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Effect of methanolic extract of *Terminalia arjuna* against *Helicobacter pylori* 26695 lipopolysaccharide-induced gastric ulcer in rats

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Abstract

Helicobacter pylori lipopolysaccharide (HP-LPS) is a potent virulence factor in the causation of gastric ulcer and gastritis. *H. pylori*-induced gastric pathology is prevalent throughout the world. Herbal medicines are attracting attention because of their traditional values, popularity and belief, as well as for their advantages such as less toxicity, affordability and medicinal value. The present study aimed to evaluate the anti-ulcer effect of a methanolic extract of *Terminalia arjuna* (TA) against HP-LPS-induced gastric damage in rats. Ulcers were induced with HP-LPS (50 μg per animal) administered orally daily for 3 days. The efficacy of TA on gastric secretory parameters such as volume of gastric juice, pH, free and total acidity, pepsin concentration, and the cytoprotective parameters such as protein-bound carbohydrate complexes in gastric juice and gastric mucosa was assessed. The protective effect of TA was also confirmed by histopathological examination of gastric mucosa. HP-LPS-induced alterations in gastric secretory parameters were altered favourably in rats treated with TA, suggesting that TA has an anti-secretory role. Furthermore, HP-LPS-induced impairments in gastric defence factors were also prevented by treatment with TA. These results suggest that the severe cellular damage and pathological changes caused by HP-LPS are mitigated by TA; these effects are comparable with those of sucralfate. The anti-ulcer effect of TA may reflect its ability to combat factors that damage the gastric mucosa, and to protect the mucosal defensive factors.

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Introduction

Helicobacter pylori was first isolated in human biopsies by Warren and Marshall in 1983, and is now considered to be a major cause of gastric ulcers, duodenal ulcers and gastritis. *H. pylori* infection is also reported to be an important cause of ulcer relapse (Parmar & Desai 1994). Approximately 40% and 80% of individuals in developed and developing countries, respectively, are infected before adulthood, making *H. pylori* one of the most common bacterial infections in humans (Mitchell 1993).

The factors implicated in the virulent actions of *H. pylori* against mucosal integrity include CagA and VacA cytotoxins, which are capable of inducing the release of pro-inflammatory cytokines, excessive production of ammonia, known for its strong toxic effect on the gastric epithelium, and the impairment of feedback inhibition of gastrin release by somatostatin (Piotrowski et al 1995; Konturek et al 1999). Cell wall lipopolysaccharide (LPS) of *H. pylori* is also involved in its virulent actions (Slomiany et al 1997, 2000). *H. pylori* LPS (HP-LPS) elicits acute mucosal inflammatory responses within 2 days, accompanied by a massive epithelial cell apoptosis, increase in mucosal expression of endothelin-1, stimulation of tumour necrosis factor α, increase in nitric oxide (NO) synthase (NOS)-2, decrease in constitutive NOS (cNOS) activity (Slomiany et al 2000), excessive nitric oxide generation, apoptotic caspase activation and a marked enhancement in gastric epithelial cell apoptosis (Slomiany et al 1997, 1998a, 1998b, 1999). Other pathogenic effects of HP-LPS relevant to the progression of the mucosal inflammatory process involve stimulation of nuclear factor (NF)-κB nuclear translocation, disturbances in mitogen-activated protein kinase cascades, and a marked up-regulation in gastric mucosal level of endothelin-1 (Slomiany et al 1999; Gupta et al 2001a; Slomiany & Slomiany 2001).

Eradication of *H. pylori* seems to cure both infection and ulcers. Successful treatment therefore leads to the resolution of gastritis and reduces the likelihood of ulcer recurrence (Germano et al 1998). The combination of a proton pump inhibitor (e.g. omeprazole) and antibiotics (i.e. ampicillin, amoxicillin, ofloxacin or tetracycline) is curative in up to 90% of patients (Korman et al 1997).

Several plants are used for the treatment of gastric ailments, including stomach ache and ulcers (Sezik et al 1997; Pertino et al 2006; Banerjee et al 2007; Lapa Fda et al 2007; Lemos et al 2007; Rao & Vijayakumar 2007). *Terminalia arjuna* (TA) Wight and Arnot, known locally as Kumbuk, has a long history of medicinal uses in India (Dwivedi & Udupa 1989), including cancer treatment (Hartwell 1982). Previous attempts to isolate medicinal agents from this tree have yielded a variety of relatively simple compounds (Prabhakar & Kumar 1988) such as flavonoids (Ramanathan et al 1992).

