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Solanum melongena Peels against Ethanol Induced Gastric Ulcer in Rats *via* Regulating Mucosal Enzymes, Oxidative Stress and Inflammatory Mediators' Pathways

Amal M. El-Feky[a], Asmaa F. Aboul Naser[b], Manal A. Hamed[b*]

[a] *Pharmacognosy Department, National Research Centre, El-Buhouth St., Dokki, Giza, Egypt.*
[b] *Department of Therapeutic Chemistry, National Research Centre, El-Buhouth St., Dokki, Giza, Egypt.*

Abstract
Peels from *Solanum melongena L.* (eggplant) contain a variety of active components that are beneficial to human health. This study aimed to determine the potential effect of eggplant peels for treating stomach ulcers in rats. Phenolics, flavonoids, and anthocyanins as bioactive compounds were quantified in the acidified alcoholic extract of eggplant peels. In addition, identification of the isolated compounds was performed by HPLC and LC-ESI-MS analyses. The biochemical evaluation was done through measuring stomach acidity (PH), gastric volume, lesion counts, glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), acid phosphatase (AP), tumour necrosis factor alpha (TNF-α) and prostaglandin E2 (PGE2). Stomach histopathological features were also monitored. Twenty-six compounds (six phenolic acids, 12 flavonoids, and 8 anthocyanins) were identified. For the first time, four compounds (kaempferol, quercetin, 5-hydroxy-3, 7, 4′-trimethoxyflavone, and kaempferol-3-*O*-glucoside) and one known compound; quercetin 3-*O*-glucoside were isolated from the eggplant peels. Rats given the alcoholic extract of *S. melongena* L. peels showed variable improvements in all biochemical parameters as well as in the histological characteristics of the stomach. In conclusion, *S. melongena* L. peels could be considered as a nutraceutical agent against stomach ulcers.

Keywords: Solanum melongena; flavonoids; anthocyanin; ethanol; stomach ulcer; oxidative stress.

1. Introduction

Ulcers are lesions of the skin or mucous membrane characterized by superficial inflammatory dead tissue, where peptic ulcers are the most common type of the various ulcer forms [1, 2]. Peptic ulcers can be developed in the duodenum (duodenal ulcer) or on the inner lining wall of stomach (gastric ulcer) [3]. The most prevalent illnesses are the gastric ulcer that could be caused by an imbalance between aggressive and defensive components [4, 5]. The aggressive factors are *Helicobacter pylori* infection, high secretion of hydrochloric acid and pepsin, malnutrition, elevation of free radicals, consumption of non-steroidal anti-inflammatory drugs and alcohol and stressful conditions, while the defensive factors are prostaglandins, mucin, nitric oxide, bicarbonate, and growth factors [6, 7].

Ethanol is a dangerous substance linked to serious disorders. The integrity of the gastric mucosa is affected by ethanol that leads to increase of mucosal permeability and bleeding [2, 8, 9]. Reactive oxygen species (ROS) and other inflammatory mediators are produced in greater amounts by neutrophils at the site of injury, which results in oxidative damage. Therefore, oxidative stress has been demonstrated to be a significant factor in the damage to the stomach mucosa.

Many treatments, such as histamine H2-receptor antagonists, antiacids, anticholinergics, and irreversible proton pump inhibitors, have been used to treat stomach and duodenal ulcers [7]. However, continued use of these drugs may lead to many side effects, including impotence, gynecomastia, hepatotoxicity, nephrotoxicity, and thrombocytopenia [10]. Due to the unpleasant side effects caused by these drugs, therefore there is an urgent need for more effective and safer treatments. Consequently, there has been an increase in attention for the development of plant-based gastroprotective medications that have less undesirable side effects [8, 9, 11-13].

*Corresponding author e-mail: manal_hamed@yahoo.com (Manal A. Hamed)
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Solanum melongena L. (eggplant) (Family; Solanaceae) is one of the most commercially and commonly grown vegetable crops worldwide [14]. Because of their high concentrations of fiber, vitamins, minerals, and other active ingredients like phenolic compounds, anthocyanins, and flavonoids, the plant and peels were named among the healthiest vegetables [15, 16].

Anthocyanins are the main pigments that exist in fruits and vegetables [17] which are part of the flavonoid class that gives plants their colors. Recently, the extraction of anthocyanins mainly derived from the peels of various fruits and vegetables for proper utilization of plant residues, minimization of industrial pollution and for environmental protection [18]**.** Many researchers have studied the roles of anthocyanins as antioxidant [19], anti-inflammatory [20], antimicrobial [21] and anticancer agents [22]. It also acts as an inhibitor of lipid oxidation through free radicals-scavenging, oxygen quenches, metal chelating and lipoxygenase inhibiting [23].

The current study was targeted to evaluate the anthocyanin, phenolic and flavonoid contents of local eggplant peels and its potential effect as antiulcer agent. The evaluation focused on stomach ulcer index, oxidative stress markers, inflammatory index, certain mucosal enzymes, and the histological architectures of the stomach.

2. Materials and Methods

2.1. Chemicals

The high analytical grade chemicals and solvents utilized in this study were manufactured by Sigma (USA) and Merck (Germany) companies. Abcam (USA) ELISA kits were used to measure TNF-α and PGE2. The slandered drug; famotidine, was graciously supplied by Amon Co., Egypt.

2.2. Plant materials

Fresh eggplants were obtained from a local market in Egypt in January 2023. The specimen of fruits was identified by Prof. Dr. Gamal Farag, Horticulture Research Center, Giza, Egypt. The peels were manually removed, air dried and powdered.

2.3. Extraction procedure

The dried powdered eggplant peels (500 g) were subjected to extraction using 70% ethyl alcohol in water (v/v) that was acidified with 3% v/v acetic acid. This process was repetitive numerous times until comprehensive extraction was accomplished. The resulting extract was concentrated using a rotary evaporator, weighed and compared with the initial weight of eggplant peels to determine the extraction yield. Aqueous acidified ethyl alcohol was used to extract anthocyanins from eggplant peels as reported by [24].

