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Original article

Anti-ulcer effect of *Ranunculus millefoliatus* on absolute alcohol-induced stomach ulceration

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ABSTRACT

Ranunculus millefoliatus (*RM*) has been reported to have a numeral of biological properties. Though, the influence of this plant extract on stomach ulceration is yet stated. Thirty rats arbitrarily alienated 5 groups: the normal group, the ulcerated control group, the omeprazole group, and 2 investigational groups. Normal and ulcerated control groups were gavage by mouth 10% Tween 20. Omeprazole group fed orally 20 mg/kg omeprazole. Investigational group's gavage of 250 mg/kg and 500 mg/kg ethanol extracted *RM* 10% Tween 20, correspondingly. Later another hour, the normal group gavage 10% Tween 20, and groups 2–5 gavage absolute ethanol. Afterward additional hours altogether rats were sacrificed. The ulcerated control group displayed extensive apparent stomach epithelial damage escorted by reduced stomachs mucus excretion and pH stomach contented. *RM* extract meaningfully condensed ethanol-induced gastric lacerations, for example, demonstrated via augmented gastric mucus and pH stomach contents, condensed ulceration expanse, decreased or lack of edema, and leucocyte penetration hypodermic coat. In stomach epithelial homogenate, *RM* extract revealed important upsurge superoxide dismutase (SOD), catalase (CAT) actions, expressively diminished malondialdehyde (MDA) level. Furthermore, *RM* extract augmented strength periodic acid–Schiff (PAS) stain stomach mucosa, besides formed up-regulation heat shock protein 70 (HSP 70) proteins down-regulation the Bcl-2-associated X p rotein (Bax) protein gastric mucosal. *RM* extract lessened the level of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and improved the quantity of interleukin-10 (IL-10). Acute toxicity greater dosage of 500 mg/kg *RM* extract organized not obvious at all toxicology symbols might improve self-protective tools against stomach epithelial abrasions. *RM* extract presented gastroprotective effects that could be due to capability upsurge pH then mucus discharge, rise SOD and CAT, decrease MDA quantity, up-regulating HSP 70 proteins, down-regulating Bax protein, and moderate provocative cytokines.

1. Introduction

Stomach ulcers are major public health difficult affects yearly millions of people worldwide. The pathogenic disorder of peptic ulcer is due to the impairment of the balance between the deteriorated and defensive factors (Hossen et al., 2021).

The ulcerogenic factors affect the gastric mucosa leading to lesions, edema and perforation. These factors include alcohol consumption, acidification of the stomach area, pepsin secretion, bacterial infection by Helicobacter Pylori, reactive oxygen species (ROS) or reactive nitrogen species (RNS) manufactured via oxidative stress, free radical, nonsteroidal anti-inflammatory drugs (Ansari et al., 2017), malnutrition, smoking and abnormal motility (Saremi et al., 2019; Al-Wajeeh et al., 2017). Preventive and protective factors against ulcers include gastric mucosa, bicarbonate as a buffer, antioxidants (vitamin and other compounds), and substances containing SH-groups. The complication of peptic ulcer over time leads to malignancy, gastrointestinal obstruction, hemorrhage, and perforation (Tarasconi et al., 2020).

Ethanol is known to mediate the gastric ulcer by inducing oxidative stress resulting in the generation of hydrogen peroxide (H_2O_2), free radical Malone dialdehyde (MDA). During the oxidative stress superoxide anion radicals are formed, which are ultimately converted into H_2O_2. Due to the deficiency of the

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antioxidants such as catalase, glutathione, glutathione reductase and glutathione peroxidase H_2O_2 will be converted to hydroxyl free radical (OH) (Li, et al., 2018). Subsequently, OH induces lipid peroxidation resulting in MDA formation. Thus, MDA and OH attack the gastric and duodenum tissues preventing the excretion of mucus forming the lesion stomach layer. Furthermore, ethanol induces construction nuclear factor kappa B (NF-κB) besides tumor necrosis factor-α (TNF-α) leading to disruption of the intestinal barrier (Aziz et al., 2019).

Omeprazole is well-known as a good drug for the treatment of gastric ulcer, through its effect as a proton pump inhibitor, preventing the secretion of proton ions (Abed et al., 2020). The therapeutic drugs that exist to date, could not achieve the desired goal because of several side effects including arrythmia, impotent, sensitivity and gynecomastia. For those reasons it is a great interest to produce new therapeutic drugs that can serve patients better.

The importance of medicinal plants has been known since ancient times. *Ranunculus millefoliatus* (*RM*) is widely distributed around Iraq, particularly in Kurdistan Region mountains. *Ranunculus* belongs to Ranunculaceae family, there are approximately 600 species of Ranunculus genus, the plant is divided into two subgenera and seventeen sections. Phytochemical studies have reported that *Ranunculus* plants possess many pharmacologically active compounds (Shin et al., 2021; Li et al., 2018; Aslam al., 2012). *Ranunculus* has been used as a folk medicine for several diseases and syndromes such, as asthma, wound healing, gout and pain (Goo, 2022). In Kurdish society, *Ranunculus* has a medicinal plant to treat rheumatism and other illnesses (Abdullah et al., 2021). To date, there is no in vivo studies about the biological activity of the *Ranunculus millefoliatus*. It was of interest to examine gastro protective influences of *Ranunculus millefoliatus* extract in rodents.

2. Material and methodology

2.1. Chemicals and reagents

The reagents and substances that used for this study were provided by Sigma-Aldrich Chemical Co (Darmstadt Germany). Omeprazole is supplied by local pharmacy. Omeprazole is dissolved in 1% carboxymethyl cellulose (CMC). 20 mg/kg (5 ml/kg) of omeprazole are given directly into the mouths of the rats using a garaged. This dose is suggested via numerous academics (Mughrabi et al., 2010).

2.2. Acute toxicity assay

Thirty-six Sprague Dawley rats (18 males and 18 females) pathogen free rats (two months old, weight (170–195 g) purchased from Animal House, Cihan University-Erbil. Rodents nourished normal rat's pellets and tap water ad libitum, kept in separate cage wide-mesh wire bases prevent coprophagy. Animals reserved in cages for 7 days to adapt. Acute toxicity study used to accomplish harmless dosage the aerial part extract of *RM*. Rats owed correspondingly three groups: vehicle (10% Tween 20), 2 g/kg, and 5 g/kg the aerial part extract of *RM*. Preceding feeding, overnight abstaining (food) practical altogether rat's groups (allow free access to tap water). Food was removed for another 3–4 h afterward treatment. Animals perceived 24–48 h later gavage of aerial part extract *RM* were checked start scientific or toxic symbols. Mortality calculated 14 days. Animals were sacrificed by inoculation of high dose of xylazine and ketamine general anesthesia on 15th days. Blood samples were collected intra-cardiac puncture serum samplings examination (renal and liver function tests). Histopathology of liver and kidney sections stained with hematoxylin and eosin stain (H & E stains) described in section 2.11.1 (Mahmood et al., 2004; Noor et al., 2006; Mahmood et al., 2007).

2.3. Ethical permission

Sprague Dawley rats (200–225 g) purchased from Experimental House, (Ethical No. Biology/25/11/2022/MAA), College of Science, Cihan University. The experiments performed according to human consulting standard "Director Care Usage Research Laboratory Animals", "Nationwide Conservatory Knowledge" "Nationwide Institution Healthiness (New York, NY, USA)".