The bark is astringent, sweet, acrid and cooling. It has aphrodisiac, demulcent, cardiotonic, styptic, antidysenteric, expectorant, alexiteric, lithontriptic and tonic activities and acts as a urinary astringent. It is useful in fractures, ulcers, urethrorrhoea, leucorrhoea, diabetes, vitiated conditions of pitta, anaemia, cardiopathy, hyperhydrosis, fatigue, asthma, bronchitis, tumours, otalgia, dysentery, inflammation, haemorrhage (internal and external), cirrhosis and hypertension (Varier 1997). It is reputed to be cardiotonic and to also possess hypotensive and hypolipidaemic activities (Vaidyaratnam 1994). Its use in wound healing has been mentioned by Sushruta *in Sushruta Samhita* (Ghanekar 1936). The bark powder is reported to exert hypocholesterolaemic and antioxidant effect in humans (Gupta et al 2001b).

The chemical constituents isolated from the plant are mainly tannins and various oleanane triterpenoids. Tannins of the leaves had been reported to have anticancer activity (Kandil & Nassar 1998) and those from the bark possessed antimutagenic effects (Kaur et al 2001).

TA bark contains high amounts of fibre, sugar, tannin, beta-sitosterol, calcium carbonate, sodium, and aglycones arjunine, arjunolic acid and arjunoids I, II, III and IV (Vaidya 1994). It also contains antioxidant polyphenolics and the flavonoids quercetin, kaempferol, pelargonidin and lutiolin (Nair et al 1996). TA has been reported to have antibacterial activity against *Escherichia coli, Klebsiella aerogenes, Proteus vulgaris* and *Pseudomonas aerogenes* (Gram-negative bacteria) (Perumal Samy et al 1998). An active principle from TA bark – the glycoside arjunaphthanoloside – showed potent antioxidant activity and inhibited NO production in LPS-stimulated rat peritoneal macrophages (Ali et al 2003a). Terminoside A, a new oleanane-type triterpene, potently inhibits NO production and decreases levels of inducible NOS (iNOS) in LPS-stimulated macrophages (Ali et al 2003b). Methanolic extracts of TA provide strong antimicrobial activity against *Salmonella typhi* (Rani & Khullar 2004).

Preliminary studies on gastric ulcers induced by 80% ethanol, diclofenac sodium and dexamethasone have indicated the protective effect of the methanolic extract of TA and revealed the presence of various phytochemical constituents such as steroids, triterpenoids, phenols, tannins, flavonoids, alkaloids and glycosides (Devi et al 2007a,b). The aim of the present study was to assess the gastroprotective effect of methanolic extract of TA against HP-LPS-induced gastric ulcer.

Materials and Methods

Plant material

The bark of TA was obtained from local drug market in Chennai, Tamil Nadu, India and was authenticated by Dr Sasikala Ethirajulu, Research officer (Botany) at the Central Research Institute for Siddha (CRIS), Arumbakkam, Chennai, and Dr S. Amerjothy, Botanist at the Department of Post-graduate and Research Studies, Presidency College, Chennai, and was compared with the voucher specimen no. 51, deposited in the herbarium of Presidency College, Chennai.

Preparation of bark extract

TA bark (2 kg) was cut into small pieces and shade dried for 7 days. The dried material was powdered and extracted with methanol using Soxhlet apparatus. The methanol extract was filtered and concentrated to a dry mass using vacuum distillation, and completely evaporated to dryness. Shiny dark-brownish-red crystals were obtained; the yield was 24%. Qualitative chemical analysis of the extract was performed as described by Kokate and colleagues (1996).

Animals

Male Sprague–Dawley rats weighing 150–200 g were obtained from the National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India. They were acclimatized to animal house conditions, were fed commercial pelleted rat chow (Hindustan Lever Ltd, Bangalore, India) and had free access to water. This study was conducted according to the ethical norms approved by Animal Ethics Committee of our institution (IAEC No. 01/038/07).

H. pylori lipopolysaccharide

A preparation of HP-LPS was a kind gift from Professor Manfred Kist, Institut für Medizinische Mikrobiologie und Hygiene, Freiburg, Germany. It was prepared from 26695 strain of *H. pylori* by the conventional method used for the preparation of LPS from Gram-negative bacteria. *H. pylori* 26695 grown in Brucella broth with 5% fetal calf serum was pelleted by centrifugation, washed twice with 0.9% NaCl, heat inactivated for 2 h in steam and washed twice with 0.9% NaCl. The pellet was then suspended in 1 mL 0.9% NaCl and stored at 4°C. It was lyophilized before use.

The effective dose of HP-LPS that induced gastric ulceration was assessed by orally administering HP-LPS dissolved in saline at doses of 10, 30, 50 or 70 μg per day for three consecutive days. Mild lesions were observed in the rats given 10 and 30 μg; severe gastric lesions were produced at the higher doses. A daily dose of 50 μg for 3 days was chosen to induce gastric ulcers.

