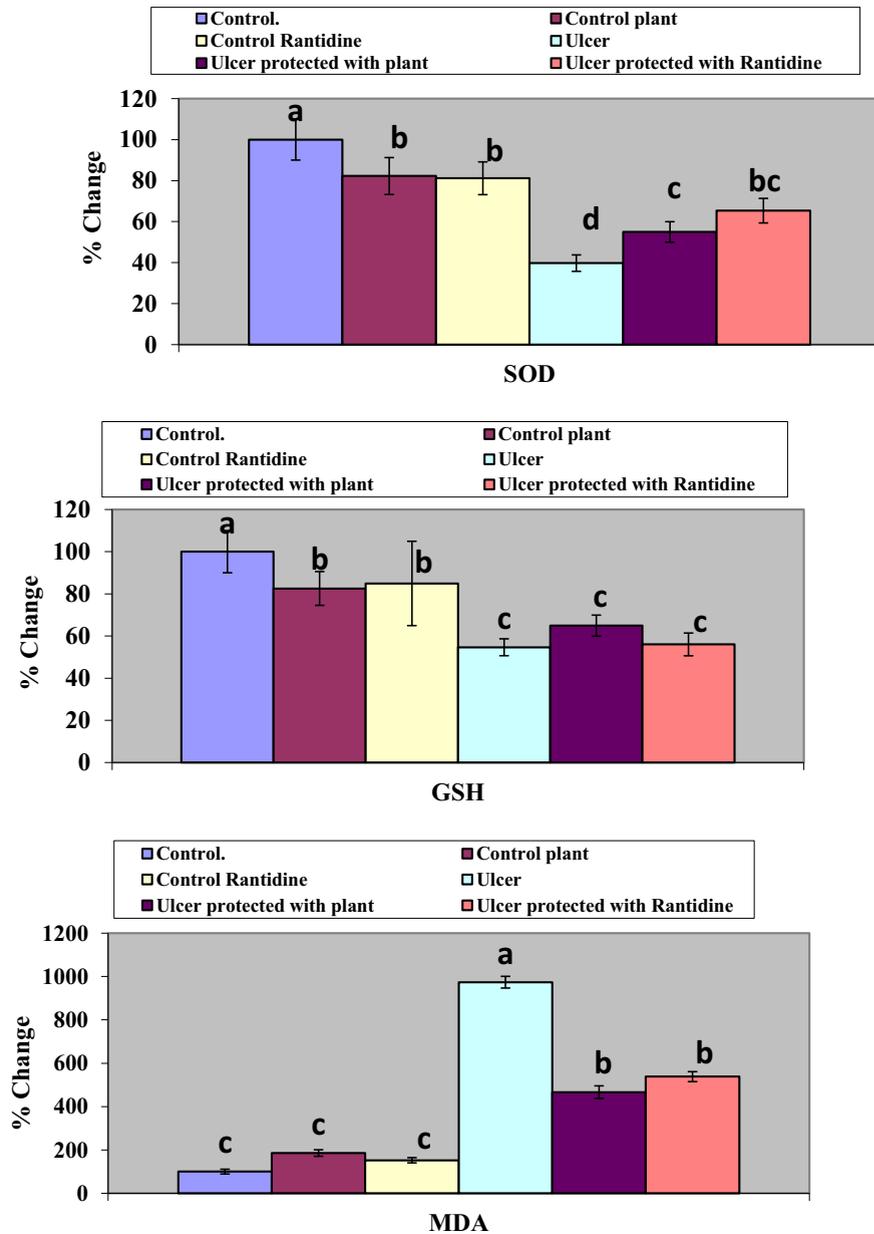
Fig. 2. Percentage changes of SOD, GSH and MDA levels versus control group. Groups having the same letters are non-significantly different, while those having different letters are significantly different at p ≤ 0.05.