2.4. Detection and quantitative determination of anthocyanins

Anthocyanins were detected through thin layer chromatography silica gel F 254 with a developing system consisting of ethyl acetate, formic acid, acetic acid, and water in a ratio of 100:11:11:26. Additionally, the total anthocyanins content in the extract of *S. melongena* L. peels was quantified using the pH differential technique and expressed as cyanidin-3-glucoside equivalents [25]. The change in UV-Vis absorbance at the maximum wavelength of 510 nm varies depending on the concentration of total anthocyanins at pH levels of 1.0 and 4.5. Anthocyanins are found in the colored flavylium cation form at pH 1.0, whereas at pH 4.5, they are in the colorless hemiketal forms [24].

2.5. Quantitative determination and identification of phenolics and flavonoids

The total phenolic content was determined in *S. melongena* L. peels extract using Folin-Ciocalteu method as reported by [26]. Content of the phenolics was expressed as gallic acid equivalent (mg GAE/gof extract). Additionally, the total flavonoid content was assigned using aluminium chloride method [27] and the obtained value was expressed as catechin equivalent (mg CE/g extract). All results were expressed as mean ± S.D. On the other hand, identification of different phenolic and flavonoid compounds was carried out using high pressure liquid chromatography (HPLC) (Shimadzu-UFLC Prominence) [28].

2.6. Characterization of anthocyanins, phenolic acids, and flavonoids using LC-ESI-MS

Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis using an XEVOTQD triple quadruple instrument with Acquity UPLC – BEHC18 column (2.1 mm x 50 mm; 1.7 µm particle size) was used to characterize anthocyanins, phenolic acids, and flavonoids in *S. melongena* L. peels extract. Using an XEVOTQD triple quadruple detector, the electrospray ionization (ESI) source was run in the negative-ion acquisition mode. From m/z 100 to 1000, the eluted chemicals' mass spectra were found. Using the Maslynx 4.1 software, the peaks and spectra were analyzed, and the retention time (Rt) and mass spectrum were compared with published data for identification [27].

2.7. Isolation of the major flavonoids

The total alcoholic peels extract (12g) was defatted with petroleum ether (40-60), then

successively extracted with ethyl acetate (5 times x 1 L) and dried in rotary evaporator under vacuum. The ethyl acetate fraction (4g) was subjected to column chromatography (Glass column, 15 cm × 4 cm) and silica gel as stationary phase, using different ratios of dichloromethane and methanol. All collected fractions were pooled according to the polarity of eluted solvents to afford 35 fractions (200 mL each). The collected fractions were examined by TLC plates and developed with chloroform: methanol (9:1, v/v), where those displaying the same chromatographic profile were combined together to give four sub-fractions. The isolated compounds which responded positively to flavonoids tests (yellowish color with ammonia vapors and AlCl3 spray reagent) were purified and identified by different spectroscopic analyses and by comparing with spectral data in the literature.

2.8. Animals and ethics

For this study, male Wistar albino rats (100-120g) were used. They came from the National Research Centre's Animal House in Cairo, Egypt. Animals were housed in a climate-controlled area with access to food and water. To ensure that the animals were not harmed during the experiment, the ethical guidelines for anaesthetic procedures and animal handling were provided by the Medical Research Ethical Committee of the National Research Centre, Cairo, Egypt (Approval no. 15904042023).

2.9. Acute toxicity study

Forty male Wistar albino rats (100-120g) were used to assess the acute toxicity of plant peels extract at concentrations of 150, 250, and 500 mg/kg b.wt. Fifteen days were spent observing the animals, where no dead rats were seen. As a result, we decided to use 250 mg/kg b.wt for the *in vivo* study.

2.10. Doses and route of administration

On an empty stomach for 24 hours, absolute ethanol was orally administered at a dose of 0.5 mL/100g b.wt. [2]. Based on the results of the acute toxicity test, 250 mg/kg b.wt/day of the peels extract was administered orally for one week. By the same manner, a dose of 20 mg/kg b.wt/day of metronidazole as a reference antiulcer drug was orally administered [29].

2.11. Experimental groups

Thirty male rats were used in this study. The animals were divided equally into five groups. Group 1 served as normal control rats. Group 2 was administered the peels extract daily for one week. Group 3 existed as the ulcerative rats' group, where they received the ethanol dose on 24 hours empty stomach and sacrificed one hour later. Groups 4 and 5 were the ulcerative rats for one hour, and then received either the peels extract or famotidine (reference drug), respectively for seven consecutive days.

2.12. Gastric secretion volume and pH value

At the end of experimental period, animals were anesthetized by intraperitoneal injection of midazolam at a dose of 10 mg /kg [30] and sacrificed by decapitation. Each rat's stomach contents were removed and centrifuged for 15 minutes at 3000 r.p.m. The supernatant volume was estimated, and its pH level was monitored by using pH meter strips.

2.13. Stomach lesion count

After the stomach content was evacuated, each stomach opened from its long curve, cleaned with saline, inflated, and the numbers of lesions counted using a magnifying lens.

2.14. Tissue homogenate preparation

A five-millilitre phosphate buffered saline solution (pH 7.4) was used to homogenize longitudinal slices weighing 0.5g from each stomach. The homogenate was centrifuged at 3000 r.p.m. for 10 min at 4 ^{o}C and kept at -80 ^{o}C for the analysis.

2.15. Biochemical assessments

Oxidative stress indicators, such as SOD [31], GSH [32], and MDA [33], were estimated in stomach tissue. Certain marker enzymes for mitochondria, cytoplasm and lysosome functions were also measured, including SDH [34], LDH [35] and AP [36], respectively. TNF-α was evaluated using ELISA Kit (Abcam, ab100765, Cambridge, USA). Additionally, PGE2 was evaluated using ELISA Kit (Abcam, ab100785, Cambridge, USA). Total protein content in stomach tissue was also measured [37].