Dose determination for *T. arjuna* against HP-LPS-induced gastric ulcers

Male Sprague–Dawley rats were divided into six groups of six rats. Gastric ulcers were induced in all groups by 3 days' treatment with HP-LPS 50 μg per day. Groups 2–5 were treated with different doses of methanolic extract of TA for 7 days before ulcer induction with HP-LPS and for the 4 days after ulcer induction. The doses of TA tested were 100, 200, 300 and 400 mg kg^{-1} body weight. TA was dissolved in water and administered orally. Group 6 rats served as reference controls and were pretreated with sucralfate (100 mg kg^{-1} dissolved in water and administered orally) for 7 days (Slomiany et al 1998b), induced with HP-LPS for 3 days (as above), followed by 4 days' further treatment with sucralfate.

Pyloric ligation

After the experimental period, rats were subjected to pyloric ligation under ether anaesthesia for the collection of gastric juice. Pyloric ligation was done by the method of Shay Komarov et al (1945). Under light anaesthesia, the abdomen was opened by a small midline incision below the xiphoid process; the pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was replaced and the abdominal wall was closed by interrupted sutures. The animals were killed 4 h after pylorus ligation. The stomach was dissected out after tying the oesophageal end and cut open. The gastric juice was then drained into a small beaker, centrifuged and the volume noted and pH measured.

The effective dose of TA was assessed by analysing the ulcer index and antisecretory parameters – volume of gastric juice, pH, free and total acidity and pepsin concentration.

Estimation of free and total acidity

This was carried out by the method of Card & Marks (1960) using gastric juice. Gastric juice (1 mL) was pipetted into a 100 mL conical flask and diluted with 9 mL distilled water. Two or three drops of Toepfer's reagent was then added and titrated with 0.01 N sodium hydroxide until all traces of red colour disappeared and the colour of the solution was yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein were then added and the titration continued until a definite red tinge appeared; the volume of alkali added was noted. The volume corresponds to total acidity. Acidity was calculated using the formula: acidity = (volume of NaOH × normality of NaOH × 100)/0.1. Acid output = volume × concentration of acid.

Estimation of pepsin concentration

Pepsin was measured according to the method of Anson (1938) using haemoglobin as substrate. The substrate (0.5 mL) was added to 0.01 mL of the homogenate and incubated at 37°C for 10 min; the enzyme reaction was terminated after 10 min by the addition of 1.0 mL 12.5% trichloroacetic acid. Tubes were shaken well, allowed to stand for 3 min and centrifuged at 2500 *g* for 15 min. A 1 mL aliquot of the supernatant was then combined with 1.0 mL water, followed by 2.0 mL 1N sodium hydroxide and 0.5 mL diluted Folin's–Ciocalteau reagent. The tubes were shaken well and the absorbance of the solution was measured after 10 min at 650 nm.

Lesion index

Lesion index was measured as the sum of the length (in mm) of each lesion according to Okabe et al (1976). The effective dose of TA extract was fixed at 300 mg kg^{-1} body weight, as this provides beneficial outcome. Its efficacy was further evaluated using this dose.

Assessment of anti-ulcer effect

Male Sprague–Dawley rats were divided into four groups of six animals. Group I were untreated normal (control) rats. Group II had ulcers induced with HP-LPS, as described above. Group III received treatment with TA before and after ulcer induction, as described above. Group IV rats were positive controls and were treated with sucralfate before and after ulcer induction, as described above. After the experimental period, the rats were killed under anaesthesia. Gastric juice and gastric mucosal tissues were collected and used for biochemical analysis.

Estimation of protein concentration

Protein concentration was estimated by the method of Lowry et al (1951). To 0.1 mL homogenate was added 0.9 mL water and 4.5 mL alkaline copper reagent, and the samples maintained at room temperature for 10 min. Then 0.5 ml Folin's reagent was added and the colour developed after 20 min was quantified spectrophotometrically at 640 nm.

Estimation of glycoconjugates

For the estimation of hexose, hexosamine and sialic acid, 200 mg tissue was weighed and the lipid extracted using a chloroform–methanol mixture in a Potter–Elvehjam homogenizer with a Teflon pestle. The extraction was repeated three times with fresh aliquots of the solvent mixture. The lipid extract was filtered through a fat-free Whatman No. 41 filter paper. The tissue retained on the filter paper was dried, and used for the estimation of hexose, hexosamine and sialic acid.

Estimation of hexose

A known amount of defatted tissue was hydrolysed with 1.0 mL 2N hydrochloric acid for 4 h at 90°C. The content was neutralized with NaOH and aliquots were used for the estimation of hexose by the method of Niebes (1972). To 0.5 mL of the neutralized sample was added 0.5 mL distilled water and 7.0 mL Orcinol reagent. The samples were then placed in a cold water bath. The contents were mixed well and the tubes were then heated for 15 min at 80°C. The colour intensity that developed in the dark after cooling the tubes was quantified spectrophotometrically at 540 nm.