3.7. Cell organelles marker enzymes

Ulcerogenic rats showed significant enhancement in LDH, SDH, AP, G-6-Pase, 5′NU levels by 1017.76, 147.59, 35.55, 50.04, 144.90% ($p < 0.0001$), respectively as compared with the control group. Ulcerogenic rats protected with plant extract and ranitidine showed significant decline by 45.70 and 32.41% for LDH, 37.61 and 35.75% for SDH, 9.07 and 10.81% for AP, 21.61 and 23.92% for G-6-Pase ($p < 0.001$), respectively as compared with the ulcerative group. Hence, we noticed that rats protected with plant extract and ranitidine exhibited improvement by 679.42, 93.13, 12.19, 30.49, 35.22 % and 362.37, 88.55%, 14.53, 35.89, 98.8% for LDH, SDH, AP, G-6-Pase and 5′ NU, respectively (Figs. 3 and 4).

3.8. Inflammatory indices

Intracellular adhesion molecule-1 (ICAM-1) and TNF-α levels, ulcerogenic rats showed significant increase by 42.37 and 100.31% ($p < 0.0001$) as compared with healthy rats, respectively. Protection with plant extract and ranitidine showed significant decline in ICAM-1 level by 17.25 and 19.86%, while the decrease in TNF-α level reached to 17.92 and 15.10% ($p < 0.01$), respectively compared to the stomach ulcerative group. Rats protected with plant extract and ranitidine exhibited improvement in ICAM-1 level by 24.73 and 28.48%, respectively, while TNF-α level improved by 35.90 and 30.26%, respectively (Fig. 5).

Concerning to IL-10 level and protein content, ulcerogenic rats showed significant decrease by 66.57 and 44.90% ($p < 0.001$), respectively as compared with the control group. Stomach ulcerogenic rats protected with plant extract and ranitidine showed significant increase in IL-10 level by 111.21 and 91.35% ($p < 0.0001$), respectively. In case of total protein content, the increase reached to 28.25 and 15.69% as compared with the ulcerative group. Therefore, protection with plant extract and ranitidine exhibited improvement in IL-10 level by 37.17 and 30.64%, respectively, while the total protein level was improved by 15.55 and 8.64%, respectively (Figs. 4 and 5).

3.9. Stomach histology

Normal rats showed intact gastric mucosa, normal distribution of gastric glands lined by mucus secreting cells with rounded nuclei and normal lamina propria (Fig. 6a, b).

Gastric mucosa of normal healthy rats administrated plants extract (Fig. 6c,d) and ranitidine drug (Fig. 6e,f) showed normal mucosal appearance, normal lamina propria and normal limits of collagen deposition.
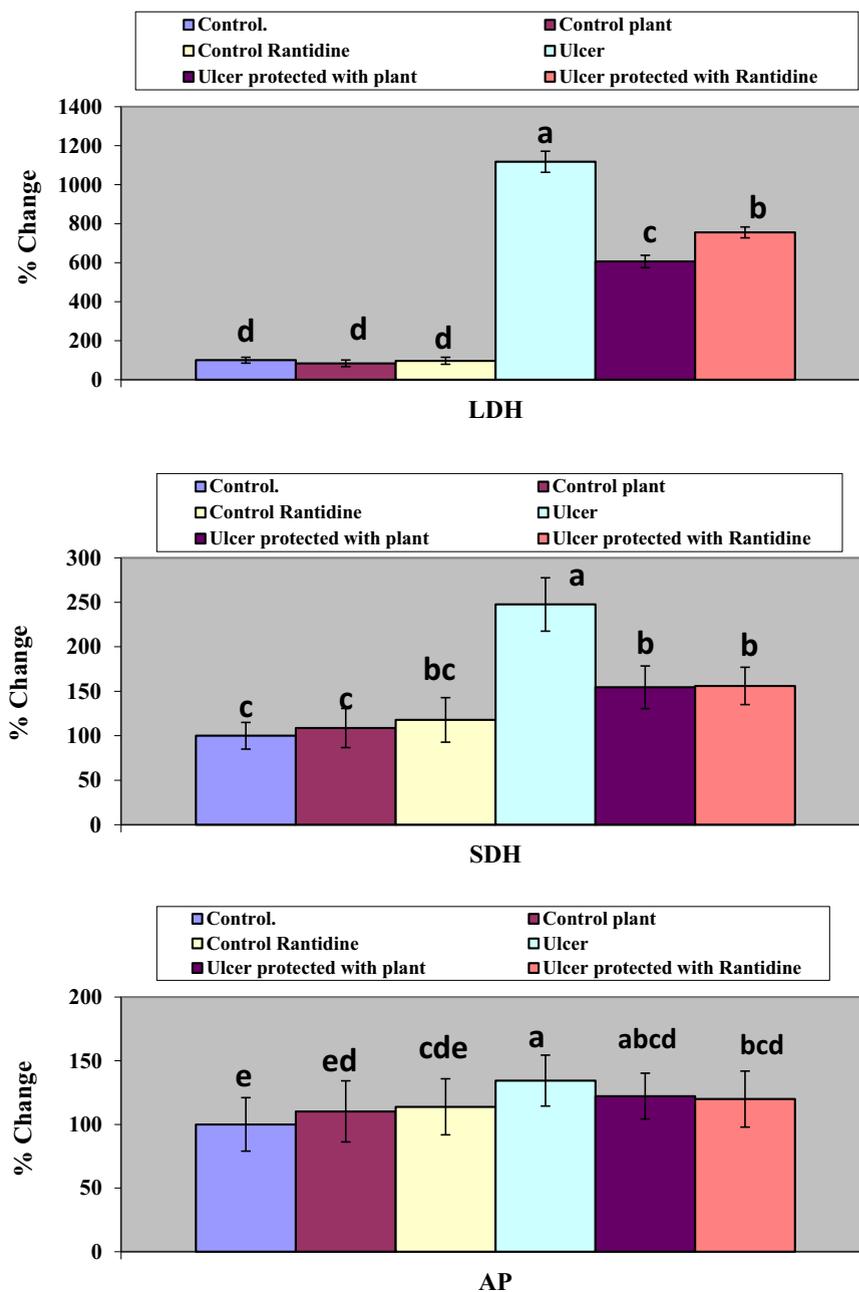
Fig. 3. Percentage changes of LDH, SDH and AP level versus control group. Groups having the same letters are non-significantly different, while those having different letters are significantly different at p ≤ 0.05.