2.16. Histopathological examination

Stomach tissues were fixed in 10% paraformaldehyde and slices of the stomach tissue were embedded in paraffin wax blocks. Haematoxylin and eosin (H&E) were applied to 5 µm thick stomach sections and subsequently inspected under a light microscope to look for any pathological alterations [38].

2.17. Statistical analysis and calculations

Statistical analysis was done by using one-way analysis of variance (ANOVA), Costat Software Computer Program accompanied by *post-hoc* test with least significance difference (LSD) between groups at $p < 0.05$.

% of change = ((Mean of control group − mean of treated group)/ mean of control group) x100.

Improvement (%) = ((Mean of treated group – Mean of ulcerative group)/ Mean of control group) x 100.

3. Results

3.1. Detection and quantitative determination of anthocyanins

The aqueous acidified ethyl alcohol extract yielded 17.8g of natural red-purple compound (3.56%w/w). Examination of the extract profile by thin-layer chromatography gave several red spots under visible light confirmed for the presence of anthocyanins. The total anthocyanins content in *S. melongena* L. peels extract was 56.4mg of cyanidin-3-glucoside/100g.

3.2. Quantification and identification of phenolics and flavonoids

The total phenolic content was determined in the peels extract as 12.337 ± 0.126 (mg GAE/g), while the total flavonoid content was assigned as 11.000 ± 0.177 (mg CE/g). On the other hand, nine phenolics and six flavonoid compounds were identified by HPLC technique. It was noticed that chlorogenic acid (2446.84ug/g) was the major detected phenolic compound, while catechin and apigenin-7-glycoside were the main identified flavonoids (530.66 &190.46 ug/g**)** (Table 1).

3.3. Characterization of phenolic acids, flavonoids, and anthocyanins

Identification and characterization of the major active constituents in *S. melongena* L. peels extract was performed using LC-ESI-MS negative ionization modes. The detected compounds were identified according to their *m/z* values and MS spectral data leading to identification of 26 compounds (6 phenolic acids, 12 flavonoids, and 8 anthocyanins) (Table 2). The phenolic acids were identified as protocatechuic acid, 5-caffeoylquinic acid, 3-5-dicaffeoylquinic acid, caffeic acid, dihydrocaffeic acid and dimethyl caffeic acid. Among them, 5-caffeoylquinic acid and 3-5-dicaffeoylquinic acid were previously reported as the major phenolic acids exist in *S. melongena* L. peels by [39].

Moreover, the flavonoids profile in the extract compromise of 2 flavonoid aglycones (quercetin and myricetin), 8 flavonoid monoglycosides (quercetin-3-glucoside, rutin, quercetin-3-rhamnoside, myricetin-3-galactoside, kaempferol-3-glucoside, kaempferol-3-O-rutinoside, luteolin-O-glucosideandapigenin-7-glucoside), and 2 flavonoid diglycosides (quercetin-3-3′-diglucoside and luteolin-O-glucoside-O-rhamnoside) (Table 2). These results were likewise informed by [39]. While quercetin, myricetin, kaempferol-3-O-rutinoside, luteolin-O-glucoside and luteolin-O-glucoside-O-rhamnoside were not reported previously in *S. melongena* L. peels.

In addition, eight anthocyanins were identified as delphinidin-3-glucoside, delphinidin-3-rutinoside, delphinidin-3-rutinoside-5-glucoside, petunidin 3-O-rutinoside, petunidin 3-O-glucoside, cyanidin-3, 5-di-O-glucoside, cyanidin-3-O- glucoside, and malvidin-3-O-glucoside (Table 2). Their identification was confirmed by comparing retention time and mass fragmentation profile with the previously available data [40].

3.4. Identification of the isolated flavonoids

Fraction eluted from dichloromethane: methanol, 95:5 showed two major purple spots gave yellow color with ammonia vapors and $AlCl_3$ spray reagent. Purification of the two detected purple spots was carried out by preparative paper chromatography using butanol: acetic acid: water, 4:2:1 to give compounds1& 2 as yellow powders (3.8 mg, R_f values 0.76 & 4.1 mg, R_f values 0.65), respectively. By direct comparison with the available flavonoid authentic, and based on melting points and co-chromatography, the structure of compounds 1&2 were identified as kaempferol (melting point 277°C) and quercetin (melting point 316°C). The HPLC analysis confirmed the presence of two isolated compounds. It is worth mentioning that kaempferol and quercetin had not been previously reported in *S. melongena* L. peels.

Compound 3 was eluted from fraction of dichloromethane: methanol, 90:10, R_f value= 0.79. After purification by TLC, the yellowish needles (8.14 mg, 0.20%) were obtained having melting point 145°C. UV λ max; MeOH (256, 348) that expressive for flavonol skeleton. MeOH/NaOMe (269, 375) showed bathochromic shift indicated for free OH group. MeOH/$AlCl_3$ (276, 288 sh., 357, 398) gave bathochromic shift directed for the presence of free OH group at C-5, MeOH/$AlCl_3$/HCl (265 sh., 275, 349, 496). MeOH/ NaOAc (253, 350) no bathochromic shift indicating for absence of OH group at C-7, MeOH/NaOAc/H_3BO_3 (254, 349). ^{1}H-NMR data (500 MHz, CD_3OD, ppm) revealed δ 6.36 (1H, d, *J*=2.1Hz, H-6), 6.73 (1H, d, *J*=2.1Hz, H-8), 8.01(1H, d, *J*=9.1Hz , H-2'), 7.14 (1H, d, J= 9.1 Hz, H-3'), 7.16 (1H, d, *J*=9.1Hz, H-5'), 8.01(1H, d, *J*=9.1 Hz, H-6'), 3.79 (3H, s, 3-OCH3), 3.81 (3H, s, 7-OCH3) and 3.84 (3H, s, 4'-OCH3). ESI MS exhibited a molecular ion at *m/z* 328 directed for molecular formula $C_{18}H_{16}O_6$. The characteristic fragments were at *m/z* 312 for $C_{18}H_{16}O_5$ and at *m/z* 121 for $C_7H_5O_2$.. By reviewing the previous literature concerning the flavonoids chemistry, the isolated compound was identified as 5-hydroxy-3, 7, 4′-trimethoxyflavone. This compound was previously isolated from the

aerial parts of *Solanum schimperianum* Hochst by [41]; nevertheless, it was first isolated from *S. melongena* L. peels in this research.