Estimation of hexosamine

A known amount of defatted tissue was hydrolysed with 1.0 mL 2N hydrochloric acid for 4 h at 90°C. The contents were neutralized with NaOH and aliquots were used for the estimation of hexosamine by the method of Wagner (1979) by the colour reaction with Ehrlich's reagent. Water was added to a known amount of the neutralized sample to make the volume up to 1.0 mL; the blank contained 1.0 mL water. Acetyl acetone reagent (6 mL) was added to each tube and the tubes were locked and heated in a boiling water bath for 30 min. They were then cooled and 2.0 mL Ehrlich's reagent was added to each. The tubes were shaken well and the colour developed was quantified spectrophotometrically at 540 nm.

Estimation of sialic acid

A known amount of the defatted tissue was hydrolysed with 0.5 mL 0.1N sulfuric acid for 60–90 min at 90°C. The hydrolysed extract was used for the analysis of sialic acid by the method of Warren (1959). The hydrolysed sample and the blank contained 0.2 mL 0.1N sulphuric acid to which was added 0.1 mL 0.2N sodium metaperiodate solution; samples were then left at room temperature for 20 min. The arsenite reagent (1 mL) was then added, followed by 3.0 mL 0.6% thiobarbituric acid reagent. Tubes were corked and heated in a boiling water bath for 15 min, then cooled. The pink colour that developed was extracted with 4.0 mL acidified butanol. The colour in the organic phase was quantified spectrophotometrically at 540 nm.

Estimation of fucose

Fucose was estimated by the method of Dische & Shettles (1948). The glycoprotein precipitated from 0.05 mL homogenate was dissolved in 1.0 mL 0.1N NaOH; 4.5 mL sulfuric acid reagent was then added, keeping the tubes cooled in water. The tubes were heated in a boiling water bath for 3 min, 0.1 mL 3% cysteine hydrochloride was added and the samples mixed well. The tubes were left in the dark at room temperature for 75 min. The absorbance was quantified spectrophotometrically at 420 nm.

Histological studies

For histological examination, gastric mucosal tissues were excised and rinsed with ice-cold 0.9% saline to remove blood and debris adhering to tissues. The tissues were then fixed in 10% formalin for 24 h. The fixative was removed by washing through running tap water overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate and embedded in paraffin wax. Sections (5 μm thick) were cut and stained with haematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under a light microscope.

Statistical analysis

Values are presented as mean ± s.d. for six rats in each group. Differences between mean values was determined by one-way analysis of variance (ANOVA) followed by Dunnett's T3 multiple comparison test, using the SPSS 10.0 software (Chicago, IL, USA). Values of $P < 0.05$ were considered significant.

Results

Figure 1 shows the dose determination for induction of gastric ulcer with HP-LPS. Mild lesions were observed at 10 and 30 μg per day; severe gastric lesions were produced at 50 and 70 μg per day. A dose of 50 μg per day was then chosen for inducing gastric lesions.

Table 1 shows the effect of different doses of TA against HP-LPS-induced gastric ulcers determined from ulcer index and secretory parameters such as volume of gastric juice, free acidity, total acidity, acid output and pepsin activity. There was a significant dose-dependent decrease in the ulcer index of rats treated with TA (100, 200, 300 and 400 mg kg^{-1}). The ulcer index decreased significantly with all doses of TA compared with untreated ulcerated rats ($P < 0.05$ for 100 mg kg^{-1}; $P < 0.001$ for 200, 300 and 400 mg kg^{-1}). The ulcer index showed that 300 mg kg^{-1} was the minimum effective dose of TA that offered significant protection, comparable to that seen with the sucralfate-treated group.

TA significantly reduced volume of gastric juice, free acidity, total acidity, acid output and pepsin concentration in a dose-dependent manner compared with control ulcerated rats. As above, 300 mg kg^{-1} TA offered desirable results, comparable with those obtained with sucralfate. This dose of TA was therefore chosen as the optimal dose against HP-LPS-induced gastric ulcer and was used for further evaluation of the gastroprotective activity of TA.

Table 2 shows the levels of protein-bound carbohydrate complexes in gastric juice and mucosa. Compared with control rats (no ulcers), control ulcerated rats showed significant decreases ($P < 0.001$) in hexose, hexosamine, sialic acid, fucose, total carbohydrate and total carbohydrate:protein (TC:P) ratio (a marker of mucin secretion) in both gastric juice and mucosa, with a subsequent increase ($P < 0.001$) in gastric juice protein levels and a decrease in gastric mucosal protein