The gastric mucosa in ulcerative rats showed a sharp deep ulcer had reached to the basement membrane lined the lamina propria. The ulcer base showed polymorphous lymphocytes. The gastric glands were hyperplastic and surrounded the ulcer. The lamina propria showed lymphocytes and polymorphonuclear leucocytes with fibrotic tissues (Fig. 7a, b).
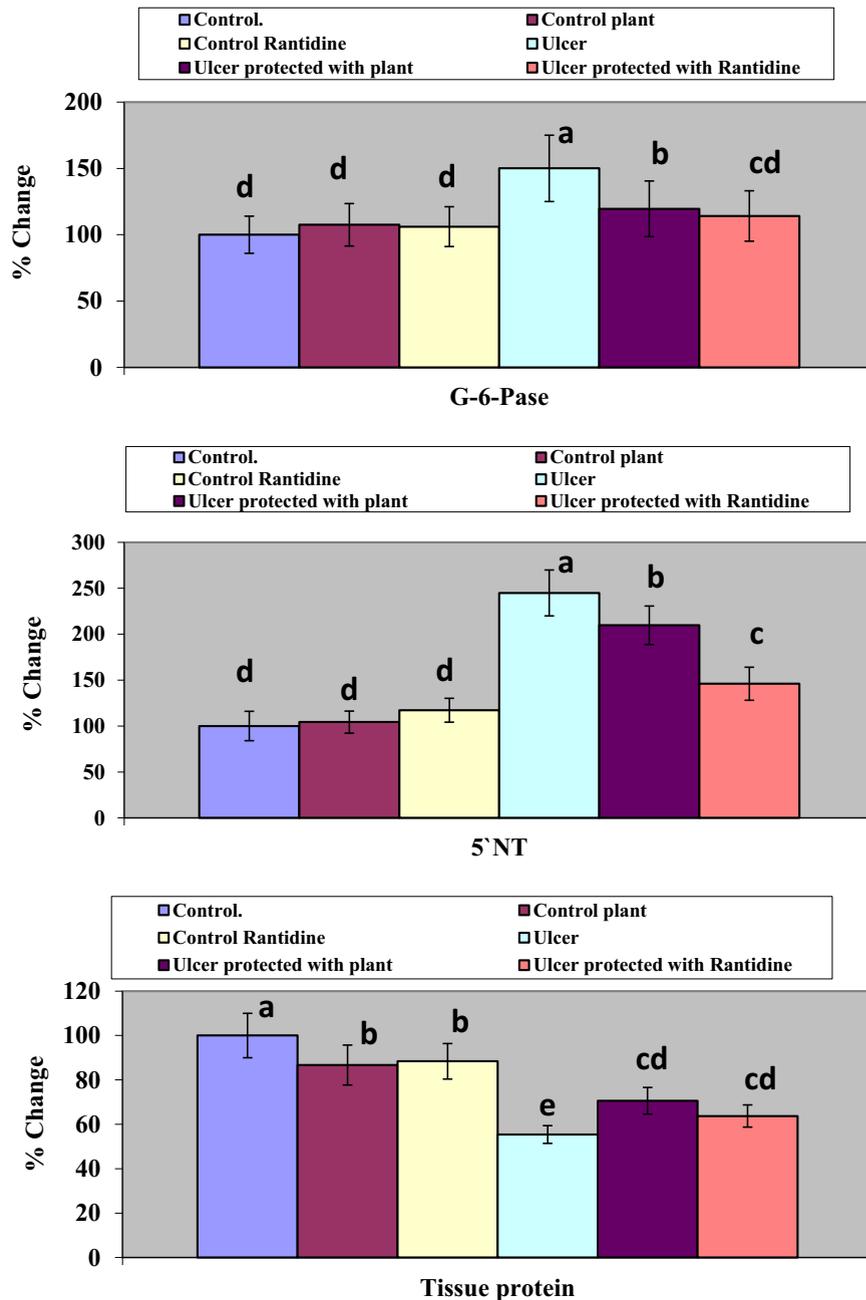
Fig. 4. Percentage changes of G-6-Pase, 5′NT and total protein levels versus control group. Groups having the same letters are non-significantly different, while those having different letters are significantly different at p ≤ 0.05.