2.4. Induction of gastric ulcers

Sprague Dawley rats kept cages wide-spread mesh bases prevent coprophagia. They fed standards diet had admission food and water. The investigational animals divided 5 groups; each group consists of 6 rats (they preserved separated cages). Abstaining programs follow: Group 1 and group 2 (normal and ulcer control) received 10% Tween 20 orally. Group 3 (omeprazole control) omeprazole oral (20 mg/kg in 10% Tween 20). Group 4 and 5 (experimental groups were gavage with the aerial part extract of RM 250 mg/kg and 500 mg/kg in 10% Tween 20, respectively. Then 60 min later altogether groups excluding normal group received orally 5 ml/kg absolute ethanol. After one hour, rats anesthetized via higher dose of general anesthesia. Subsequently rats sacrifice, blood withdrew from intracardial perforation. Sera separated biochemical experiments (Abdullah et al., 2012; Mughrabi et al., 2011).

2.5. Gross assessments of stomachs

The stomachs open greater curvature. Then the tissues stomach rinsed ice-called buffer saline. Lacerations and edema that occur on stomach epithelium indicate an injury. The gastric ulcer looked similar to stomach's axis. The gastric ulcer was photographically documented injured gastric part evaluated Image J software. Inhibition percentage (I %) assessed using formula.

I% = UAcontrol– UAtreated / UAcontrol X 100 (Bashah and Abdulla, 2009; Wasman et al., 2012).

2.6. Gastric pH assessment

Specimen's gastric contents collected the concentration of pH gastric liquid measured using pH meter and titration 0.1 N NaOH solution (Ketuly et al., 2011).

2.7. Quantification of gastric secretion

Mucosa of the stomachs washed ice-cold phosphate-buffered saline. Gastric mucosa was gently cleaned using clean slide. Stomach's secretion weightiness assessed by accurate electric balance (Mahmood et al., 2006).

2.8. Preparation of gastric wall homogenate

Glandular gastric parts washed cold phosphate buffered saline with help of homogenized slices stomach epithelium homogenize using (10% w/v) ice-cold PBS containing mammalian's protease enzyme inhibitor mixture. Gastric homogenate was centrifuge 1000x g at 4 °C. Newly isolated gastric liquid was used to determine SOD, CAT, prostaglandin E2 and MDA. Measurement approved according to constructer's instructions (Hussaini et al., 2013; Rouhollahi et al., 2014).

2.9. Antioxidant assessment of gastric homogenates

The concentration of SOD and CAT stomach epithelial homogenate were measured using a marketable kit (Cayman Chemical Company, Ann Arbor, MI, USA) (Abdulla et al., 2010).

2.10. MDA Measurement in gastric homogenates

The amount of MDA stomach homogenates determined via Thiobarbituric Acid Reactive Substances (TBARS), according to the manufacturer (Elabscience, Wuhan, China) (Sidahmed, et al., 2013).

2.11. Histological evaluation

2.11.1. Hematoxylin and eosin stain (H & E stains)

Small pieces (about 1–2 ml) of each gastric glandular epithelium were sectioned. Then they were immediately fixed 10% buffered formalin room temperature one day. The gastric tissues were desiccated by ethanol via shadowing, the clearance was carried out by xylene, paraffin penetration using paraffin tissue processing machine. Tissues implanted paraffin 5 µm section immobilizing on slides (Moghadamtousi et al., 2014; Gwaram et al., 2016).

2.11.2. Periodic acid Schiff stain (PAS stain)

In order to evaluate stomach secretion and distinguish between acid and basic glycoprotein in mucus, sections of the stomach's glandular tissues (5 m thick) were stained using PAS staining. That was performed by following company's orders (Golbabapour et al., 2013; Salga et al., 2012).

2.12. Immunohistochemically staining

Stomach sections with 3–4 µm thickness immunohistochmical stained utilizing antibodies heat shock protein 70 HSP 70 (1:100) and Bcl-2-associated X protein Bax (1:50) (Santa Cruz USA). Examination of the immunohistochemical stained-positive cells using microscope. The percentage positive cells were estimated (Sidahmed et al., 2015; Omar et al., 2017).

2.13. Measurement proinflammatory cytokines

The immunological tests for TNF-α, IL-6, and IL-10 were performed using ELISA kit (Elabscience, Wuhan, China), following the manual procedure provided by the manufacturer. Cytokine measurements were carried out using the recombinant sanitized cytokines (Shareef et al., 2022a).

2.14. Statistical analysis

The results of this study were statistically analyzed by GraphPad prism 8.0, using one-way (ANOVA) analysis. The procedure was followed the Tukey's post-hoc trial. Kolmogorov–Smirnov examination used normalcy test. Standards stated as Mean ± SEM. Values $p < 0.05$ indicate statistically substantial.

3. Results

3.1. Acute toxicity observations

The rats fed aerial part of *Ranunculus millefoliatus* extract 2 g/kg besides 5 g/kg up to 14 days. Altogether rats active no evident sym-

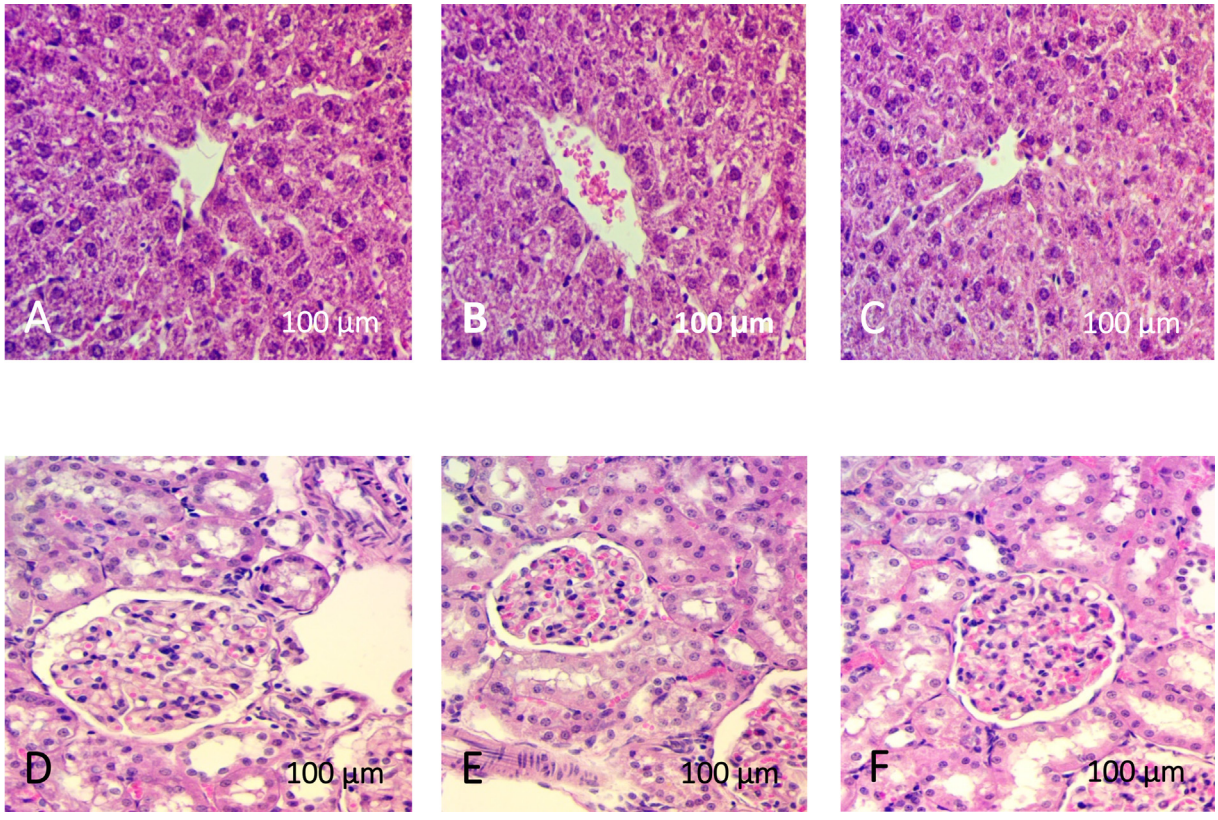

Fig. 1. Rats' kidney and liver histological sections were examined for acute toxicity. Figure A, B and C present liver tissues, figure D, E and F present kidney tissues. Normal groups pretreated 10% Tween 20 (A, D). Rats pretreated 2 g/kg aerial part of *RM* (C, E). Rats pretreated 5 g/kg aerial part of *Ranunculus millefoliatus* (D, F). No organizational alterations detected amongst treated groups as well as normal group.

