

Protective and Therapeutic Effects of *Argyreia speciosa* against Ethanol-Induced Gastric Ulcer in Rats

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The protective and therapeutic effects of *Argyreia speciosa* Sweet (Convolvulaceae) against ethanol-induced gastric ulcer in rats were evaluated. Ethanolic and water extracts of the aerial plant parts (200 mg/kg body weight) were orally administered daily for seven days prior to or after ulceration with one oral dose of 1 mL absolute ethanol on 24-h empty stomachs. Rats were divided into eleven groups. Group 1 served as control. To groups 2 and 3 each extract was administered. Groups 4 to 6 received each extract or ranitidine (100 mg/kg body weight) prior to ulcer induction. Groups 7 to 9 received each extract or ranitidine post ulcer induction. Groups 10 and 11 were gastric ulcerative rats after one hour and one week of ethanol induction. The evaluation was done through measuring ulcer indices: stomach acidity and volume, lesion counts, mucus, and prostaglandin E₂ contents. Oxidative stress marker, *i. e.* malondialdehyde, glutathione, and superoxide dismutase, were estimated. Certain marker enzymes for different cell organelles, *i. e.* succinate and lactate dehydrogenases, glucose-6-phosphatase, acid phosphatase, and 5'-nucleotidase, were evaluated. The work was extended to determine the collagen content and the histopathological assessment of the stomach. Gastric ulcer exhibited a significant elevation of the ulcer index, antioxidant levels, collagen content, and the marker enzymes. The water extract attenuated these increments and was more potent as a protective agent, while the ethanol extract exhibited stronger therapeutic potency. In conclusion, *A. speciosa* acted as antiulcer agent. More detailed studies are required to identify the compounds responsible for the pharmacological effect.

Key words: Gastric Ulcer, Ethanol, *Argyreia speciosa*

Introduction

Gastric ulcers affect a large portion of the world's population and is induced by several factors including stress, alcohol consumption, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs (Arun and Asha, 2008). The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors).

Today, there are two main approaches for treating gastric ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection (Valle, 2005). Development of tolerance and incidence of

relapses as well as side effects of clinical evaluation reduce treatment efficacy. This has been the basis for the development of new antiulcer drugs, which includes herbal drugs (Moraes *et al.*, 2008). Nowadays 80% of the populations of most developing countries rely on herbal medicines for their primary health care needs (Mukherjee and Wahil, 2006). The World Health Organization estimates that out of a total of 422,000 flowering plants reported from the world more than 50,000 are used for medicinal purposes (Poonam *et al.*, 2009).

Argyreia speciosa Sweet (Convolvulaceae), commonly known as Elephant Creeper, is a woody climber distributed in many countries up to an altitude of 300 m. The seeds are a rich source of ergoline alkaloids, while the roots are reported to be a tonic, aphrodisiac, bitter, and diuretic, and are used

against rheumatism, gonorrhoea, chronic ulcer, and in the treatment of neurological disorders (Hanumanthachar *et al.*, 2007). Shukla *et al.* (1999) reported also the antifungal activity of *A. speciosa*, while Gokhale *et al.* (2002) claimed its anti-inflammatory and antiarthritic activity. In addition, *A. speciosa* showed antimicrobial (Habbu *et al.*, 2009) and immunomodulatory effects (Gokhale *et al.*, 2003).

In this study the protective and therapeutic effects of *A. speciosa* water and ethanol extracts were evaluated against gastric ulcer induced by ethanol in rats. The evaluation was done through measuring stomach ulcer indices, oxidative stress markers, certain marker enzymes, and through histopathological analysis of the gastric mucosa.

Material and Methods

Chemicals

All chemicals were of analytical grade and products of Sigma (St. Louis, MO, USA), Merck (Munich, Germany), and BDH (Dorset, England).

Plant collection

A. speciosa Sweet (Convolvulaceae) aerial parts were collected from the Suez Desert and identified by Dr. Ibrahim El-Garf, Department of Taxonomy, Faculty of Science, Cairo University, Cairo Egypt. A voucher specimen (ASL-2010) was deposited at the Phytochemistry and Plant Systematic Department, National Research Center, Dokki, Egypt, as a reference. Dried aerial plant parts were ground in a grinder with 2-mm diameter mesh. Five hundred g of the dry powder were kept in a tightly closed container until needed.

Plant extraction

The dried powdered plant material (500 g) was extracted exhaustively in a Soxhlet apparatus with 95% ethanol for 72 h. Another 500 g were extracted with double distilled water in a Soxhlet apparatus for 72 h. After complete extraction, the solvents were evaporated to dryness under vacuum at 40 °C yielding semisolid free ethanol and water extract residues (7 and 15% of dry weight, respectively) (Sharififar *et al.*, 2009).