Protection with plant extract showed shallow superficial erosions and mild infiltrations by lymphocytes and polymorphonuclear leucocytes in the lamina propria (Fig. 7c, d). Protection by Ranitidine drug recorded superficial erosions widening by mild chronic inflammatory cells, lymphocytes and polymorphonuclear leucocytes (Fig. 7e, f).
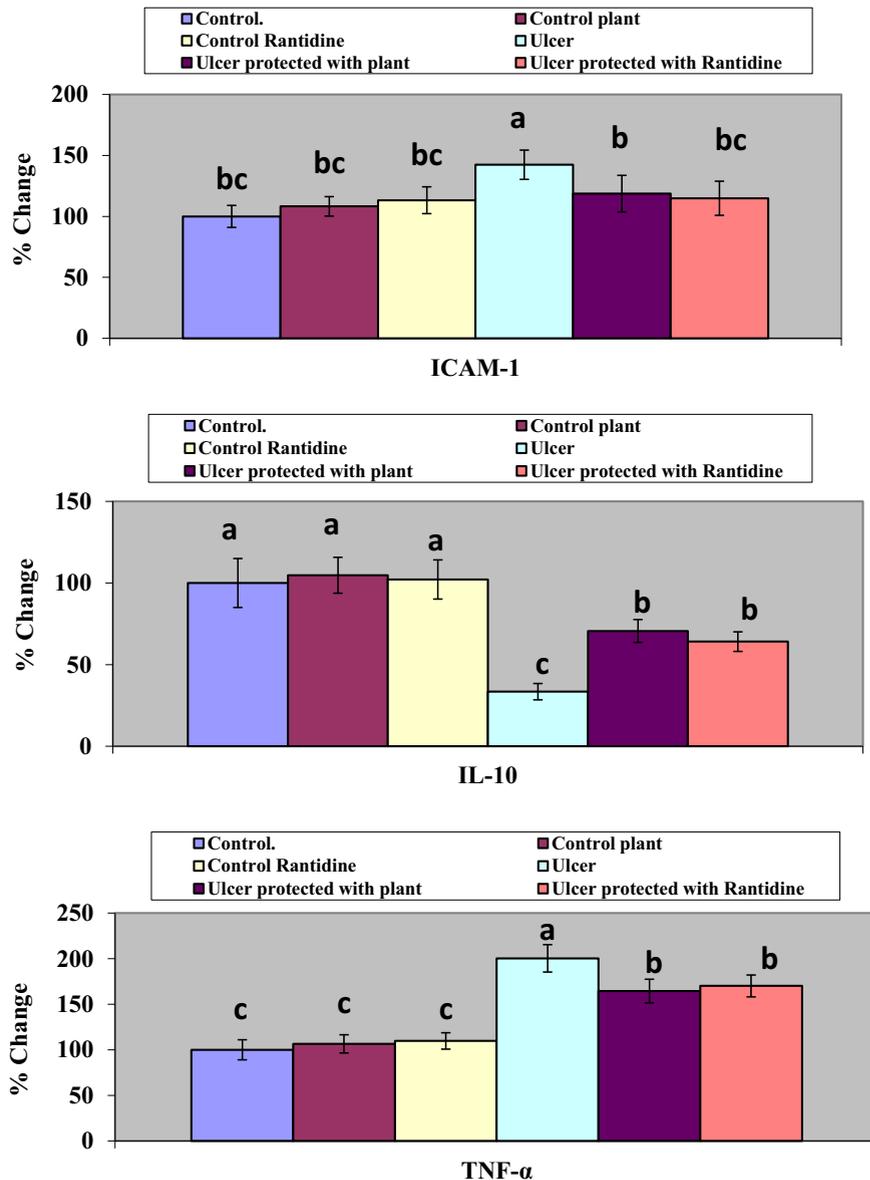
Fig. 5. Percentage changes of ICAM-1, IL-10 and TNF-α levels versus control group. Groups having the same letters are non-significantly different, while those having different letters are significantly different at p ≤ 0.05.