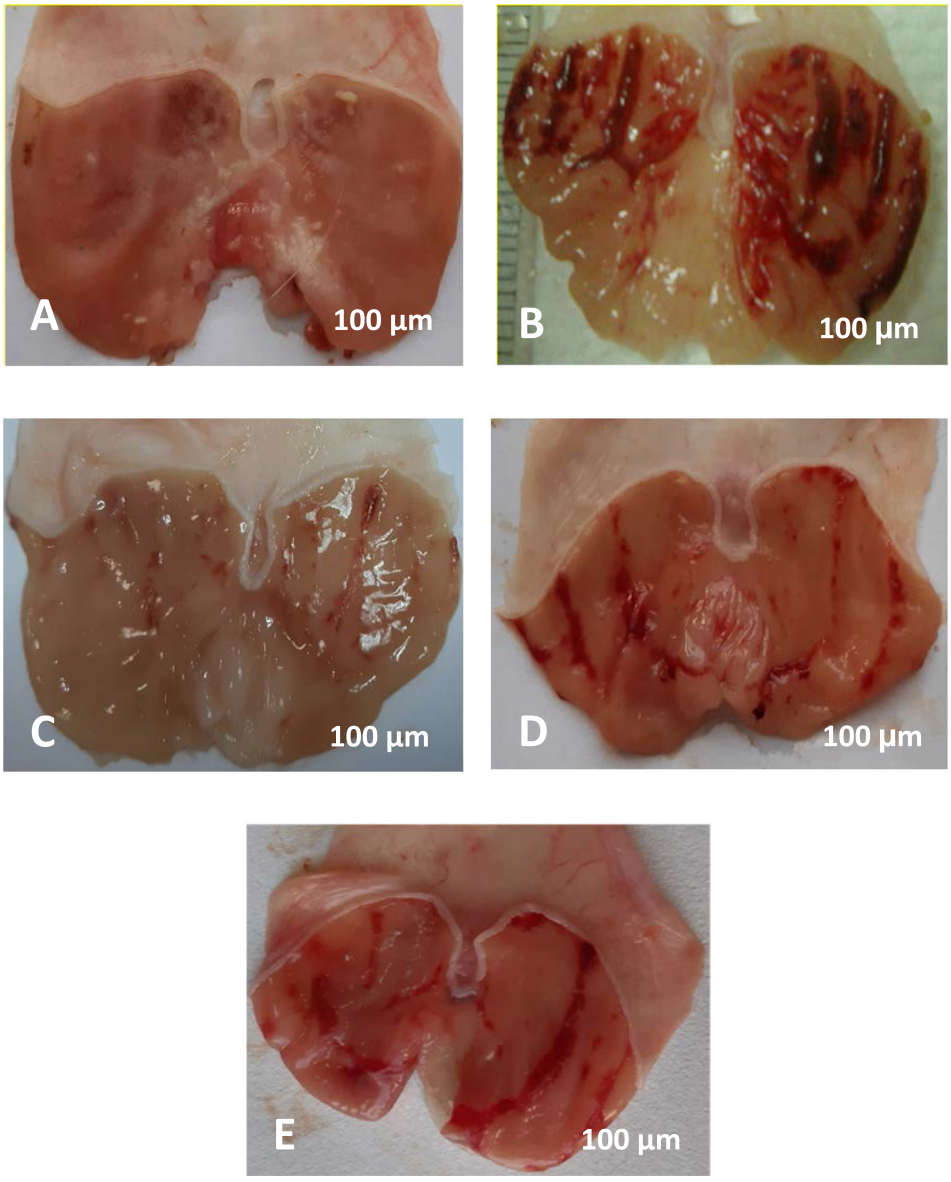

Fig. 2. Effect aerial part extract of *Ranunculus millefoliatus* on macroscopic appearance of alcohol-produced stomach damage. A. Negative group obtainable unbroken gastric epithelial surface. B. Ulcer control group displayed extensive hemorrhagic lacerations of stomach epithelium. C. Omeprazole group demonstrated slight injuries to stomach mucosa. D. & E. Experimental groups showing reduction in gastric ulcer.

Table 1
Effect of aerial part extract of *Ranunculus millefoliatus* on the pH gastric, mucus weigh, ulcerated part as well as % of inhibition ulcer expanse stomachs.

Groups	Pre-feeding	pH (acidity)	Mucus weight (gram)	Damaged Area (mm^2)	Inhibition (%)
1	10% Tween 20	7.01 ± 0.07^{a}	2.42 ± 0.02^{a}		–
2	10% Tween 20	3.08 ± 0.09^{d}	0.87 ± 0.03^{c}	698.50 ± 8.33^{c}	–
3	20 mg/kg	6.47 ± 0.08^{b}	2.80 ± 0.06^{a}	107.33 ± 1.86^{a}	84.63
4	250 mg/kg	5.19 ± 0.12^{c}	1.89 ± 0.12^{b}	142.00 ± 3.12^{b}	79.67
5	500 mg/kg	6.17 ± 0.12^{b}	2.27 ± 0.02^{a}	106.17 ± 3.55^{a}	84.80

bol hepatotoxicity or nephrotoxicity. No obvious indication of toxicity in experimental groups (Fig. 1). Additionally, Serum biochemical investigation seemed usual (statistics not presented, nevertheless accessible upon demand).

3.2. Effect of Ranunculus millefoliatus on gross assessment of stomach

The results of this study show that inducing of peptic ulcer with absolute ethanol in rats causes massive damage in stomach and severe gastric lesion of as in Fig. 2B. It can be observed that rats treated with Omeprazole and *RM* extract suffer less gastric lesion compared to rats without treatment Fig. 2 C, D and E.

3.3. Effect of Ranunculus millefoliatus on gastric mucus content

The result of this experiment shows that the mucus weight of rats treated with aerial part of *Ranunculus millefoliatus (RM)* meaningfully inferior than ulcer control group (G2). Mucus secretion

ulcer control rats was significantly decreased due to the ethanol treatment. Mucus weight ulcerated control rat (0.87 ± 0.03 g) was significantly lower than those of normal group (G1), Omeprazole group (G3), aerial part of *RM* 250 mg/kg (G4) and 500 mg/kg (G5), they were 2.42 ± 0.02 g, 2.80 ± 0.06 g, 1.89 ± 0.12 g and 2.27 ± 0.02 g.

Standards stated as Mean ± SM. Mean with diverse superscripts expressively differences. Significant was considered at $P < 0.05$.