Compound 4 was eluted from fraction of dichloromethane: methanol, 70:30 to give one major spot, R_f value= 0.82. Purification on preparative TLC yielded (10.15 mg, 0.25%) of yellow needles with melting point 228 °C. UV λ max; MeOH (265, 350) that suggestive for flavonol structure**.** MeOH+NaOMe (275, 325 sh., 395) caused bathochromic shift in band I which signposted the existence of free OH at C-4′. MeOH+$AlCl_3$ (276, 305 sh., 350, 399) resulted in bathochromic shift confirming the presence of free OH group at C-3 and/or C-5, MeOH+$AlCl_3$/HCl (275, 304 sh., 347, 395 sh.). On the other hand, MeOH+NaOAc (275, 318 sh., 392) showed bathochromic shift in band II directed for the occurrence of free OH group at C-7, MeOH+NaOAc/ H_3BO_3 (265, 350). ^{1}H-NMR analysis (500 MHz, CD_3OD, ppm) represented δ 6.26 (1H, d, *J*=2.2Hz, H-6), 6.51(1H, d, *J*=2.2Hz, H-8), 8.02(1H, d, *J*=8.1Hz, H-2'), 6.99 (1H, d, *J*=8.1Hz, H-3'), 6.90 (1H, d, *J*=8.1Hz, H-5'), 8.00 (1H, d, *J*=8.1Hz, H-6') and 5.34 (H-1``). ESI MS showed a molecular ion at *m/z* 448 with molecular formula of $C_{21}H_{20}O_{11}$. The characteristic fragmentations are *m/z* 286point to aglycone moiety, *m/z* 153 for $C_7H_5O_4$ and *m/z* 121for $C_7H_5O_2$. According to the obtained data and by comparing with previous records [42], the isolated compound was identified as kaempferol-3-O-glucoside. This compound was previously identified from *S. melongena* L. peels [39], but it is isolated in a pure form for the first time in this study.

Table 1: HPLC analysis of phenolics and flavonoids in *S. melongena* L. peels extract.

Concentration (ug/g)			
Phenolics		Flavonoids	
Gallic acid	50.84	Rutin	124.65
Protocatechuic acid	142.82		
p-hydroxybenzoic acid	65.29	Hesperidin	ND
Gentisic acid	ND	Apigenin-7-glucoside	190.46
Chlorogenic acid	2446.84	Diodzein	ND
Caffeic acid	139.22	Genistin	ND
Syringic acid	4.90		
Vanillic acid	129.75	Quercetin	78.45
Ferulic acid	11.73		
Sinapic acid	ND	Kaempferol	98.35
p-coumaric acid	18.14		
Cinnamic acid	ND	Chrysin	117.26
Rosmarinic acid	ND	Catechin	530.66

ND: Not detected.

3.5. Ulcer index

Rats in the control group had a gastric secretion volume of 0.20 ml. In comparison to the control group, the ulcerative rats' gastric secretion volume increased by 1150.00%. Rats with stomach ulcers treated with the peels extract or the reference drug recorded significant reduction in the gastric secretion volume by 76.00 and 78.00%, respectively as compared to the gastric ulcerative rats with percentages of improvement reached to 950 and 975%, respectively.

In contrast, the ulcerogenic rats' pH levels dramatically dropped by 33.85% as compared to the control group (Table 3). Comparing with the gastric ulcerative group, the treated rats and the famotidine-treated group showed negligible increases by 27.50 and 23.25%, respectively. These results led to healing improvement values by 18.46 and 15.38%, respectively.

Additionally, the ulcerogenic rats displayed an average of eight lesions/ stomach. Gastric ulcerative rats treated with the peels extract or famotidine showed substantial decreases in lesions count by 62.50 and 68.75%, respectively as compared to the gastric ulcerative rats.

3.6. Oxidative stress markers

After giving normal rats the peels extract, non-significant alterations in oxidative stress markers and total protein levels were seen. Rats with stomach ulcers significantly decreased the GSH levels by 65.10%. Rats treated with the peels extract or famotidine showed significant increases in GSH levels by 75.40 and 101.03%, respectively as compared to the gastric ulcerative rats. This demonstrated a level of improvement reached to 26.32 and 35.26%, respectively.

As compared to the healthy group, the ulcerogenic rats showed a significant decline in SOD level of 60.57%. In comparison with the stomach ulcerative group, the SOD level was increased by 72.42 and 85.72% after treatment with the peels extract or famotidine, respectively. Therefore, treatments

showed an improvement value amounting to 28.54 and 33.45%, respectively.

MDA level of the ulcerogenic rats was significantly increase by 351.35% as compared to normal rats. The MDA levels of the treated rats with the peels extract or famotidine were significantly decreased by 33.86 and 40.0%, respectively comparing with those of the gastric ulcerative rats. As a result, MDA recorded an improvement value of 152.81 and 180.52%, respectively.

The total protein level was increased by 304.14% after ethanol induction as compared to the control group. It exhibited reduction levels by 59.81 and 68.59% after treatments with the peels extract or famotidine, respectively as compared to the gastric ulcerative rats with improvement levels reached to 241.75 and 277.20%, respectively (Table 4 and Fig. 1).

Table 2: LC-ESI-MS analysis of the phenolic compounds in S. melongena L. peels extract.