Phytochemical screening

All extracts were tested for sterols (Nadal, 1971), flavonoids (Seikel, 1962), carbohydrates, amino acids, tannins, alkaloids (Trease and Evans, 1989), and saponins (Wall *et al.*, 1954).

Animals

Male Wistar albino rats (100–120 g) were selected for this study. They were obtained from the Animal House, National Research Center, Dokki, Egypt. All animals were kept in a controlled environment of air and temperature with access to water and diet *ad libitum*.

Ethics

Anesthetic procedures and handling of animals complied with the ethical guidelines of the Medical Ethical Committee of the National Research Center, Dokki, Egypt (approval no. 09210).

Doses and route of administration

Absolute ethanol was orally administered at a dose of 0.5 mL/100 g body weight on 24-h empty stomachs (Mard *et al.*, 2008). *A. speciosa* extracts were orally given at a dose of 200 mg/kg body weight daily for a week (Gokhale *et al.*, 2003). Ranitidine as a reference antiulcer drug was orally administered at a dose of 100 mg/kg body weight daily for a week (Mard *et al.*, 2008).

Experimental groups

Eighty eight male normal healthy Wistar albino rats were divided into 11 equal groups. Group 1 consisted of untreated control rats. To groups 2 and 3 ethanol and water extracts of *A. speciosa* were administered. Groups 4 to 6 (protective groups) received one of the two plant extracts or ranitidine daily for 7 d prior to an oral dose of absolute ethanol on 24-h empty stomachs and were sacrificed 1 h later. Groups 7 to 9 (therapeutic groups) received one oral dose of absolute ethanol on 24-h empty stomachs, and were treated with either of the two plant extracts or ranitidine daily for 7 d. Group 10 received the ethanol dose on 24-h empty stomachs, was sacrificed after 1 h, and served as the gastric ulcerative rats for the protective groups. Group 11 received the ethanol dose, was not further treated for 7 d, and served as the gastric ulcerative rats for the therapeutic group.

Sample preparations and biochemical assays

Gastric lesion counts

A stomach was removed, opened from the long curvature, washed with normal saline, expanded, and fixed on the dissection plate, and lesion num-

bers were counted using a magnifying lens (Szelenyi and Thieme, 1978).

Gastric total acidity

A stomach was removed, the gastric content was collected and centrifuged at 3000 x g for 15 min. The supernatant volume (in μL) was measured, and the total acidity was determined by titration with 0.1 M NaOH using 2% phenolphthalein as an indicator. The results were expressed as molar equivalent (mEq)/L (Guedes *et al.*, 2008).

Mucus assay

The glandular portion of the stomach was separated, weighed, and transferred immediately to 10 mL of 0.1% (w/v) alcian blue solution. After staining for 2 h, the excess dye was removed by two successive rinses with 0.25 M sucrose solution. The dye complex with the gastric wall mucus was extracted with 0.5 M MgCl_2 . The blue extract was vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3000 x g for 10 min, and the absorbance of the aqueous layer was read at 580 nm. The quantity of alcian blue (in μg) extracted per g of wet glandular tissue was calculated from a standard curve prepared using various concentrations of alcian blue (Banerjee *et al.*, 2008).

Determination of prostaglandin E_2 (PGE_2) content in gastric mucosa

The tissue specimens (1 g) were homogenized in 5 mL homogenization buffer containing 0.1 M phosphate, 1 mM EDTA, and 10 μM indomethacin at pH 7.4. The samples were centrifuged at 3000 x g for 15 min, and the supernatants were used for the determination of PGE_2 using an enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA). PGE_2 was expressed as ng/mg protein (Yamaguchi *et al.*, 2008).

Oxidative stress markers and total protein content

Stomach tissue was homogenized in normal physiological saline solution (0.9% NaCl) (1:5 w/v). The homogenate was centrifuged at 4 °C for 5 min at 3000 x g, and the supernatant was used for the determination of marker enzyme activities and oxidative stress markers.

Malondialdehyde (MDA) was determined as an indicator of lipid peroxidation according to Buege and Aust (1978). Glutathione (GSH) was assayed according to Moron *et al.* (1979). Super-oxide dismutase (SOD) was assayed according

to Nishikimi *et al.* (1972). Total protein (mg/g tissue) was determined by the method of Bradford (1976).

Cell organelle marker enzymes

Succinate dehydrogenase (SDH) (mitochondria marker) was assayed according to Rice and Shelton (1957) and lactate dehydrogenase (LDH) (cytoplasm marker) according to Babson and Babson (1973). Activities of the three enzymes glucose-6-phosphatase (G-6-Pase) (microsome marker), acid phosphatase (AP) (lysosome marker), and 5'-nucleotidase (5'NT) (plasma membrane marker) were determined by measuring the release of inorganic phosphate (Swanson, 1955; Wattiaux and De Duve, 1956; Bodansky and Schwartz, 1963).