3.4. Effect of Ranunculus millefoliatus on pH of the stomach

The pH measurements of the gastric mucus of the rodent experiments show that the pretreatment of the rats with omeprazole (G3) and aerial part extracts of *RM* (250 mg/kg G4 and 500 mg/kg G5) strongly reduced ulceration and caused less acidity of the stomach. Thus, the pH of the gastric mucus of G3, G4 and G5 (6.47 ± 0.08, 5.19 ± 0.12 and 6.17 ± 0.12) were significantly higher than that of ulcer control group (G2), which was 3.08 ± 0.09. The pH of G5 and G3 seems to be nearly similar. Based on the Table 1.., it appears that the effect of extract is dose dependent. The pH of normal group was neutral.

3.5. Effect of Ranunculus millefoliatus on histology of the stomach H&E stain

Normal Group of rats (G1) exhibited intact gastric mucosa (Fig. 3A), whereas ulcerated control group displayed wide-ranging injured gastric epithelium edema infiltration in subcutaneous coat (Fig. 3B). Rat groups pretreated with aerial part extract of *RM* showed improved prevention of mucosal ulceration and reduced ulcerated area. The was also reduced infiltration of submucosal layer (Fig. 3D, 3E).

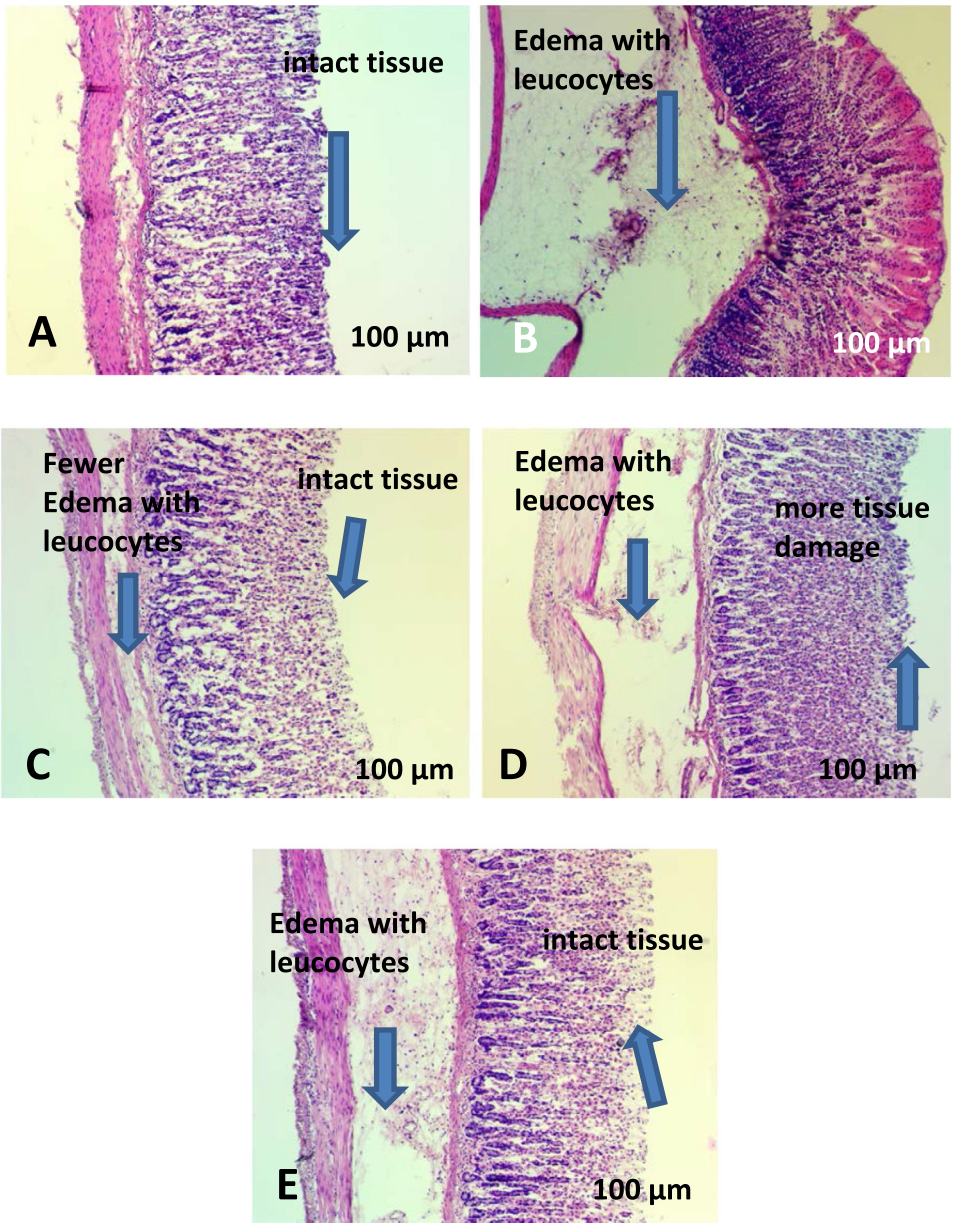

Fig. 3. Effect of aerial part extract of *Ranunculus millefoliatus* on histology structures of stomach against ethanol-induced mucosa damages (H & E stained 10x). A. Normal group (G1) showed intact stomach epithelium. B. Ulcerated control group (G2) show widespread epithelial damage, edema leucocytes infiltration of submucosal coating. C. Reference group (G3) observing minor mucosa injury. D & E Investigational (G4, G5) groups exhibition decrease stomach epithelial injury.