4. Discussion

Ethanol-induced gastric ulcers serve as a well known ulcerogenic agent. It is metabolized in the body and releases superoxide anion and hydroperoxy free radicals (Umamaheswari et al., 2007). In the current study, ethanol increased acid secretion and decrease gastric acidity. Pre-treatment with the plant extract decreased gastric acid secretions vs. stomach ulcer group. Therefore and in agreement with the present study, substances which suppress gastric acid secretion have the ability to accelerate
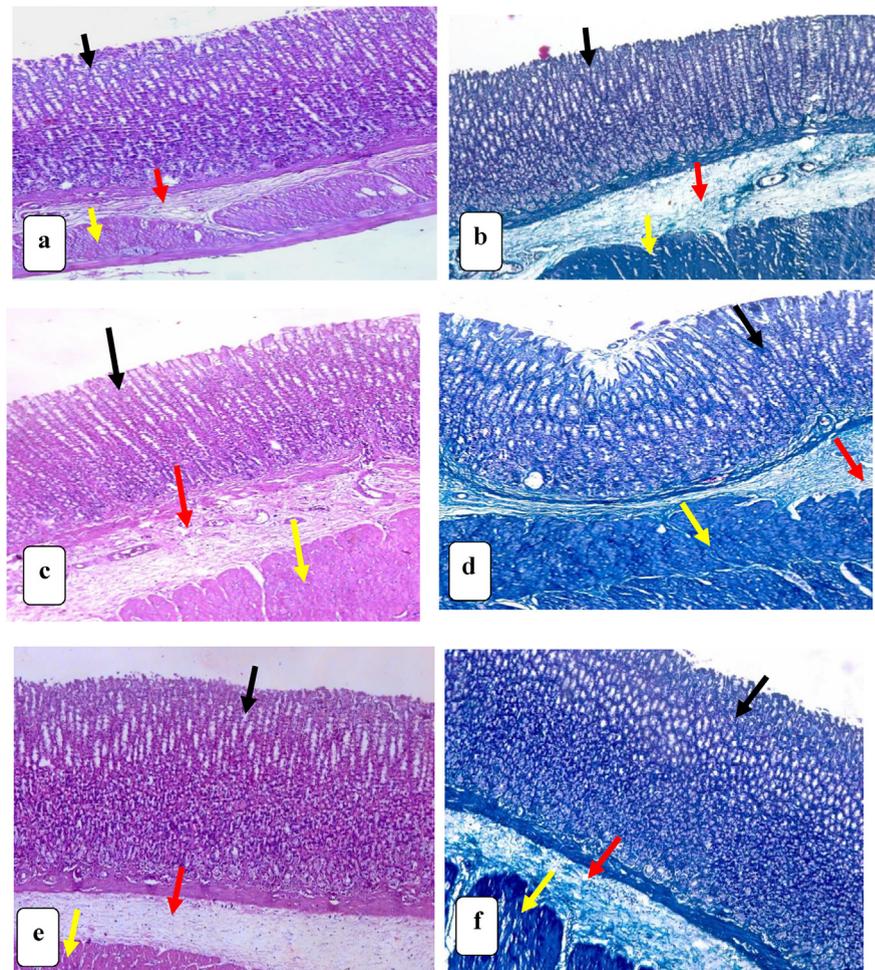
Fig. 6. (a, b): Photographs of normal stomach showing no injuries to the gastric mucosa with normal mucosa (black arrow), submucosal (red arrow), and muscle (yellow arrow) layers (H&E, ×100, ×200). (c, d): Stomach of normal rats administered with plant extract showing almost normal gastric mucosa with no disruption to the surface epithelium (black arrow), with mild edema and no leucocytes infiltration of the submucosal layer (red arrow) (×100, ×200). (e,f):Stomach of normal rats administered with ranitidine drug showing mild hyperplastic gastric mucosa with no disruption to the surface epithelium (black arrow), with no leucocytes infiltration of the submucosal layer (red arrow), (×100, ×200). (a, c, e) stained sections with Hematoxylin & Eosin, (b, d, f) stained sections with Masson's Trichrom).