Histopathological study

Stomach portions were cut, fixed in 10% paraformaldehyde, and embedded in paraffin wax blocks. Tissue sections of 5 μm thickness were stained with hematoxylin and eosin (H&E) and Masson's trichrome, then examined under a light microscope for determination of pathological changes (Hirsch *et al.*, 1997).

The collagen content was determined in Masson's trichrome sections and expressed as the volume of collagen in the ulcer tissue: collagen content (%) = number of blue points on 10 successive fields (1 cm^2 eye piece reticule)/number of points in the reticule \cdot 100 (Asad *et al.*, 2001).

Statistical analysis

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 using least significance difference between groups at $p < 0.05$.

Results

Phytochemical constituents

Phytochemical screening of an *A. speciosa* water extract revealed the presence of flavonoids, alkaloids, and saponins. High contents of carbohydrates and amino acids were also detected. The ethanol extract was rich in steroids, tannins, flavonoids, and alkaloids. Low concentrations of carbohydrate and amino acids were also present (Table I).

Table I. Phytochemical screening of *Argyreia speciosa* extracts.

Constituent	Water extract	Ethanol extract
Steroids	-	++
Flavonoids	+	++
Tannins	-	++
Saponins	+	-
Alkaloids	+	++
Carbohydrates	++++	+
Amino acids	++++	+

(+++++) Abundant; (++) rich; (+) present; (-) absent.

Effect of A. speciosa extracts on gastric ulcer indices

Normal control rats treated with water or ethanol extracts, respectively, exhibited insignificant changes in the gastric volume, total acidity, and mucus content. No lesions were detected after application of extracts (results not shown).

Protection of the ulcerative stomach with water or ethanol extracts caused a significant decrease in the gastric volume by 87 and 15%, respectively. Total gastric acidity significantly decreased by 54 and 42%, while lesion counts decreased by 33 and 31%, respectively. The gastric mucus content was significantly increased by 35 and 25%. Ranitidine as a reference drug provoked a significant decrease in the gastric volume, total gastric acidity, and lesion counts (49, 35, and 31%, respectively), while the mucus content was significantly increased by 39% (Table II). Treatment of the ulcerative stomach with water and ethanol extracts resulted in a significant decrease in the gastric volume by 21 and 27%, respectively. Total gastric acidity showed a significant decrease by 29 and 41%, while lesion counts decreased by 13 and 23%. The gastric mucus content was increased by 12 and 16%. Treatment with ranitidine produced a significant decrease in the gastric volume, total gastric acidity, and lesion counts by 25, 46 and 45%, respectively, while the mucus content was significantly increased by 20% (Table III).

Effect of A. speciosa extracts on inflammatory mediators

The prostaglandin E₂ and collagen content were not changed significantly after treatment of normal control rats with *A. speciosa* extracts (results not shown).

Stomach protection by water and ethanol extracts led to a significant increase in the PGE₂

content by 121 and 20%, while collagen deposition was increased by 16 and 21%, respectively. Ranitidine caused a significant increase in the PGE₂ content by 144%, and of collagen deposition by 13% (Table IV). Treatment with water and ethanol extracts caused a significant increase in the PGE₂ content by 24 and 60% and of collagen deposition by 31 and 21%, respectively. Ranitidine treatment increased the PGE₂ content by 54% and the collagen deposition by 10% (Table V).

Effect of A. speciosa extracts on oxidative stress markers and protein content

In normal healthy rats administered with water and ethanol extracts, the levels of glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD) as well as total protein content did not change significantly (results not shown).

Gastric ulcer protected with *A. speciosa* water extract exhibited a significant decrease in GSH, MDA, and SOD as well as protein content by 79, 69, 89, and 71%, respectively. Protection with the ethanol extract resulted in a significant decrease in GSH, MDA, SOD, and protein content by 51, 42, 75, and 46%, respectively. Ranitidine produced a significant decrease in the oxidative stress markers and protein content by 83, 40, 63, and 50%, respectively (Table VI). Treatment of gastric ulcer by the water extract resulted in a significant decrease in the oxidative stress markers by 90, 49 and 67%, while stomach protein level increased by 23%, but this was not significant. The ethanol extract caused a significant decrease in the oxidative stress markers by 89, 59, and 63%, while total protein content significantly increased by 61%. Ranitidine caused a significant decrease in the oxidative stress markers by 90, 40, and 69%, and also a significant increase in the total protein level by 68% (Table VII).