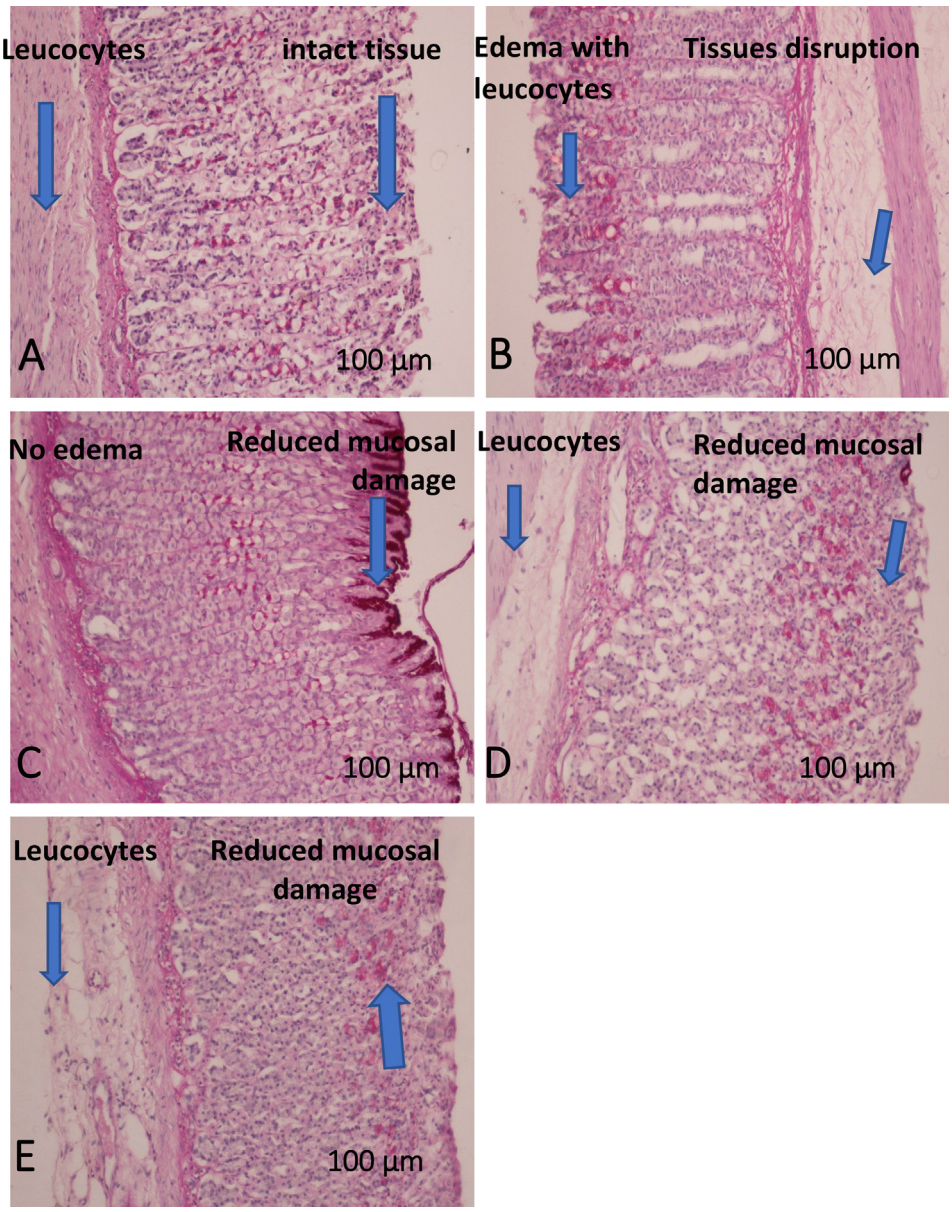

Fig. 4. Effect of aerial part extract of *Ranunculus millefoliatus*. Periodic Acid Schiff stains of gastric glycoproteins secretion in absolute alcohol-produced stomach injuries rats. A. Normal group (G1) demonstration standard PAS-stained stomach epithelium. B. Ulcer control group (G2) showed lessening or non-appearance PAS stains of gastric epithelium extensive mucosa damages. C. Omeprazole group (G3) exhibition high PAS stains intensity. D & E (G4, G5) Experimental groups presented moderate up-take of PAS stains.

3.6. Periodic acid Schiff (PAS) stain

Results revealed pretreated rats with aerial part extract of *RM* exhibited stronger PAS-stain strength of glycoprotein likened to ulcerated rats (Fig. 4). The ulcer control group displayed severe stomach tissue disruption, edema, and leucocytes. Whereas, the tissue damage and edema were significantly less in case of treated rats (Fig. 4 D and E).

3.7. Immunohistochemically stains

Immunohistochemically experiment of gastric tissues the experimental animals revealed that the countenance heat shock protein 70 (HSP 70) rats treated with omeprazole as well as *Ranunculus millefoliatus* extract (G3, G4 and G5) is significantly higher compared to ulcerated control group (G2) shown Fig. 5. HSP 70 expression appeared to be intense brown colored in Fig. 5 C, D and E. whereas, there is slightly brown colored gastric tissue of ulcer control rats (Fig. 5 B). Manifestation Bcl-2 associated X protein (Bax) gastric tissues of ulcerated control rats (Fig. 6 B) are significantly higher associated rats fed omeprazole and *RM* extract (Fig. 6 C, D and E). It was observed that the intensity of the brown colored gastric tissues of ulcer control rats considerably higher than those of treated rats with omeprazole and *RM* extract. Intensity of the HSP 70 and Bax was measured using Image J software.

3.8. Effects of Ranunculus millefoliatus on endogenous antioxidants in gastric tissue homogenate

The investigation of the status endogenous antioxidant enzymes namely, SOD besides CAT showed that levels of both

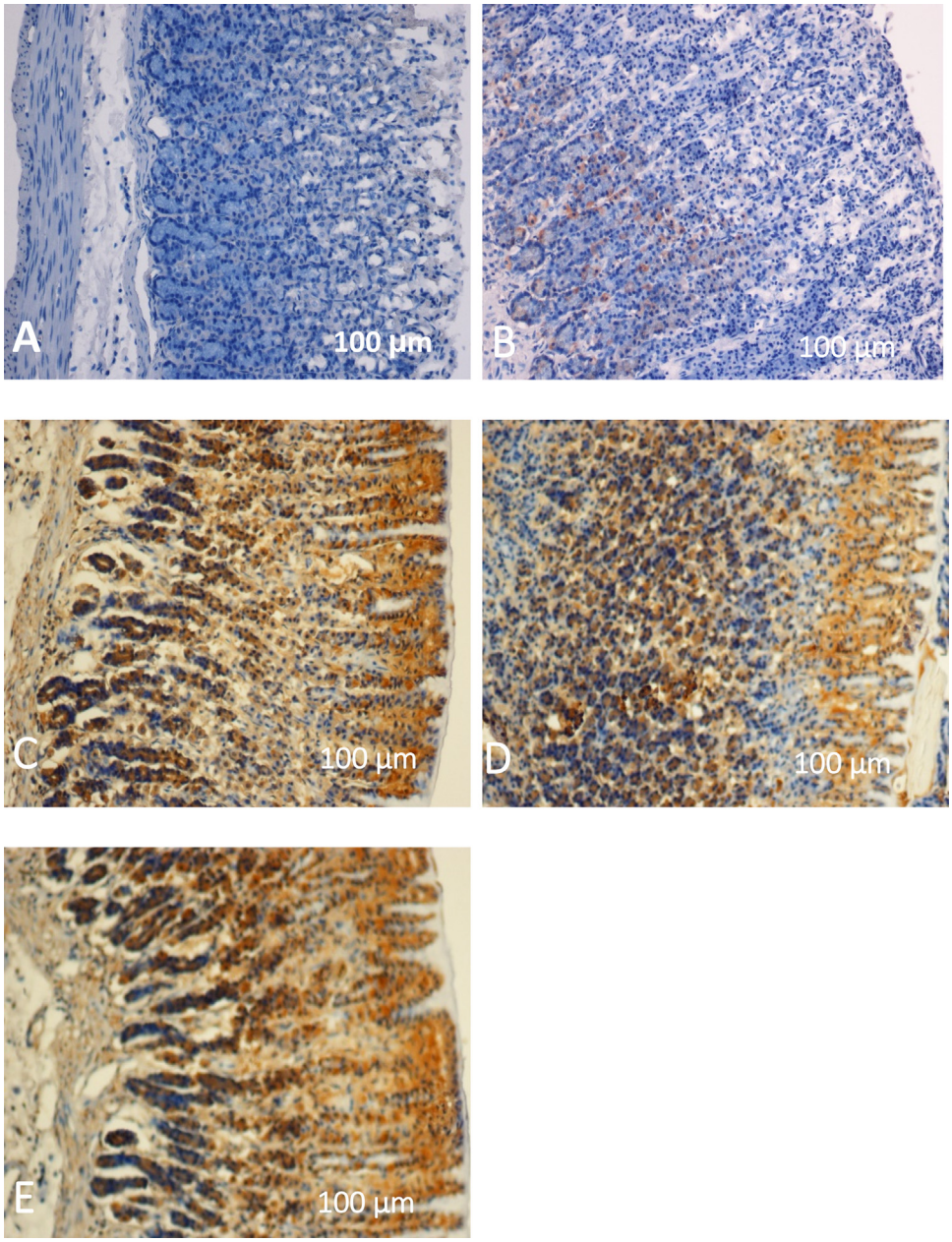

Fig. 5. Effect's aerial part extract *Ranunculus millefoliatus* on appearance HSP 70 proteins-stained gastric mucosa ethanol-induced stomach damages (HSP 70 stain 20x). A. Normal control group presented absence HSP 70 protein-stained stomach epithelia. B. Ulcerated control group exhibited down-regulation HSP 70 protein-stained stomach epithelia. C. Omeprazole group displayed over-regulation SP 70 protein-stained stomach epithelium. D. & E. Investigational group revealed over-regulation of HSP 70 protein stains gastric epithelia.

enzymes are considerably decreased in gastric homogenate of ulcer control rats likened rats fed omeprazole and *RM* extract (Fig. 7). The level of SOD stomach homogenate treated rats with omeprazole then *RM* extracts seems to be similar. Whereas, amount CAT stomach homogenate rats treated *RM* extract (250 mg/kg) is significantly lower than that of rats treated with omeprazole and *RM* (500 mg/kg) (Fig. 7).