Class	Identified compounds.	Molecular Formula	Molecular Weight	Observed (*m/z*)	Product ions
Phenolic Acids	Protocatechuic acid *	$C_7H_6O_4$	154	153	153,109
	5-Caffeoylquinic acid	$C_{16}H_{18}O_9$	354	353	191, 179, 127, 111
	3-5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516	515	353, 335, 191, 179
	Caffeic acid *	$C_9H_8O_4$	180	179	161, 135
	Dihydrocaffeic acid *	$C_9H_{10}O_4$	182	181	135, 121, 109
	Dimethyl caffeic acid *	$C_{11}H_{12}O_4$	208	207	163, 131, 103
Flavonoids	Quercetin *	$C_{15}H_{10}O_7$	302	301	153, 229, 257
	Myricetin *	$C_{15}H_{10}O_8$	318	317	153, 217, 245
	Quercetin-3-3′-diglucoside	$C_{27}H_{30}O_{17}$	626	625	463, 300, 271, 255, 243
	Quercetin-3-glucoside	$C_{21}H_{20}O_{12}$	464	463	300, 271, 255, 151
	Rutin	$C_{27}H_{30}O_{16}$	610	609	300, 271, 179, 134
	Quercetin-3-rhamnoside	$C_{21}H_{20}O_{11}$	448	447	271, 255, 151
	Myricetin-3-galactoside	$C_{21}H_{20}O_{13}$	480	479	316, 270, 179, 151
	Kaempferol-3-glucoside	$C_{21}H_{20}O_{11}$	448	447	284, 267, 255, 227
	Kaempferol-3-O-rutinoside *	$C_{27}H_{30}O_{15}$	594	593	449, 431, 413, 395, 353
	Luteolin-7-O-glucoside *	$C_{21}H_{20}O_{11}$	448	447	287, 147, 129
	Luteolin-O-glucoside-O-rhamnoside *	$C_{27}H_{30}O_{15}$	594	593	449, 287, 147
	Apigenin-7-glucoside*	$C_{21}H_{20}O_{10}$	432	431	270, 153, 119
Anthocyanins	Delphinidin-3-glucoside	$C_{21}H_{21}O_{12}$	465	464	303
	Delphinidin-3-rutinoside	$C_{27}H_{31}O_{16}$	611	610	449, 299, 119
	Delphinidin-3-rutinoside-5-glucoside	$C_{33}H_{41}O_{21}$	773	772	611, 465, 303
	Petunidin 3-O-rutinoside	$C_{27}H_{31}O_{15}$	595	594	314, 299, 119
	Petunidin 3-O-glucoside	$C_{22}H_{23}O_{12}$	479	478	314, 299, 271, 243, 125
	Cyanidin-3, 5-di-O-glucoside *	$C_{27}H_{31}O_{16}$	610	609	287, 299, 329, 431, 449, 611
	Cyanidin-3-O- glucoside	$C_{21}H_{21}O_{11}$	498	497	287
	Malvidin-3-O-glucoside *	$C_{23}H_{25}O_{12}$	493	492	331, 493

* Compounds are identified for the first time.

3.7. Inflammatory markers

Following administration of the peels extract to normal rats, negligible alterations in TNF-α and PGE2 levels were seen. The TNF-α level was significantly increased by 203.28% in gastric ulcerative rats as compared to the control group. Whereas after treatments with the peels extract or famotidine, a significant decrease in TNF-α level (386.22 and 43.30%) were recorded as compared to the stomach ulcerative group. Therefore, we noticed improvement in TNF-α level by 117.13 and 131.32%, respectively.

In contrast, the PGE2 level was significantly inhibited by 64.04% in the ulcerogenic animals as compared to the control group. In comparison to the gastric ulcerative group, the PGE2 level was increased by 44.87 and 68.55% after treatment with the peels extract or famotidine, respectively. Treatment with the peels extract or the reference drug showed improvements by 16.13 and 24.65%, respectively (Table 5 and Fig. 1).

Table 3: Gastric ulcer index in all studied groups.

Groups	Gastric solution volume (mL)	(PH)	Ulcer lesion count
Control	0.20 ^{c} ± 0.04	6.50 ^{a} ± 0.50	---
Control +*S. melongena*	0.18 ^{c} ± 0.05 (-10.00)	6.35 ^{a} ± 0.63 (-2.30)	---
Ulcer	2.50 ^{a} ± 0.42 (+1150.00)	4.30 ^{c} ± 0.75 (-33.85)	8.00 ^{a} ± 0.83 ---
Ulcer + *S. melongena*	0.60 ^{b} ± 0.08 [-76.00]	5.50 ^{b} ± 0. 55 [+27.90]	3.00 ^{b} ± 0.30 [-65.51]
Ulcer + Famotidine	0.55 ^{b} ± 0.07 [-78.00]	5.30 ^{b} ± 0.50 [+23.25]	2.50 ^{b} ± 0.25 [-72.41]

- All data are presented as the mean ± SD of six rats per group.
- The significant level ($p < 0.05$) exists in groups with different letters, while similar letters indicate non-significant level.
- The values in brackets represent percentage changes over the control group.
- The values in parenthesis represent percentage changes over the ulcer group.

Table 4: Oxidative stress markers and protein content in all studied groups.

Groups	GSH (µg/g tissue)	MDA (mmol/mg protein)	SOD (U/g tissue)	Tissue protein (µg/g)
Control	36.10 a ± 1.68	9.60 c ± 1.04	75.98 a ± 3.42	23.16 c ± 2.01
Control +*S. melongena*	34.02 a ± 2.05 (-5.76)	9.10 c ± 0.75 (-5.28)	77.51 a ± 3.86 (-2.01)	25.63 c ± 1.91 (+10.66)
Ulcer	12.60 c ± 1.35 (-65.10)	43.33 a ± 4.52 (+351.35)	29.81 c ± 5.70 (-60.75)	93.60 a ± 5.04 (+304.14)
Ulcer + *S. melongena*	22.10 b ± 2.85 [+75.40]	28.66 b ± 5.13 [-33.86]	51.40 b ± 5.02 [+72.42]	37.61 b ± 5.87 [-59.81]
Ulcer + Famotidine	25.33 b ± 2.08 [+101.03]	26.00 c ± 4.00 [-40.00]	55.23 b ± 3.90 [+85.27]	29.40 c ± 5.57 [-68.59]

- All data are presented as the mean ± SD of six rats per group.
- The significant level ($p < 0.05$) exists in groups with different letters, while similar letters indicate non-significant level.
- The values in brackets represent percentage changes over the control group.
- The values in parenthesis represent percentage changes over the ulcer group.