Effect of A. speciosa extracts on cell organelle marker enzymes

No changes in the activities of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), acid phosphatase (AP), glucose-6-phosphatase (G-6-Pase), and 5'-nucleotidase (5'NT), respectively, were observed in control rats treated with the water extract, while treatment with the ethanol extract caused a significant decrease in AP and G-6-Pase activities (results not shown).

Table II. Protective effect of *Argyrea speciosa* extracts on gastric ulcer markers.

Parameter	Normal control	Ulcer (one hour)	Ulcer protected with water extract	Ulcer protected with ethanol extract	Ulcer protected with ranitidine
Gastric volume	134 ± 35.77 ^d	1462 ± 305.81 ^a	190 ± 97.16 ^d	1245 ± 280.50 ^b	744 ± 183.52 ^c
Total acidity	17.60 ± 2.30 ^{bc}	39 ± 11.40 ^a	18 ± 5.70 ^{bc}	22.50 ± 5.70 ^b	25.40 ± 9.78 ^b
Lesion counts	-	14 ± 1.58 ^a	9.40 ± 1.14 ^b	10.20 ± 1.48 ^b	9.60 ± 0.54 ^b
Mucus	322 ± 19.23 ^a	203 ± 4.69 ^d	274.40 ± 3.64 ^b	254.40 ± 5.85 ^c	283 ± 5.70 ^b

Data are means ± SD of eight rats in each group; gastric volume is expressed in μL , total acidity as molar equivalent (mEq)/L, and mucus as $\mu\text{g/g}$ tissue. Unshared superscript letters between groups indicate significantly different values at $p < 0.0001$.

Table III. Therapeutic effect of *Argyrea speciosa* extracts on gastric ulcer markers.

Parameter	Normal control	Ulcer (one week)	Ulcer treated with water extract	Ulcer treated with ethanol extract	Ulcer treated with ranitidine
Gastric volume	134 ± 35.77 ^b	182 ± 17.88 ^a	144 ± 11.41 ^b	132.40 ± 7.50 ^b	136 ± 11.40 ^b
Total acidity	17.60 ± 2.30 ^{ab}	22 ± 5.70 ^a	15.60 ± 3.64 ^{bc}	13 ± 2.12 ^{bc}	11.80 ± 2.38 ^c
Lesion counts	-	8 ± 1.14 ^a	6.60 ± 0.89 ^{ab}	5.80 ± 0.84 ^b	4.20 ± 0.83 ^c
Mucus	322 ± 19.23 ^a	228.40 ± 18.51 ^c	244.60 ± 15.51 ^{bc}	253.20 ± 11.56 ^b	261.60 ± 9.44 ^b

Data are means ± SD of eight rats in each group; gastric volume is expressed in μL , total acidity as molar equivalent (mEq)/L, and mucus as $\mu\text{g/g}$ tissue. Unshared superscript letters between groups indicate significantly different values at $p < 0.0001$.

Table IV. Protective effect of *Argyrea speciosa* extracts on inflammatory mediators of gastric ulcer.

Parameter	Normal control	Ulcer (one hour)	Ulcer protected with water extract	Ulcer protected with ethanol extract	Ulcer protected with ranitidine
Prostaglandin E ₂	54.71 ± 3.45 ^a	20.94 ± 2.61 ^c	46.31 ± 6.71 ^b	25.21 ± 4.69 ^c	51.12 ± 8.89 ^{ab}
Collagen content	5.81 ± 0.83 ^c	40.60 ± 3.28 ^a	16.20 ± 1.30 ^c	21.00 ± 1.58 ^b	13.20 ± 1.30 ^d

Data are means ± SD of eight rats in each group; PGE₂ content is expressed as ng/mg protein, and collagen content (%) = no. of points in ten fields/no. of points in the reticule · 100. Unshared superscript letters between groups indicate significantly different values at $p < 0.0001$.

Table V. Therapeutic effect of *Argyrea speciosa* extracts on inflammatory mediators of gastric ulcer.

Parameter	Normal control	Ulcer (one week)	Ulcer treated with water extract	Ulcer treated with ethanol extract	Ulcer treated with ranitidine
Prostaglandin E ₂	54.71 ± 3.45 ^a	28.66 ± 5.12 ^d	35.54 ± 5.43 ^c	45.74 ± 6.84 ^b	44.10 ± 7.30 ^b
Collagen content	5.81 ± 0.83 ^c	41.40 ± 2.41 ^a	31.20 ± 1.14 ^b	21.60 ± 1.30 ^c	12 ± 1.58 ^d

Data are means ± SD of eight rats in each group; PGE₂ content is expressed as ng/mg protein, and collagen content (%) = no. of points in ten fields/no. of points in the reticule · 100. Unshared superscript letters between groups indicate significantly different values at $p < 0.0001$.