3.9. Influences of Ranunculus millefoliatus on malonedialdehyde (MDA) in gastric tissue homogenate

The investigation of the oxidative stress status showed that quantity MDA stomach tissue homogenate of ulcer control rats (G2) is significantly higher compared to those that treated with omeprazole, and *RM* extract (G3, G4 and G5) as shown in Fig. 8. The level of MDA in rats treated with omeprazole (G3) and *RM* extract (G5, 500 mg/kg) seems to be similar, whereas, it is significantly higher in rats treated with *RM* extract (G4 250 mg/kg) than those of G3 and G5.

3.10. Effect aerial part extract of Ranunculus millefoliatus in cytokines level

Results data of the current experiment about influence aerial part extract of *RM* on the cytokine parameters of in blood reveal that rats fed with omeprazole and *RM* extract had considerably lower TNF-a and IL-6 levels in gastric homogenate (Fig. 9). TNF-a and IL-6, G3, G4, and G5) compared with those of ulcer control rats

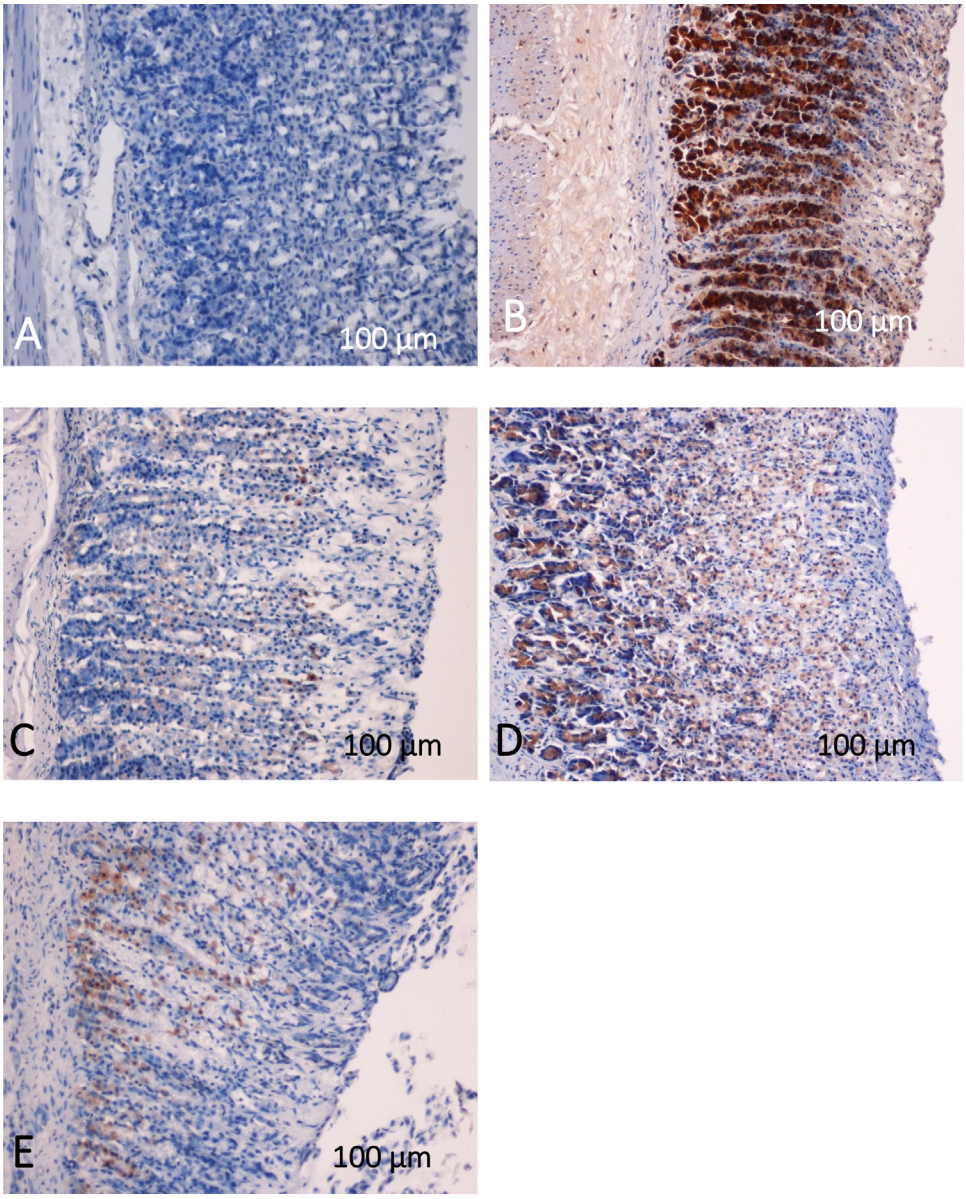

Fig. 6. Effect of the *Ranunculus millefoliatus* on microscopic images of the stomach mucosa based on the representation of the Bax protein (Bax stain 20x). A. Normal control group showed non-appearance Bax protein-stained stomach epithelium. B. Ulcerated control group displayed over-regulation Bax proteins-stained stomach mucosa. C. Omeprazole group showing down-regulation Bax protein-stained gastric epithelium. D. & E. Investigational group presented down-regulation Bax proteins-stained gastric epithelium.

(Fig. 9 TNF-a and IL-6, G2). Whereas amount IL-10 in gastric homogenates of rats fed with omeprazole and *RM* extract is significantly lower (Fig. 9 IL-10 G3, G4, and G5) in comparison to that of ulcer control (Fig. 9 IL-10, G2).

4. Discussions

The existing experiment was taking place with verbal acute toxicity test *Ranunculus millefoliatus* experimental rats, outcome exposed harmless no illness besides mortality throughout the entire tentative time greater dosage of 500 mg/kg *RM*. Continuously, countless co-investigations by numerous researchers utilizing diverse therapeutic constituents displayed harmless no symbol influence specified (Al-Batran et al., 2013; AlWajeeh et al., 2017a; Abbas, M.A. et al., 2021; Shareef et al., 2022a).