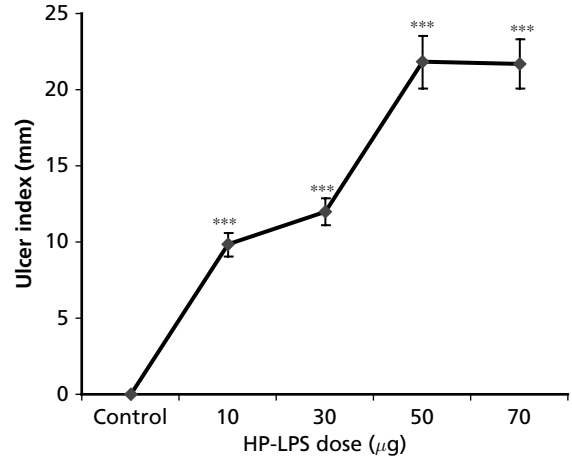

Figure 1 Determination of the dose of HP-LPS for induction of gastric lesions in rats. Data are mean ± s.d. for six animals in each group. ***$P < 0.001$ control vs all groups.

Table 1 Determination of the effective dose of methanolic extract of *Terminalia arjuna* (TA) in rats with HP-LPS-induced ulcers, using ulcer index and anti-secretory parameters

Treatment	Ulcer index	Vol. gastric juice	Free acidity	Total acidity	Acid output	Pepsin concn
Control	$20.50 \pm 1.87^{b\ddagger}$	$3.15 \pm 0.24^{b\ddagger}$	$53.67 \pm 2.73^{b\ddagger}$	$88.50 \pm 3.78^{b\ddagger}$	$278.68 \pm 22.63^{b\ddagger}$	$243.83 \pm 19.33^{b\ddagger}$
TA 100 mg	$16.00 \pm 1.41^{a*,\ b\ddagger}$	$2.93 \pm 0.16^{aNS,\ b\ddagger}$	$50.67 \pm 2.16^{aNS,\ b\ddagger}$	$79.33 \pm 4.72^{a*,\ b\ddagger}$	$232.80 \pm 20.28^{a*,\ b\ddagger}$	$199.00 \pm 16.43^{a*,\ b*}$
TA 200 mg	$8.08 \pm 1.80^{a\ddagger,\ b\dagger}$	$2.30 \pm 0.24^{a\dagger,\ b\dagger}$	$41.50 \pm 2.43^{a\ddagger,\ b\dagger}$	$73.17 \pm 3.31^{a\ddagger,\ b\ddagger}$	$168.90 \pm 24.49^{a\ddagger,\ b\dagger}$	$187.83 \pm 17.70^{a\dagger,\ bNS}$
TA 300 mg	$1.57 \pm 0.10^{a\ddagger,\ b\ddagger}$	$1.73 \pm 0.14^{a\ddagger,bNS}$	$31.50 \pm 2.07^{a\ddagger,\ bNS}$	$63.17 \pm 4.92^{a\ddagger,\ bNS}$	$109.20 \pm 8.59^{a\ddagger,\ bNS}$	$166.00 \pm 12.02^{a\ddagger,bNS}$
TA 400 mg	$1.58 \pm 0.16^{a\ddagger,\ b\ddagger}$	$1.70 \pm 0.14^{a\ddagger,bNS}$	$33.17 \pm 1.47^{a\ddagger,bNS}$	$63.67 \pm 3.01^{a\ddagger,bNS}$	$108.02 \pm 7.12^{a\ddagger,\ bNS}$	$167.50 \pm 11.71^{a\ddagger,bNS}$
Sucralfate 100 mg	$5.50 \pm 0.45^{a\ddagger}$	$1.58 \pm 0.15^{a\ddagger}$	$32.50 \pm 3.02^{a\ddagger}$	$59.83 \pm 2.93^{a\ddagger}$	$94.75 \pm 10.38^{a\ddagger}$	$160.00 \pm 13.04^{a\ddagger}$
F value	343.75	81.68	102.11	49.95	118.23	25.17

Data are mean ± s.d. for six animals in each group. Units are: ulcer index (mm); volume of gastric juice (ml $100\,g^{-1}\,4\,h^{-1}$); free acidity (mEq L^{-1} $100\,g^{-1}$); total acidity (mEq^{-1} L $100\,g^{-1}$); acid output (mEq $100\,g^{-1}\,4\,h^{-1}$); pepsin activity (μmol tyrosine liberated $mL)^{-1}$. [a]All groups vs HP-LPS controls; [b]All groups vs rats treated with sucralfate. $*P < 0.05$; $^{\dagger}P < 0.01$; $^{\ddagger}P < 0.001$; NS, not significant. The F value signifies the differences between means and represents significant alterations in a particular parameter.