3.8. Cell organelles markers

SDH, LDH, and AP mucosal enzymes showed slight changes after administration of the peels extract to the normal rats. In comparison to the control group, ulcerogenic rats displayed a substantial drop in SDH and LDH by 63.44 and 48.10%, respectively, and a significant increase in AP levels by 131.16%. Treatments with the peels extract or famotidine resulted in a noticeably improvement in SDH level by 25.85 and 31.20%, respectively as compared to the gastric ulcerative rats. By the same manner, we noticed amelioration in LDH and AP levels after treatment with the peels extract or famotidine. The improvement levels reached 9.50 and 17.11% for LDH and 90.94 and 94.60% for AP, respectively (Table 6 and Fig. 2).

Table 5: Inflammatory markers in all studied groups.

Groups	TNF-α (Pg/ml)	PGE2 (ng/ml)
Control	75.21 c ± 7.80	7.87 a ± 0.10
Control +*S. melongena*	79.33 c ± 4.04 (+5.47)	7.50 a ± 0.15 (-4.70)
Ulcer	228.10 a ± 12.30 (+203.28)	2.83 c ± 0.07 (-64.04)
Ulcer + *S. melongena*	140.00 b ± 10.00 [-38.62]	4.10 b ± 0.30 [+44.87]
Ulcer + Famotidine	129.33 b ± 8.14 [-43.30]	4.77 b ± 0.20 [+68.55]

- All data are presented as the mean ± SD of six rats per group.
- The significant level ($p < 0.05$) exists in groups with different letters, while similar letters indicate non-significant level.
- The values in brackets represent percentage changes over the control group.
- The values in parenthesis represent percentage changes over the ulcer group.

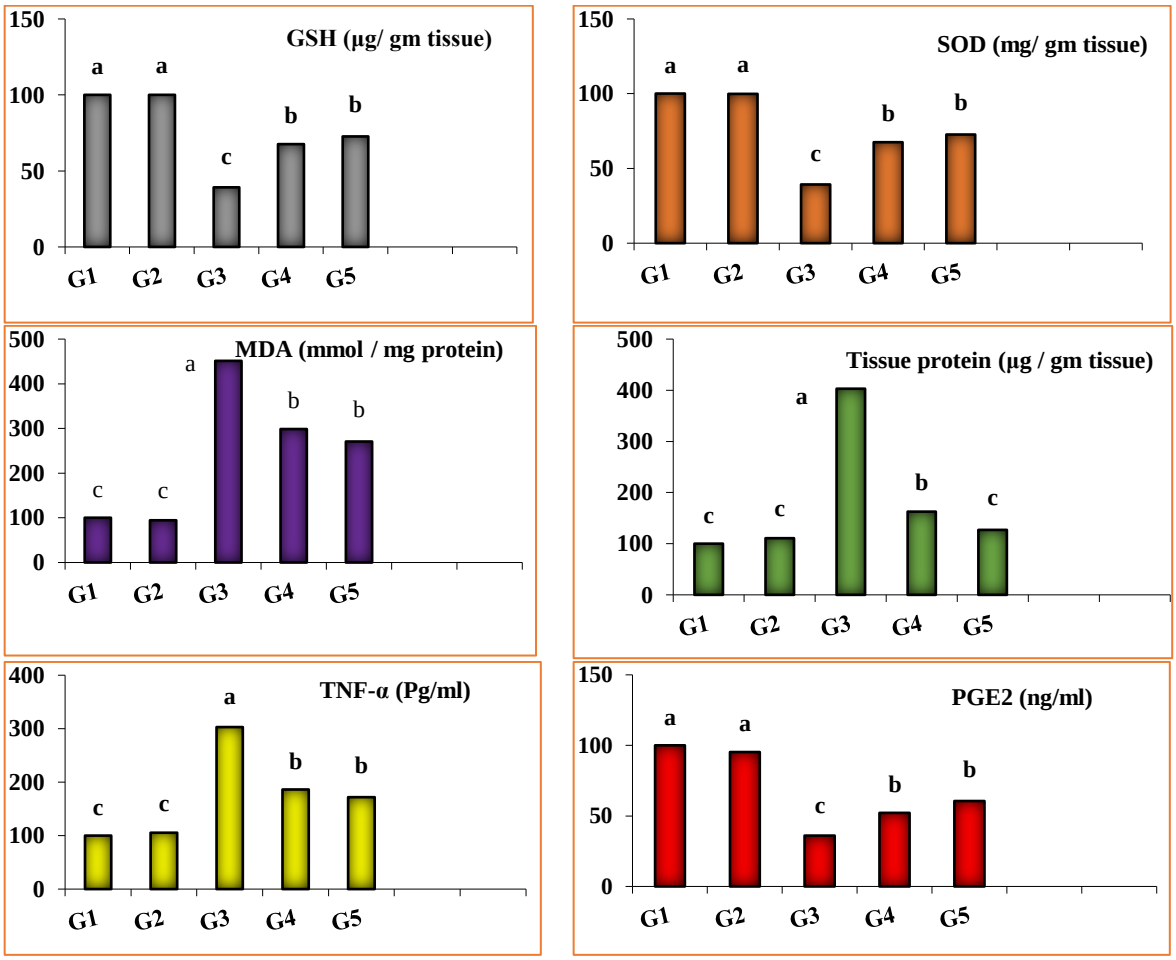

Fig. 1. Percentage change of oxidative stress markers, protein content and inflammatory index. G1: Control group, G2: Control + *S. melongena*, G3: Ulcer group, G4: Ulcer + *S. melongena*, G5: Ulcer + famotidine.