the healing process of the gastric lesions and inhibit the formation of mucosal injury (Brzozowski et al., 2000; Katary and Salahuddin, 2017).

Regarding to the oxidative stress markers, significant increase in malondialdehyde and significant decrease in SOD and GHS levels were recorded. These results are in agreement with the results of Tandon et al. (2004) and Shetty et al. (2008). The authors explained this observation according to stress causes stimulation of stomach leading to actual schemia. The ischemic condition caused an increase in the level of H_2O_2 by the action of SOD, which, in conjugation with O_2 generates OH. Hydroxyl radicals thus generated oxidizes important cellular constituents such as structural and
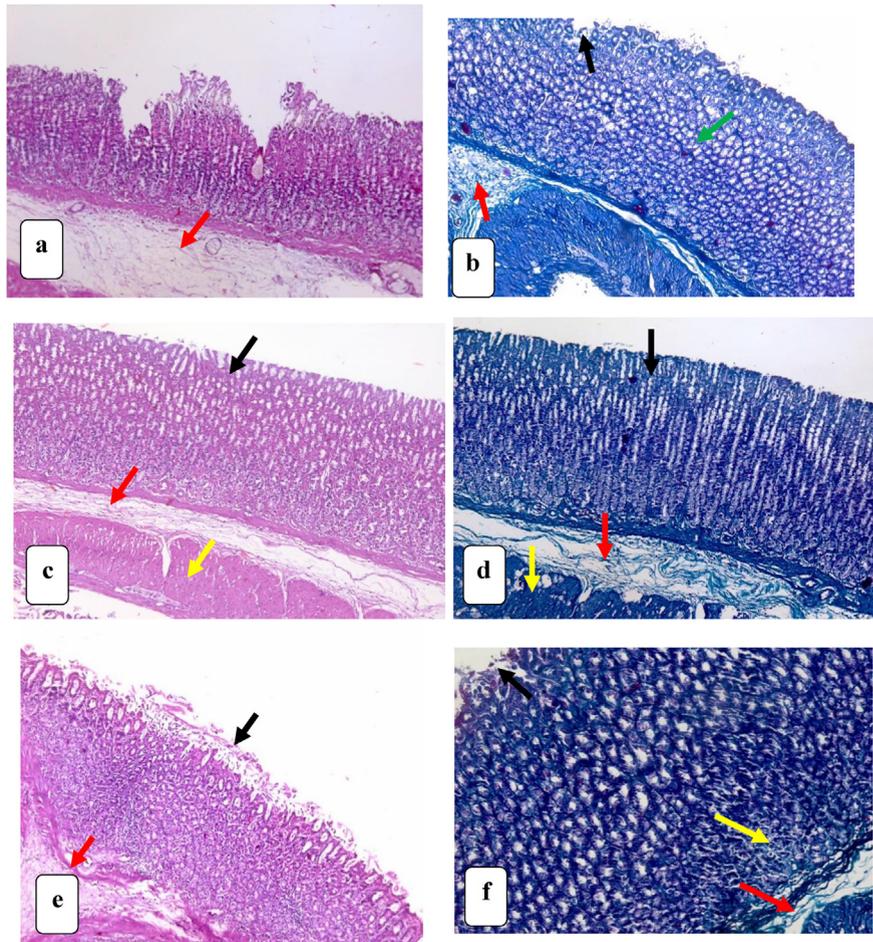
Fig. 7. (a, b) Photograph of ulcerative stomach showing hyperplasia of gastric mucosa (green arrow) erosion (black arrow), edema of lamina propria and infiltration with lymphocyte and polymorphs cells (red arrow) (×100, ×200). (c, d):Ulcerative stomach protected plant extract showing intact (no disruption of the surface epithelium) and healed gastric ulcer with almost normal gastric mucosa (black arrow) with no edema, mild leucocytes infiltration of the submucosal layer (red arrow) and normal muscle layer (yellow arrow), (×100, ×200). (e, f): Ulcerative stomach protected with ranitidine drug showing intact (no disruption to the surface epithelium) and healed gastric ulcer (black arrow) with leucocyte infiltrate in the intermucosal layers (yellow arrow) with no edema and mild leucocytes infiltration of the submucosal layer (red arrow), (×100, ×200). (a, c, e) stained sections with Hematoxylin & Eosin, (b, d, f) stained sections with Masson's Trichrom).