Current research obviously confirmed that oral gavage of *RM* had the capability to display gastro-protective ability on ethanol-produced stomach ulceration. *RM* protected stomach epithelium harmful influence ethanol. Ethanol-produced stomach ulceration is famous (Salama et al., 2016; Sarema et al., 2019; Ibrahim et al., 2022). Perfect look similar to indications acute gastric ulceration occurs in people. The toxicity exerted by ethanol induces gastric ulceration investigational representations lengthened hemorrhagic band laceration, submucosal edema WBC permeation, and mucosal tissue injury (Omar et al., 2017; Salga et al., 2017). Herbal and medicinal plants utilized folk medication for gastric ulcerated therapies. Abundant experiments by many co-searchers have pronounced on using remedial plants for anti-ulcer influences in rats (Nordin et al., 2014; Al-Wajeeh et al., 2016; Shareef et al., 2022b). Induction serious hemorrhage ulceration gastric by ethanol gavage modest technique evaluating anti-ulcerated achievement since absolute ethanol merely penetrates the stomach epithelium, therefore producing stomach damage (Moghadamtousi et al., 2014; Fahmy et al., 2020). Outcomes obvi-

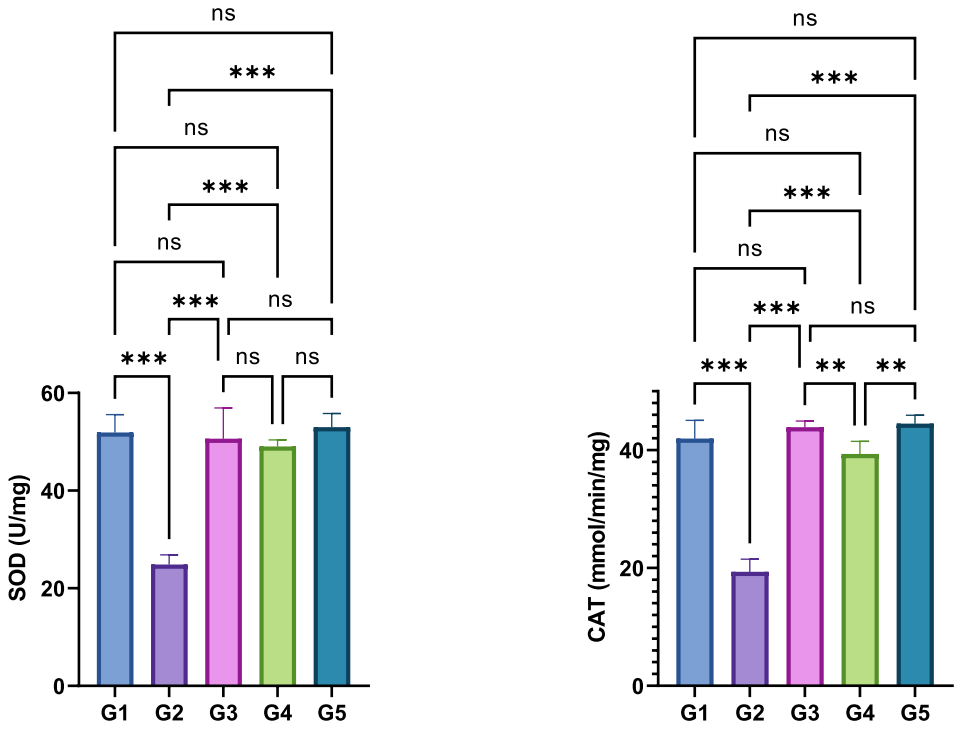

Fig. 7. The effect *Ranunculus millefoliatus* extract on SOD and CAT quantities gastric homogenates of rats with ethanol induced puptic ulcer. G1, normal group; G2 Ulcerated control group; G3 Omeprazole group; G4, rats treated with *RM* extract (250 mg/kg); (G5), rats fed *RM* extract (500 mg/kg). p values presented *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

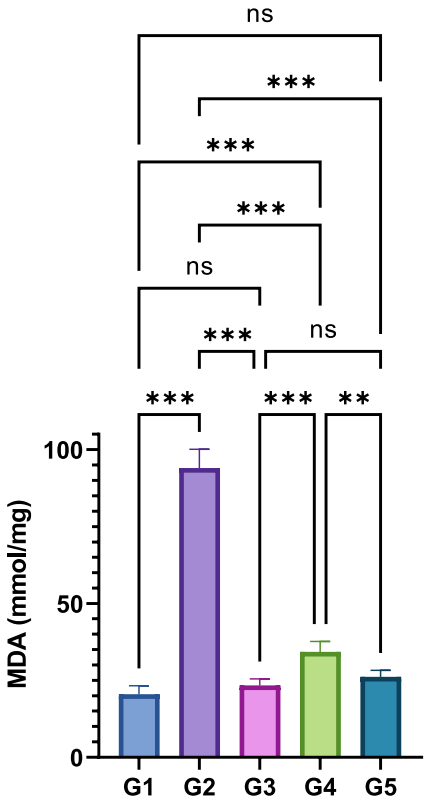

Fig. 8. The effect *Ranunculus millefoliatus RM* extract on MDA amount stomach homogenates rats with ethanol induced peptic ulcer. G1, normal group; G2 Ulcerated control group; G3 Omeprazole group; G4, rats treated with *RM* extract (250 mg/kg); (G5), rats fed *RM* extract (500 mg/kg). p values existing *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

ously specify toxic action by ethanol. Rats who received absolute ethanol visibly exhibited severely disturbed gastric mucosa and hemorrhagic damage abrasions, extended in epithelium and edema in the subepithelial coat with WBC penetration. *RM* suggestively protected gastric mucosal damage. The degree of defence obvious gross and histopathology staining, enhancement lesions likened ulcer control group perfectly important. Similar outcomes described by huge researchers utilizing different remedial florae extracts (Nordin et al., 2014; Sidahmed et al., 2019).

Stomach mucus secretion shows an important part of stomach epithelial defence alongside endogenous aggressive factors, for example, enzymes, acids, and chemicals. Results of this investigation confirmed verbal gavage *RM* defends gastric epithelial via discharging extra mucous against extensive damage via ethanol. In specific, *RM* has revealed substantial antioxidant activity formerly (Abdullah et al., 2012; Aboul Naser et al., 2020).

Oxidative stress raised intracellular amounts of reactive oxygen species (ROS). Particles of energetic physiology part of cellular homeostasis (Hossen et al., 2021). Rats fed with *RM* showed increased pH and mucus secretion of stomach content and presented gastro protection likened ulcer control group. Line with the results of the present experiment, several academics using divers medicinal plants showed increased pH and mucus secretion of stomach content (Golbabapour et al., 2013; Sidahmad et al., 2015; Al- Wajeeh et al., 2017; Shirode et al., 2020).

The result of this experiment revealed rats fed *RM* significantly increased SOD and CAT and decreased lipid peroxidation (MDA). Similarly, an upsurge in SOD and CAT and decrease in MDA have been reported by several researchers using divers medicinal plants (Aboul Naser et al., 2020; Shareef et al., 2022a). In the ulcer control group, histopathological examination of gastric mucosa showed severe hemorrhagic damage with increased leucocyte permeation and edema of the submucosal layer.

Similarly, experimental rats fed with omeprazole or *RM* exposed gastroprotective properties (Al-Wajeeh et al., 2016;