Table 6: Mucosal enzymes in all studied groups

Groups	SDH (mmol/mg protein)	LDH (mmol/mg protein)	AP (mmol/mg protein)
Control	6.73 a ± 0.21	5.26 a ± 0.21	17.23 c ± 0.83
Control +*S. melongena*	6.33 a ± 050 (-5.94)	5.13 a ± 0.15 (-2.47)	17.13 c ± 0.32 (-0.58)
Ulcer	2.46 c ± 0.80 (-63.44)	2.73 c ± 0.38 (-48.10)	39.83 a ± 2.20 (+131.16)
Ulcer + *S. melongena*	4.20 c ± 0.30 [+70.73]	3.23 b ± 0.15 [+18.31]	24.16 b ± 1.50 [-39.34]
Ulcer + Famotidine	4.56 c ± 0.60 [+85.36]	3.63 b ± 0.20 [+32.96]	23.53 b ± 1.48 [-40.92]

- All data are presented as the mean ± SD of six rats per group.
- The significant level ($p < 0.05$) exists in groups with different letters, while similar letters indicate non-significant level.
- The values in brackets represent percentage changes over the control group.
- The values in parenthesis represent percentage changes over the ulcer group.

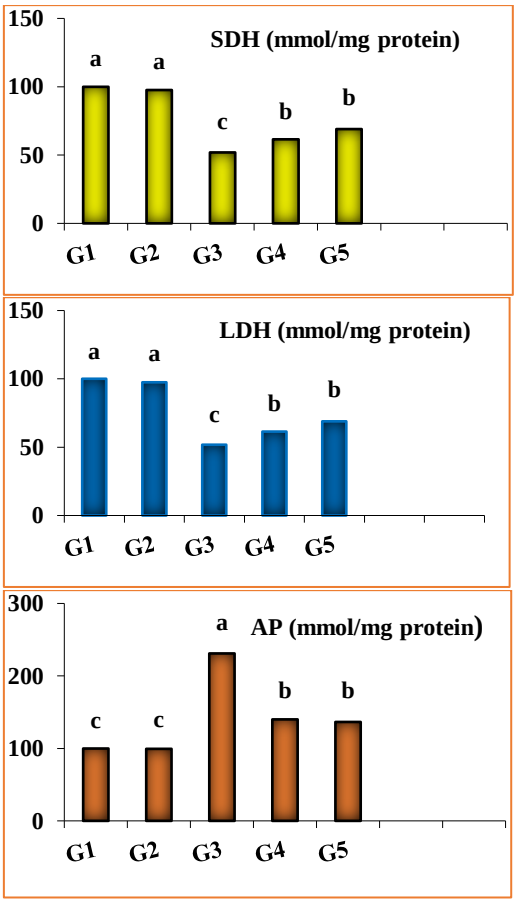

Fig. 2. Percentage change of cell organelles marker enzymes; SDH, LDH and AP. G1: Control group, G2: Control + *S. melongena*, G3: Ulcer group, G4: Ulcer + *S. melongena*, G5: Ulcer + famotidine.

3.9. Histopathological observations

The histopathological features of the gastric mucosa of control and control showed normal stomach layers and normal mucosal layers (represented by * in Fig. 3A and B). Additionally, the submucosal epithelium was normal with intact

basement membrane (arrows) (Fig. 3A and B). Fig. 3C demonstrated ulcerative gastritis. We noted that the basement membrane was severely affected due to the extensive ulceration. The ulcer formation was represented by deep mucosal layer and destruction reaching the submucosal layer (arrow). In gastric ulcerative rats treated with the peels extract, figure 3D displayed normal stomach layers with the absence of ulcer and inflammatory cells as well as some blood congestion (arrow). Figure 3E showed gastric ulcerative rats treated with famotidine. It showed healed ulcer with moderately developed mucosal lining layer with less thickness than normal (arrows).

4. Discussion

Ethanol-induced gastric mucosal injury is one of the most often used experimental models for evaluating the effects of antiulcer drugs [45]. The ulcer indices, including pH level and lesion counts, are used as a predictor of stomach ulcers following alcohol exposure [46]. Reduced hydrogen ion concentration in gastric juice causes stomach injury because the pH level determines both the acidity and volume of the gastric output. The observed decrease in stomach PH, rise in gastric output, and number of lesions were all in line with our findings. As noted by [8, 9], the imbalance between the generation of free radicals and their capacity for scavenging may also be the reason for the rise in gastric secretion and decrease in pH level in ulcerative stomach. Furthermore, it was reported by [47-49] that the advancement of ulcers and the acceleration of the lipid peroxidation process are linked to elevated ROS and antioxidant depletion. In this study, the injection of ethanol raised the MDA content in the stomach tissues of ulcerogenic rats while lowering the levels of SOD and GSH.

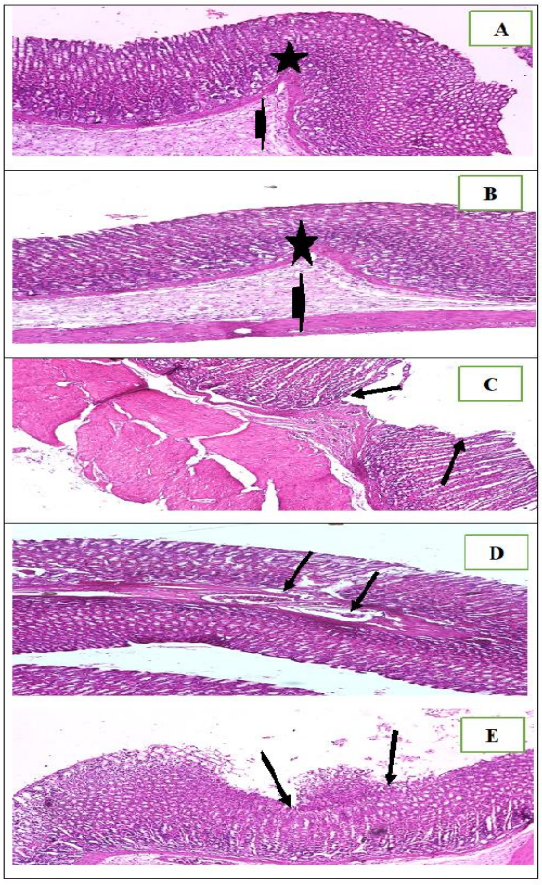

Fig. 3. Photomicrographs of the stomach mucosa of different studied groups stained with haematoxylin and eosin (H&E) and 400 x magnification power.