Ulcerative stomach protected with the water extract showed a significant decrease in the activities of all marker enzymes (35, 41, 12, 25, and 57%, respectively). The ethanol extract decreased the marker enzyme activities by 34, 34, 23, 23, and 50%, respectively, while ranitidine decreased

SDH, LDH, AP, G-6-Pase, and 5'NT activities by 41, 60, 15, 10 and 24%, respectively (Table VIII). Treatment with the water extract caused a significant decrease in SDH, LDH, AP, G-6-Pase, and 5'NT activities by 18, 34, 38, 86, and 30%, respectively. The ethanol extract treatment recorded

Table VI. Protective effect of *Argyreia speciosa* extracts on oxidative stress markers and protein content in gastric ulcer.

Parameter	Normal control	Ulcer (one hour)	Ulcer protected with water extract	Ulcer protected with ethanol extract	Ulcer protected with ranitidine
Glutathione	20.21 ± 4.51 ^c	126.87 ± 18.21 ^a	26.95 ± 3.38 ^c	61.67 ± 26.90 ^b	21.57 ± 23.12 ^c
Malondialdehyde	0.89 ± 0.19 ^c	4.29 ± 1.12 ^a	1.35 ± 0.18 ^c	2.49 ± 0.32 ^b	2.56 ± 1.13 ^b
Superoxide dismutase	9.76 ± 1.77 ^d	43.91 ± 11.85 ^a	18.78 ± 5.28 ^{bc}	11.17 ± 4.15 ^{cd}	16.36 ± 3.02 ^b
Total protein	40.36 ± 3.24 ^a	47.88 ± 9.00 ^a	13.78 ± 2.23 ^c	25.86 ± 2.09 ^b	23.83 ± 6.85 ^b

Data are means ± SD of eight rats in each group; data are expressed as $\mu\text{g}/\text{mg}$ protein for glutathione, $\mu\text{mol}/\text{mg}$ protein for malondialdehyde and superoxide dismutase, and mg/g liver for total protein. Unshared superscript letters between groups indicate significantly different values at $p < 0.0001$.

Table VII. Therapeutic effect *Argyreia speciosa* extracts on oxidative stress markers and protein content in gastric ulcer.

Parameter	Normal control	Ulcer (one week)	Ulcer treated with water extract	Ulcer treated with ethanol extract	Ulcer treated with ranitidine
Glutathione	20.21 ± 4.51 ^b	228.10 ± 118.49 ^a	23.28 ± 5.36 ^b	24.43 ± 5.19 ^b	22.13 ± 5.02 ^b
Malondialdehyde	0.89 ± 0.19 ^{cd}	2.46 ± 0.73 ^a	1.26 ± 0.16 ^{bc}	1.01 ± 0.38 ^{cd}	1.48 ± 0.67 ^b
Superoxide dismutase	9.76 ± 1.77 ^{bcd}	32.30 ± 4.11 ^a	10.80 ± 3.88 ^{bc}	11.83 ± 4.04 ^b	10.09 ± 3.8 ^{bcd}
Total protein	40.36 ± 3.24 ^a	24.40 ± 6.31 ^b	30.08 ± 8.21 ^b	39.17 ± 6.42 ^a	41.11 ± 8.68 ^a

Data are means ± SD of eight rats in each group; data are expressed as $\mu\text{g}/\text{mg}$ protein for glutathione, $\mu\text{mol}/\text{mg}$ protein for malondialdehyde and superoxide dismutase, and mg/g liver for total protein. Unshared superscript letters between groups indicate significantly different values at $p < 0.0001$.

Table VIII. Protective effect of *Argyreia speciosa* extracts on cell organelle marker enzymes in gastric ulcer.

Parameter	Normal control	Ulcer (one hour)	Ulcer protected with water extract	Ulcer protected with ethanol extract	Ulcer protected with ranitidine
Succinate dehydrogenase	4.16 ± 0.89 ^{cd}	9.7 ± 2.15 ^a	6.26 ± 2.36 ^b	6.41 ± 2.19 ^b	5.74 ± 2.44 ^{bc}
Lactate dehydrogenase	57.89 ± 17.94 ^d	233.10 ± 57.5 ^a	138.66 ± 10.3 ^b	154.1 ± 19.58 ^b	92.62 ± 20.34 ^c
Acid phosphatase	83.16 ± 15.37 ^c	148.94 ± 21.32 ^a	130.5 ± 14.49 ^{ab}	91.34 ± 17.49 ^c	126.22 ± 35.90 ^d
Glucose-6-phosphatase	155.09 ± 16.84 ^b	232.7 ± 47.12 ^a	175.18 ± 32.3 ^b	178.7 ± 25.4 ^b	182.22 ± 27.40 ^{ab}
5'-Nucleotidase	180.8 ± 11.6 ^c	442.28 ± 12.4 ^a	189.94 ± 57.3 ^c	223.4 ± 23.14 ^c	337.10 ± 11.24 ^b