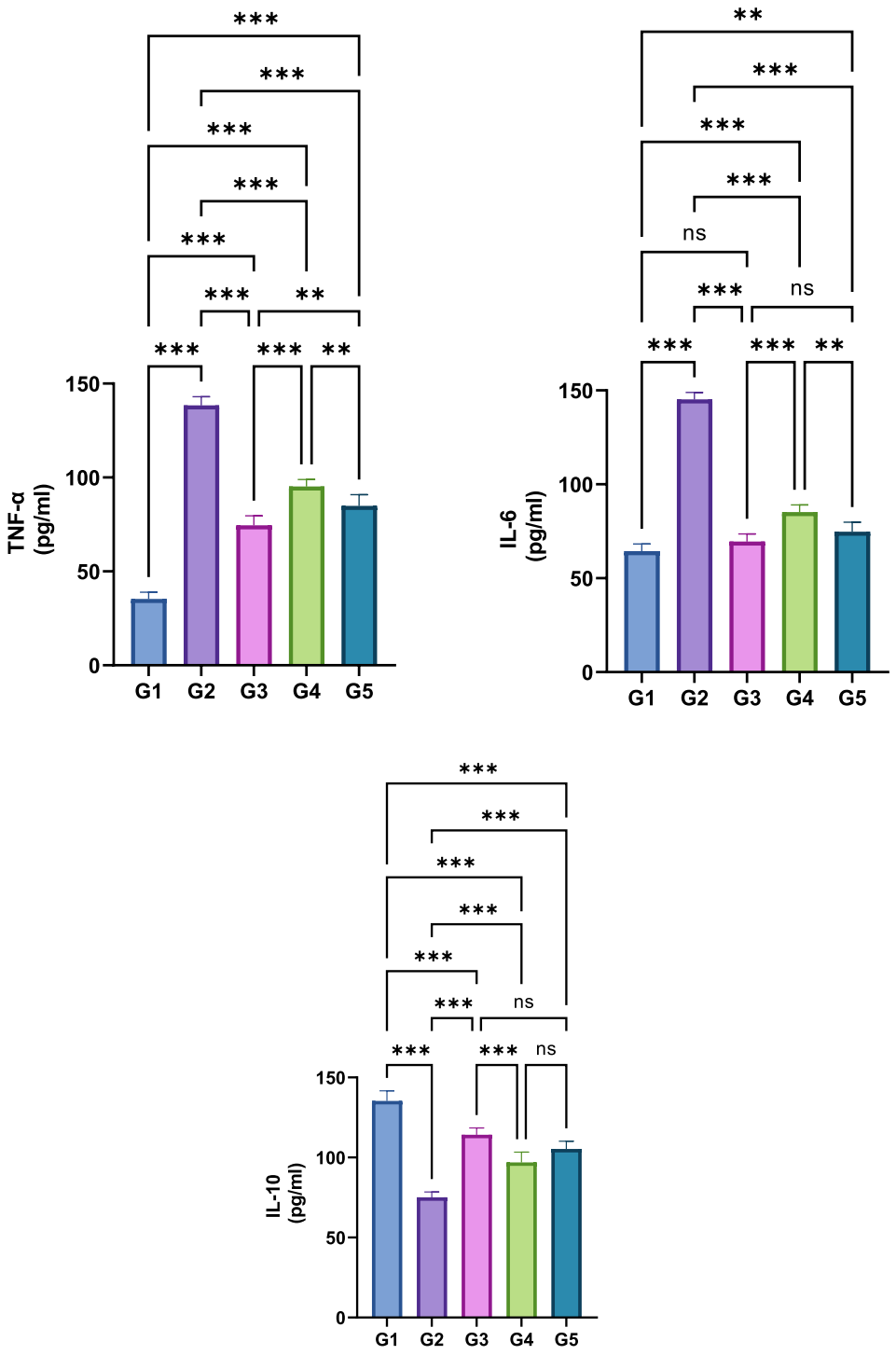

Fig. 9. Influence of *Ranunculus millefoliatus* on TNF-a, IL-6, IL-10 amounts gastric tissue homogenate ethanol-produced ulcerated. G1, normal control group, G2 Ulcerated control group, G3 omeprazole group, G4 treated with *RM* extract (250 mg/kg), G5 fed *RM* extract (500 mg/kg). Principles prevailing *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Makelele et al., 2020). The outcome of this experiment displayed investigational rats nourished *RM* demonstrated augmented PAS stains gastric slices compared ulcerated control group. Correspondingly, many co-researchers utilizing diverse remedial herbs described improved strength PAS stained gastric slices in investigational rats (Omer et al., 2017; Abbas et al., 2021). Similar to the outcomes of existing research, numerous academics utilizing various remedial plants testified the gastroprotection against absolute alcohol produced stomach ulceration (Rouhollahi et al., 2014; Ofusori et al., 2020).

In existing experiment, absolute alcohol amplified construction reactive oxygen species (ROS), repressed presence HSP 70 proteins improved the existence pro-apoptotic protein. Oxidative pressure damages lipids, proteins, DNA, causing lipid peroxidation, cells decrease, material injury (Saremi et al., 2019). HSP 70 proteins protect stomach mucosa from oxidative strain formed via ethanol. Heat shock protein avoids partial denature protein assembly. Over-regulation HSP 70 investigational groups nourished *RM*-led defence gastric epithelium. With consistency results of our experiment, abundant investigators have reported overexpression HSP

70 and then down-expression of Bax proteins against ethanol induced stomach ulceration in rats possibly through fading of ROS facilitated gastric oxidative stress (Nazarbahjat et al., 2016).

The competence of HSP 70 avoids the manufacture of oxidative pressure via absolute alcohol. Significant gastro protective encouragements of HSP 70 have been pronounced by copious co-investigators (Al-Wajeeh et al., 2016; El-Shiekh et al., 2021). HSP 70 typically started cytoplasm, nucleus, mitochondria, cell membranes, and extracellular interplanetary. HSP 70 presence rises parts of cellular tightness and protects alongside damage. In gastric ulceration, HSP 70 protection includes hopeful usual protein building through the removal of damaged protein (Ibrahim et al., 2016; Rahman et al., 2020). Bax protein member Bcl-2 family, associated guideline programmed cell death mitochondrial damages (Moawad et al., 2019). Absolute alcohol can cause the creation of apoptosis gastric epithelium via the over-expression of pro-apoptotic proteins. Experimental rats fed with *RM* presented down-appearance Bax protein over-countenance HSP 70 proteins in stomach tissue slices likened ulcer control group. These outcomes are dependable consequences of the earlier investigation, confirmed beginning HSP70 protein escorted via damage Bax protein rat's protection gastric epithelium beside damages stimulated via absolute alcohol (Zhou et al., 2020; El-Din et al., 2021). TNF-α, IL-6 and IL-10 play an important part in absolute alcohol persuaded stomach ulceration by polymorph nuclear neutrophil infiltration in the submucosal layer (Zhou et al., 2020). In the existing study, experimental rats fed with *RM* or omeprazole condensed quantity TNF-α and IL-6, enhancing IL-10 associated ulcerated control group. IL-6 excites neutrophils, monocytes, as well as lymphocytes at the position of inflammation (Li et al., 2018; Armah et al., 2021). IL-6 prompts the production of the greatest acute phase protein provocative responses. *RM* enhanced gastro protection through anti-inflammatory mechanisms. Underlying machinery success *RM* could be by free radical scavenging and reducing construction single oxygen defends gastric mucosa against oxidative stress, excites stomach healing anti-inflammatory contrivances. Also, antioxidant and anti-apoptotic role through regulation of Bax and variation pro-inflammatory cytokines.

5. Conclusions

Founded outcomes present experiment, *Ranunculus millefoliatus* exposed meaningfully gastro protective influence absolute alcohol-produced gastric lesion investigational animals documented via macroscopic and histological investigation. Produced important rise in gastric mucus excretion, rise in pH stomach contented, condensed edema inflammatory cell permeation submucosal layer gastric partition. Gastric homogenate, *RM* meaningfully increases SOD and CAT activities, expressively reducing MDA amount. Besides, causes upsurge uptake of PAS stain intensity and overexpression of HSP 70 protein and down-appearance Bax protein gastric epithelial slices in investigational animals. Protective significances are mostly because of antioxidant anti-inflammatory peacekeeper's gastric epithelium. Appliance action *RM* strength complete free radical scavenging quenching development single oxygen, thus defensive the stomach mucosa by decreasing oxidative stress absolute ethanol in the stomach, encouraging stomach mucosal reconstruction and anti-inflammatory mechanisms.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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