These outcomes were consistent with the earlier research findings of [48-51]. Furthermore, ethanol-induced gastric ulcer exhibits damage of stomach mucosa that led to increase the mucosal permeability and enzymes leakage [2]. These results were confirmed in the present study by the observed decrease in SDH and LDH enzymes. In agreement

with the present study, Fahmi et al. [8] stated that the gastric ulcer showed a great liability of lysosomal membranes which lead to aggressive AP enzyme release. Li et al. [52] also stated that a severe necrosis was found in some areas of the gastric mucosa. This ulcerogenic condition led to an increase in the levels of inflammatory markers and proinflammatory cytokines, and a decrease in the anti-inflammatory cytokines [50, 51].

According to [53], PGE2 controls the secretion of stomach mucus, stimulates the formation of bicarbonate and blood flow, and quickens the healing process for ulcers. As a result, the decreased PGE2 and increase in TNF-α level may be considered as significant indicators of mucosal ulceration. Additionally, the change in total protein content might serve as a valuable indicator for the severity of cellular dysfunction as clearly shown in the present study [2, 6, 8, 9].

According to histological findings by [54] that confirmed our observations, ethanol damages the gastrointestinal mucosa, disrupts the cellular membranes of the mucosa, and harmfully affects the microvessels of the stomach, all of which led to bleeding and necrosis. Furthermore, ethanol causes severe submucosal edema and damages epithelial cells [52].

Recently, phenolic and flavonoid compounds have gained great attention because of their various and vital activities, which could be utilized as potent agents against many complaints [55]. Flavonoids can be classified into 13 groups based on various substitutions and have a basic C6-C3-C6 structure. The flavonols, flavones, isoflavones, flavanones, flavanols, and anthocyanidins are among those that have great biological activities [56]. Additionally, flavonoids have antioxidants [57], anti-inflammatory [58], and anticancer activities [59].

Multiple investigations have shown that flavonoids have a curative impact on the epithelium of the intestines [60, 61], which include maintaining the integrity of the intestinal barrier, absorbing the lipid and carbohydrate, modulating enzyme activity, controlling stomach secretions, regulating the immune system, and interacting with pathogenic microbes [62]. Like how histamine (H2)-receptor antagonists' act, flavonoids could potentially provide anti-ulcer benefits by decreasing gastric acid release [63-65]. Flavonoids have also been demonstrated to drastically lower the ulcer index and lower gastrointestinal acidity in peptic ulcers [66].

According to [67 and 68], anthocyanins administration prevents the development of macroscopic lesions in the gastric mucosa and decreases inflammatory biomarkers, the size of the macroscopic lesion, and neutrophil inflow. They also increase antioxidant defenses by preventing GSH depletion and increasing SOD activity**.**

The phenolic acids, flavonoids, and anthocyanins compounds are highly present in *S. melongena* L. peels more than that present in the flesh of fruits and vegetables [39, 69]. In a study conducted by [39], it was found that 3, 5-dicaffeoylquinic acid in *S. melongena* L. peels have concentration around ten times greater than that presented the pulp, this finding is due to assumption that the peels are in close contact with the environmental stressful conditions and the fruit could modify the phenolic content to avoid injury exposure. Compatible with previous literature, the major identified anthocyanins in peels of *S. melongena* L. are characterized as glycosides of delphinidin [70]. The obtained results agreed with [71] and [72], who stated that delphinidin-3-glucoside, delphinidin-3-rutinoside and delphinidin-3-rutinoside-5-glucoside were the predominant anthocyanins in *S. melongena* L. peels. Also, in another study conducted by [55], petunidin 3-*O*-glucoside, petunidin 3-*O*-rutinoside and cyanidin-3-*O*- glucoside were characterized with high concentration in *S. melongena* L. peels. Interestingly, cyanidin-3, 5-di-*O*-glucoside and malvidin-3-*O*-glucoside were the two new anthocyanins identified for the first time in this study.

All these data confirmed the findings of [73] who stated that the diversity of biologically active compounds found in plants potentiate the treatment of a variety of ailments. In the current study, noticeable improvements were observed in the oxidative stress index, mucosal enzymes, inflammatory markers, and stomach histological structures after treatment of gastric ulcerative rats with *S. melongena* peels extract.

5. Conclusions

For the first time, kaempferol, quercetin, 5-hydroxy-3, 7, 4′-trimethoxyflavone, and kaempferol 3-*O*-glucoside were isolated from *Solanum melongena* peels alcoholic extract. Rats with stomach ulcers induced *via* ethanol were successfully treated with the peels extract of *S. melongena* L. A clear therapeutic effect was shown by the observed improvements in ulcer indices, antioxidant levels, certain mucosal marker enzymes, inflammatory factors, and the histological picture of the stomach mucosa. The peel extract's therapeutic potential is confirmed by the phenolic acids, flavonoids, and anthocyanins components present in it. To identify the active component (s) responsible for its anti-gastric ulcer potential, an extensive phytochemical analysis of the alcoholic extract of *S. melongena* peels is required.

6. Conflicts of interest

The authors declared no conflict of interest.

7. Formatting of funding sources

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