Table 2 Effect of methanolic extract of *Terminalia arjuna* (TA) on the levels of protein-bound carbohydrate complexes in gastric juice and gastric mucosa of rats with HP-LPS-induced ulcers

Parameter	Group I (control)	Group II (HP-LPS)	Group III (HP-LPS + TA)	Group IV (HP-LPS + SUC)	F value
Protein-bound carbohydrate complexes in gastric juice (in $\mu g\ mL^{-1}$ gastric juice)					
Hexose	405.14 ± 25.64	$277.29 \pm 28.23^{\ddagger}$	$403.66 \pm 22.09^{a\ddagger,bNS}$	$395.68 \pm 23.26^{\ddagger}$	37.45
Hexosamine	174.58 ± 15.58	$118.03 \pm 11.85^{\ddagger}$	$182.11 \pm 14.48^{a\ddagger,bNS}$	$164.5 \pm 14.03^{\ddagger}$	25.15
Sialic acid	40.38 ± 3.17	$27.94 \pm 1.98^{\ddagger}$	$40.17 \pm 4.04^{a\dagger,bNS}$	$36.38 \pm 3.72^{\dagger}$	18.42
Fucose	45.02 ± 2.22	$31.34 \pm 2.33^{\ddagger}$	$44.09 \pm 2.82^{a\ddagger,bNS}$	$42.64 \pm 2.23^{\ddagger}$	41.74
TC	665.12 ± 16.01	$454.59 \pm 29.95^{\ddagger}$	$670.03 \pm 24.87^{a\ddagger,bNS}$	$639.21 \pm 27.61^{\ddagger}$	99.81
Protein (P)	272.46 ± 12.65	$392.11 \pm 25.25^{\ddagger}$	$282.53 \pm 13.2^{a\ddagger,bNS}$	$273.86 \pm 22.57^{\ddagger}$	54.66
TC:P ratio	2.45 ± 0.12	$1.16 \pm 0.13^{\ddagger}$	$2.46 \pm 0.23^{a\ddagger,bNS}$	$2.26 \pm 0.1^{\ddagger}$	98.52
Protein-bound carbohydrate complexes in gastric mucosa (in mg g^{-1} gastric mucosa)					
Hexose	14.35 ± 0.95	$7.9 \pm 0.47^{\ddagger}$	$15.82 \pm 1.28^{a\ddagger,b*}$	$13.05 \pm 1.09^{\ddagger}$	71.8
Hexosamine	8.78 ± 0.42	$4.53 \pm 0.22^{\ddagger}$	$8.95 \pm 0.31^{a\ddagger,bNS}$	$8.62 \pm 0.4^{\ddagger}$	228.13
Sialic acid	1.78 ± 0.05	$0.81 \pm 0.07^{\ddagger}$	$1.84 \pm 0.18^{a\ddagger,bNS}$	$1.71 \pm 0.11^{\ddagger}$	111.25
Fucose	3.45 ± 0.3	$2.22 \pm 0.15^{\ddagger}$	$3.58 \pm 0.23^{a\ddagger,bNS}$	$3.42 \pm 3.42^{\ddagger}$	35.33
TC	28.37 ± 1.11	$15.46 \pm 0.6^{\ddagger}$	$30.19 \pm 1.4^{a\ddagger,b*}$	$26.79 \pm 1.54^{\ddagger}$	178.75
P	22.37 ± 1.28	$17.0 \pm 0.96^{\ddagger}$	$24.07 \pm 1.83^{a\ddagger,bNS}$	$23.25 \pm 1.53^{\ddagger}$	29.66
TC:P ratio	1.27 ± 0.08	$0.91 \pm 0.07^{\ddagger}$	$1.3 \pm 0.09^{a*,\ bNS}$	$1.12 \pm 0.11^{\ddagger}$	24.59

Values are expressed as mean ± s.d. for six animals in each group. Groups were compared as follows: I vs II; II vs III; II vs IV; III vs IV. [a]vs group II; [b]vs group IV. $*P < 0.05$; $^{\dagger}P < 0.01$; $^{\ddagger}P < 0.001$; NS, not significant. The F value signifies the differences between means and represents significant alterations in a particular parameter. SUC, sucralfate; TC, total carbohydrate.

levels. Compared with the control ulcerated rats, rats treated with TA showed a highly significant increase ($P < 0.001$) in hexose, hexosamine, sialic acid, fucose, total carbohydrate and TC:P ratio in both gastric juice and mucosa, with a concomitant decrease ($P < 0.001$) in gastric juice protein levels and an increase in gastric mucosal protein levels. Rats treated with sucralfate also registered a highly significant increase ($P < 0.001$) in hexose, hexosamine, sialic acid, fucose, total carbohydrate and TC:P ratio, with a concomitant decrease ($P < 0.001$) in protein levels compared with the untreated ulcerated rats. Gastric juice parameters were similar in rats treated with TA or sucralfate. However, non-significant alterations in these parameters in gastric mucosa were noted in TA-treated rats compared with sucralfate-treated rats, except for hexose ($P < 0.05$) and total carbohydrate levels ($P < 0.05$).