Data are means ± SD of eight rats in each group; data are expressed in $\mu\text{mol}/(\text{min mg protein})$. Unshared superscript letters between groups indicate significantly different values at $p < 0.0001$.

significant inhibition in the marker enzymes by 24, 58, 42, 92, and 37%, while ranitidine showed diminution by 19, 34, 38, 93, and 36%, respectively (Table IX).

Effect of A. speciosa extracts on stomach histopathology

Normal gastric mucosa contained crypts of overlying gastric glands lined by mucus secreting cells with rounded nuclei. The lamina propria was intact, infiltrated by scattered lymphocytes, blood vessels, and fibrous tissue (Figs. 1a, d). Treatment with either of the plant extracts had no effect on the histology (Figs. 1b, c, e, f).

After one hour of ethanol delivery, ulcers were visible (Figs. 1g, h). Deep ulcer extending to the basement membrane was seen. The thickened ulcer base showed polymorphous lymphocytic fibrin. The gastric glands were hyperplastic and surrounded the ulcer. The lamina propria contained few lymphocytes and polymorphonuclear leucocytes. One week after ulcer induction, narrow ulcers reaching the basement membrane were seen. Mild hyperplasia of the gastric glands was noticed. The lamina propria was infiltrated by few chronic inflammatory cells (Figs. 1i, j).

A. speciosa extracts produced shallow superficial gastric erosion in the mucosa. The lamina

Table IX. Therapeutic effect of *Argyrea speciosa* extracts on cell organelle marker enzymes in gastric ulcer.

Parameter	Normal control	Ulcer (one week)	Ulcer treated with water ex- tract	Ulcer treated with ethanol extract	Ulcer treated with ranitidine
Succinate dehydrogenase	4.16 ± 0.89 ^{cd}	5.73 ± 0.90 ^a	3.99 ± 1.16 ^b	4.34 ± 1.06 ^b	4.67 ± 2.30 ^{bc}
Lactate dehydrogenase	57.89 ± 17.94 ^c	145.85 ± 26.53 ^a	61.91 ± 16.46 ^c	61.45 ± 8.82 ^c	96.42 ± 12.36 ^b
Acid phosphatase	83.16 ± 15.37 ^b	147.46 ± 19.31 ^a	96.16 ± 25.69 ^b	85.63 ± 16.69 ^b	91.69 ± 24.70 ^b
Glucose-6-phosphatase	155.09 ± 16.84 ^{ab}	185.41 ± 10.62 ^a	177.03 ± 45.32 ^{ab}	142.2 ± 44.71 ^{bc}	167.4 ± 20.90 ^{ab}
5'-Nucleotidase	180.8 ± 11.6 ^{cd}	379.4 ± 91.17 ^a	264.04 ± 28.8 ^b	240.09 ± 27.36 ^{bc}	242.89 ± 13.2 ^{bc}

Data are means ± SD of eight rats in each group; data are expressed in $\mu\text{mol}/(\text{min mg protein})$. Unshared super-script letters between groups indicate significantly different values at $p < 0.0001$.