functional proteins and membrane lipids. Lipid peroxidation causes loss of membrane fluidity, impaired ion transport and membrane integrity that leads to cellular functions loss (Kwiecien et al., 2002). In parallel with our results, ethanol consumption lead to a reduction in gastric glutathione (Loguercio et al., 1993). Malmezat et al. (2000) attributed the decrease of GSH to the increase of GSH peroxidase and GSH transferase (the enzyme responsible for catalyzing oxidized glutathione formation and responsible for the conjugation of toxic compounds with GSH, respectively). In addition, Koc et al. (2008) and Liu et al. (2011) observed significant

decrease in SOD levels in gastric ulcer which give an additional support of our results.

Ingestion of ethanol induced the inflammatory response by over expression of the pro-inflammatory cytokine (TNF-α) and the decline of the anti-inflammatory cytokine (IL-10). These findings are in line with the reports of Verma and Kumar (2016). In addition, adhesion molecule as ICAM-1 plays an important roles in the recruitment of neutrophils to sites of inflammation and leading to tissue injury (Watanabe et al., 1997). We noticed that pre-treatment with plant extract normalized the alterations of these parameters.

With respect to mucosal enzymes, ethanol treatment recorded significant elevation of their activities. This was in accordance with the observation of Motawi et al. (2012) and Abd-Alla et al. (2016) who recorded the same pattern of elevation in SDH and LDH level after intoxication of rats with ethanol. Hirokawa et al. (1998) attributed this elevation to the increase of mitochondrial permeability and depolarization. In addition, Brzozowski et al. (2005) consider LDH elevation as a sensitive indicator of mucosal damage. Moreover, a great liability of lysosomal membranes and enzymes release were observed in gastroduodenal ulcer (Rodrigues et al., 1998). Glucose-6-phosphatase and 5′-nucleotidase also recorded significant increase after ethanol ulceration (Ozeki et al., 1987; Ishihara et al., 2010). They explained this phenomenon of enzyme leakage to the endoplasmic reticulum and plasma membrane damage *via* ethanol exposure.

The decrease in total protein content is considered to be a useful tool for identifying cellular dysfunction in many diseases as shown in this study. Therefore, treatments that cause enhancement of protein synthesis has been considered as auto-healing process that accelerates the regeneration process (Sharma and Shukla, 2010).