Figure 2 shows macroscopic images of stomachs from each experimental group. Stomachs from untreated control rats had a normal appearance (Figure 2A). HP-LPS administration caused severe gastric damage, visible from the outside of the stomach as thick reddish-black lines; gastric lesions were found in the mucosa, consisting of elongated bands, parallel to the long axis of the stomach (Figure 2B). Stomachs from rats treated with TA or sucralfate had a normal macroscopic appearance (Figures 2C and 2D, respectively).

Figure 3 shows the histopathological appearance of gastric mucosa from control and experimental rats. The gastric mucosa of the control rats shows normal morphology, with perfect intact structures and regular epithelial lining (Figure 3A). The gastric mucosa of rats treated with HP-LPS shows ulceration, denuded mucosa, inflammation and congested vessels

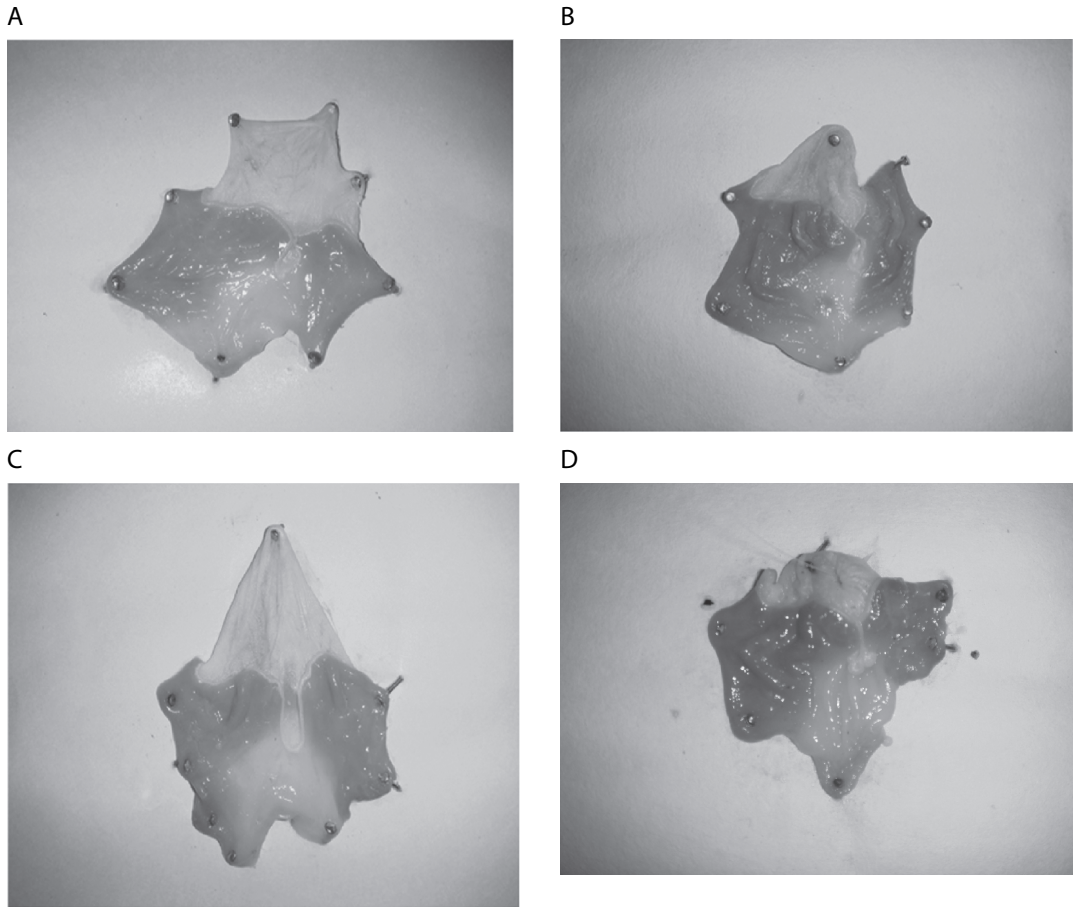

Figure 2 Macroscopic examination of stomachs from experimental rats. Stomachs from control rats had normal appearance (A); stomachs from rats with HP-LPS-induced ulcers showed severe gastric damage, visible from the outside of the stomach as thick reddish-black lines, and gastric lesions as elongated bands, parallel to the long axis of the stomach (B). Stomachs from rats with HP-LPS-induced ulcers treated with TA or sucralfate had normal macroscopic appearance (C and D, respectively).

(Figure 3B). The gastric mucosa of rats treated with TA shows no ulcers, instead exhibiting regenerating epithelium, with mild oedema in the submucosa and mild inflammation (Figure 3C). The gastric mucosa of rats treated with sucralfate shows slight regeneration of superficial epithelium and inflammation of the submucosa (Figure 3D).