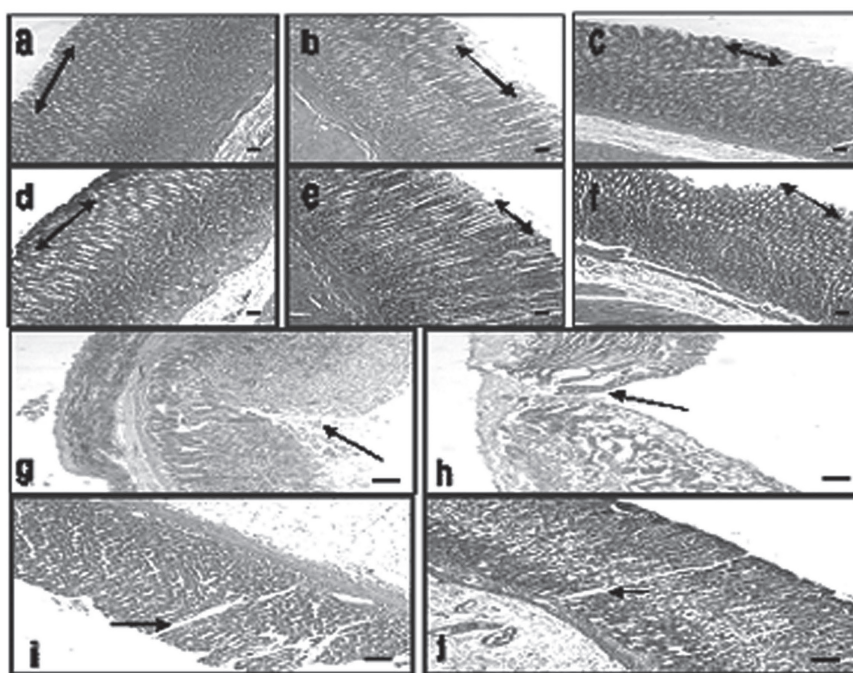


Fig. 1. Photomicrograph of rat gastric mucosa stained with hematoxylin and eosin (H&E) and Masson's trichrome. (a, d) Control, (b, e) treatment with *A. speciosa* water extract and (c, f) ethanol extract, (g, h) ulcerative gastric mucosa after one hour of ethanol induction, (i, j) ulcerative gastric mucosa after one week of ethanol induction. Arrows indicate deep ulcer reaching to the basement membrane and lining the lamina propria. Small arrows indicate narrow ulcers extending to the basement membrane. Double head arrows indicate smooth gastric surface. Bars: 20 μm .

propria showed some infiltration by lymphocytes and polymorphonuclear leucocytes (Figs. 2a, b, d, e). Ranitidine prophylaxis resulted in hyperplastic mucosa with superficial erosions. The lamina propria was widened by some chronic inflammatory cells, lymphocytes, and polymorphonuclear leucocytes (Figs. 2c, f).

After treatment of ulcer with water and ethanol extracts of *A. speciosa* the mucosa was intact with superficial erosions and healed base ulcer membrane. The lamina propria was infiltrated by some chronic inflammatory cells (Figs. 2g, h, j, k). Treatment with ranitidine showed healed mucosa with scanty superficial erosions and hyperplasia of the

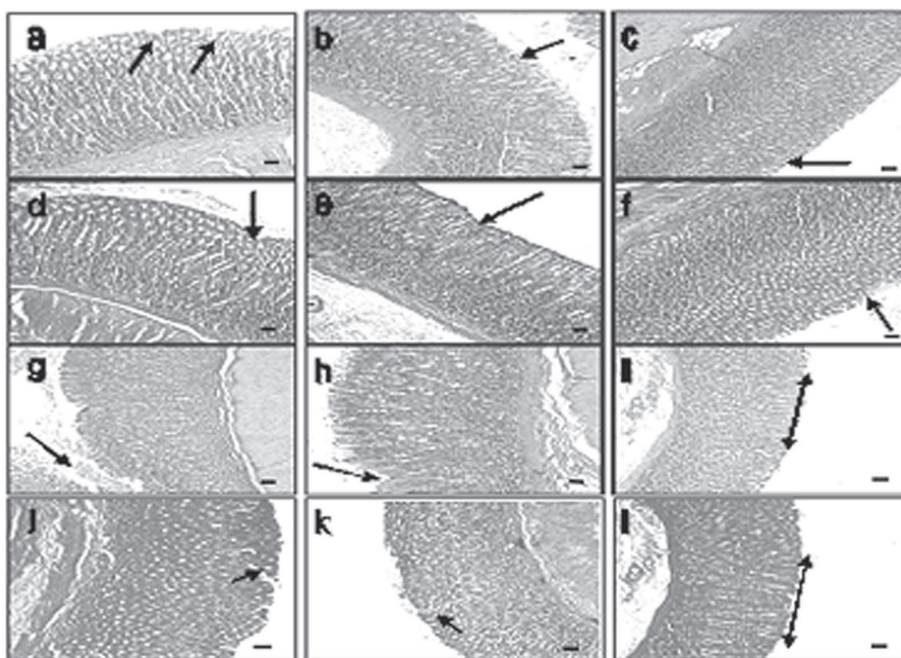


Fig. 2. Photomicrograph of protected and treated ulcerative gastric mucosa stained with hematoxylin and eosin (H&E) and Masson's trichrome. (a, d) Ulcerative mucosa protected by *A. speciosa* water extract, (b, e) ethanol extract and (c, f) ranitidine. (g, j) Ulcerative mucosa treated by *A. speciosa* water extract, (h, k) ethanol extract and (i, l) ranitidine. Arrows indicate shallow erosions. Small arrows indicate superficial erosions with healed base ulcer membrane. Double head arrows indicate smooth gastric surface. Bars: 20 μ m.

gastric glands. The lamina propria was infiltrated by some chronic inflammatory cells (Figs. 2i, l).