Regarding to the histological changes in gastric mucosa upon ulceration by ethanol, deep ulcer reached to the basement membrane was recorded with severe lymphocytes infiltration and polymorpho nuclear leucocytes with high degree of fibrosis. These observations were in parallel with the results of Motawi et al. (2012); Abd-Alla et al. (2016); Katary and Salahuddin (2017). Plants that exhibited anti-ulcer activity showed the presence of flavonoids and tannins that have been previously reported for their anti-ulcer activities. Qualitative phytochemical analysis of the active extract revealed the presence of sesquiterpene lactones (pseudoguaianolides, eudesmanolide and xanthanolides type), coumarins, alkaloids, tannins, flavonoids, triterpenoids, diterpene glycosides, essential oils and coumarins which have been reported as cytoprotective agents (Liu et al., 2010; Foudah et al., 2015). Our present work on *P. undulata* revealed the presence of carbohydrates (total hydrolysable carbohydrates were 24.29% on dry weight basis and soluble sugar content being 15.98%) as well as proteins in considerable percentage (9.55% on dry weight basis) which give an additional support for its protective effect. These constituents acted as

a mucilage lining the stomach inner wall and prevent the adverse effect of ethanol and free radicals.

Essential fatty as α-Linolenic acid and linoleic acid have shown a beneficial effect on the skin ulcer (Huang et al., 2018).The major mechanisms of linolenic acid ($C_{18:3}$) and linoleic acid ($C_{18:2}$) for attenuating skin inflammation was done by the competition of the inflammatory arachidonic acid and the inhibition of proinflammatory eicosanoid acid production (Huang et al., 2018). On the other hand, these fatty acids can consider as regulators that affect the synthesis and activity of cytokines for promoting wound healing (Huang et al., 2018). The protective role of *P. undulata* could be attributed to the synergistic effect of these fatty acids to the presence of a mixture of other organic acids such as: capric, lauric, linoleic, myristic, oleic, palmitic, stearic. This protection could be slightly attributed to the presence of sterols and unsaturated fatty acids in high content of *P. undulata*. Huff et al. (2012) reported that, using of plant food supplement containing fatty acids, plant sterols, lignans, and minerals, might have efficacy in treatment and prevention of gastric ulcers.

Palmitic was found to be the major saturated fatty acids in the current investigated plant. It was reported that extract was rich in palmitic acid showed gastroprotective activity against HCl/ethanol-induced gastriculcer in rats (Hamdi et al., 2018).

In the current work, the extract contained 24.56 % of crude fibers. Ferguson et al. (2000) postulated the role of fibers as buffers that reduce bile acids concentrations. Less abdominal bloating and a decrease of discomfort and pain in the gastrointestinal tract could be attributed to the supplementation of fibers.

There is a great need of protein and some micronutrients in the recovery process (Vomero and Colpo, 2004). In our current study the value of zinc and selenium were 17.00 and 3.54 ppm, respectively. To accelerate the gastric ulcer healing process, in addition to protein, there are specific micronutrients such as zinc, which is important to maintain the immune system function and response to oxidative stress (Ferguson et al., 2000). The protective effect of selenium against gastric ulcer is mediated through prevention of lipid peroxidation, increase of non-enzymatic reduced glutathione, activation of radical scavenging enzymes, prostaglandin E2 generation, and increase in anti-inflammatory activity (Kim et al., 2011; Abbas and Sakr, 2013). Selenium (Se^{2+}) is a rare antioxidant element and may reduce infection complications and improve healing (Ferguson et al., 2000) and its content was not previously measured in *P. undulata*. Many studies have shown that the antioxidants are significantly strengthen the gastric walls and protect tissue from oxidative damage (Rajesh et al., 2009).

In conclusion, the results of the present study support the traditional use of *P. undulata* as anti-gastriculcer agent and may serve as a new material for further

development of a safe phytochemical, anti-inflammatory and gastroprotective ulcer-genic agent.

Declarations

Author contribution statement

Abdelgawad A. Fahmi, Mariam Abdur-Rahman,Manal A. Hamed: Conceived and designed the experiments, Wrote the paper.

Asmaa F. Aboul Naser, Mohamed I. Nasr; Performed the experiments, Analyzed and interpreted the data.

Howaida I. Abd-Alla, Nagwa M. M. Shalaby: Conceived and designed the experiment, Performed the experiments, Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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