Discussion

Intake of ethanol is always associated with excess generation of reactive oxygen species that can lead to mucosal damage (Repetto and Llesuy, 2002) which is associated with a decrease in the mucus content and progressive lesion areas, as was observed in this study. Moraes *et al.* (2008) pointed out that mucus is an important protective factor for the gastric mucosa, capable of acting as an antioxidant agent and reducing mucosal damage. Moreover, the protective properties of the mucus barrier depend not only on the gel structure but also on the thickness of the layer covering the mucosal surface (Moraes *et al.*, 2008).

In the present study gastric ulceration by ethanol also resulted in an increase in gastric volume, total acidity, and prostaglandin E_2 content. Hydrochloric acid released from the surface of epithelial cells plays a role in the mucosal defensive mechanisms, and prostaglandins are involved

in the regulation of a variety of gastrointestinal functions, including blood flow, acid, mucus, and hydrochloric acid secretion (Gracioso *et al.*, 2002).

The present study revealed a significant increase in malondialdehyde, superoxide dismutase activity, and glutathione. High gastric mucosal malondialdehyde levels in patients with peptic ulcer and gastritis are thought to reflect free radical-mediated gastric mucosal damage (Demir *et al.*, 2003). In agreement with our results Tandon *et al.* (2004) and Shetty *et al.* (2008) observed a significant elevation of superoxide dismutase in gastric ulcer. Stress causes stimulation of the stomach leading to local hypoxia or actual "ischemia". The ischemic condition causes an increase in the level of H_2O_2 by the action of superoxide dismutase, which, in conjunction with O_2 , generates OH^\cdot . Thus, hydroxyl radicals oxidize important cellular constituents such as structural and functional proteins and membrane lipids. Lipid peroxidation causes loss of membrane fluidity, impairs ion transport and membrane integrity, and finally loss of cellular functions (Tandon *et al.*, 2004).

In contrast to many investigations, glutathione (GSH) in the present study was significantly elevated in the gastric ulcer group. The GSH status is dependent on the relative activity of many enzymes (Malmezzat *et al.*, 2000). The increased activity of enzymes involved in GSH synthesis (γ -glutamyl-cysteine synthetase) and GSH reduction (glutathione reductase) can lead to an increased GSH concentration. Conversely, increased activities of GSH peroxidase and GSH transferase, the enzyme responsible for the conjugation of toxic compounds with GSH, lead to decreased GSH concentration. This is in accordance with the observed decrease of GSH peroxidase and GSH transferase in indomethacin-induced gastric ulcer (Koc *et al.*, 2008), ethanol-induced mucosal injury (Rao *et al.*, 2004), and in stress ulcer (Liu *et al.*, 2011) which may give additional support to our results.

An increase in total protein content can be considered a useful index of the severity of cellular dysfunction in many diseases (Sharma and Shukla, 2011) as clearly shown in our studies. Ethanol treatment caused considerable elevation of mucosal enzyme activities. This is in parallel with the observation of Serebrianskaia *et al.* (1992) who showed marked activation of succinate dehydrogenase. SDH elevation was attributed to the increase of mitochondrial permeability and damage (Hirokawa *et al.*, 1998; Ishihara *et al.*, 2010). Brzozowski *et al.* (2005) observed the same phenomenon in case of lactate dehydrogenase. Erosive gastropathy and gastroduodenal ulceration cause damage to lysosomal membranes and the release of autoaggressive enzymes (Rodrigues *et al.*, 1998). Gastric ulcer mucosa is also mediated via endoplasmic reticulum and plasma membrane

stress, respectively, which leads to enzyme leakage and damage to their membranes (Ozeki *et al.*, 1987; Ishihara *et al.*, 2010). This parallels the increase in AP, G-6-Pase, and 5'NT activities which we observed.

Ulcer healing is a complex process and entails several distinct repair mechanisms. An increase in mucus production usually assists the healing process. Further, prostaglandins also stimulate mucus and cellular growth/repair and regulate the pH value at the gastric surface (Banerjee *et al.*, 2008). These observations are in line with the elevation of the prostaglandin E₂ level and the reduction in mucus production, gastric volume, acidity, lesion counts, collagen deposition, antioxidant levels, and mucosal enzymes by the therapeutic actions of the *A. speciosa* extracts, in particular the ethanol extract, found in our study. Therefore, the results reinforced the presence of antisecretory, antioxidant, and antiulcerogenic effects of *A. speciosa* extracts which was confirmed by the observed histopathological changes of the gastric mucosa.

In conclusion, *A. speciosa* extracts succeeded in protecting and treating gastric ulcer induced by ethanol in rats. The water extract produced a more potent protective effect, while the ethanol extract was more therapeutically active. Further studies are needed to identify the compounds responsible for the pharmacological effects and for clinical and pharmaceutical applications.

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