Discussion

In the present study, a reduction in ulcer index clearly points towards a beneficial effect of TA against HP-LPS-induced gastric ulceration. Patients with high gastric acid production typically develop antrum-predominant gastritis and are at increased risk for duodenal ulcer. In contrast, patients with low gastric acid secretion frequently develop pangastritis, which may progress to chronic atrophic gastritis and carcinoma (Gschwantler & Dragosics 2000).

Maximal acid output is significantly increased in patients with *H. pylori* infection and duodenal ulceration, indicating a role for acid in the pathogenesis of mucosal ulceration. Low pH has been shown to enhance *H. pylori*-induced NF-κB nuclear binding (O'Toole et al 2005). *H. pylori* increases basal gastrin levels, basal acid output, meal-stimulated maximal acid output and 24 h intragastric acidity. The effects on gastric acid production depend on the distribution of gastritis in the stomach (Loffeld & van der Hulst 2002).

Sucralfate markedly suppresses *H. pylori* infection and the accompanying hypersecretion of acid. These effects are likely to be important mechanisms by which the drug promotes healing of duodenal ulcers (Banerjee et al 1996).

H. pylori LPS can stimulate acid secretion, which may contribute to mucosal damage of the stomach and duodenum. Another mechanism by which *H. pylori* LPS can stimulate acid secretion at the glandular level derives from data showing that it can increase histamine release from rat enterochromaffin-like cells (Kidd et al 1997). LPS purified from the known gastric pathogen *H. pylori* has this secretory property greatly impaired and, depending on the strain of the bacterium, directly stimulates both pepsinogen (Moran et al 1998; Young et al 1992) and acid secretion, potentially contributing to gastrointestinal pathology. LPS from *H. pylori* SS1 stimulates acid secretion, whereas other LPS preparations do not, which is probably related to differences in the molecular

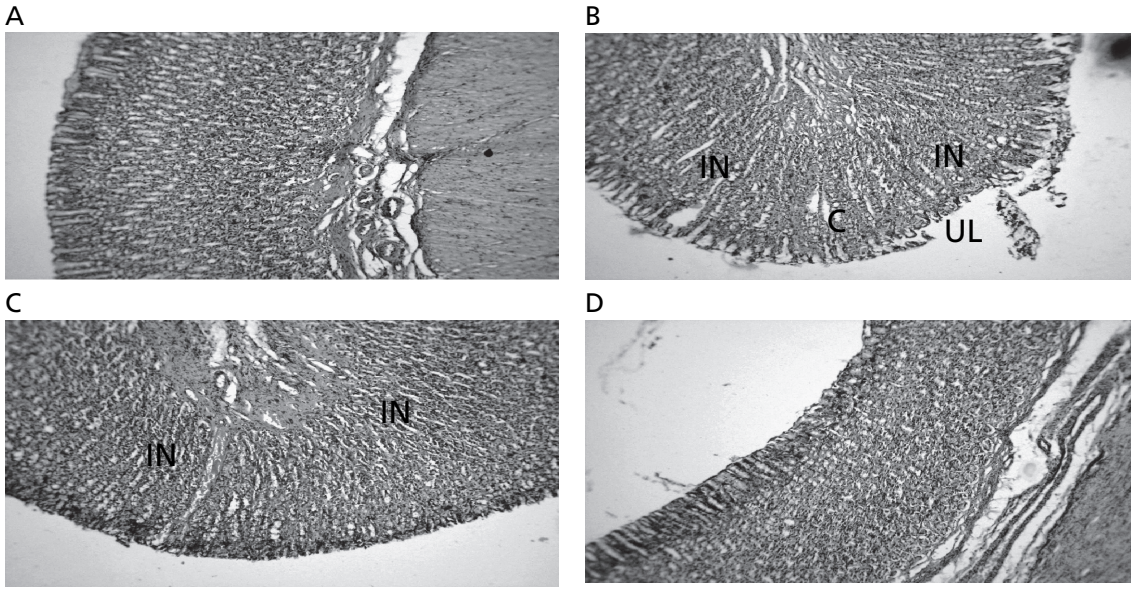

Figure 3 Histopathological examination of gastric mucosa from control and experimental animals (×100, stained with haematoxylin and eosin). Normal gastric mucosa showed perfect intact structures and regular epithelial lining (A). Gastric mucosa from rats with HP-LPS-induced ulcers exhibited ulcer (UL), denuded mucosa, inflammation (IN) and congested (C) vessels (B). Gastric mucosa from rats with HP-LPS-induced ulcers treated with TA had no ulcers and exhibited regenerating epithelium, mild oedema of the submucosa and mild inflammation (C). Similarly, the gastric mucosa of rats treated with sucralfate revealed slight regeneration of superficial epithelium and inflammation in the submucosa (D).

structure of the LPS preparations (Padol et al 2001). In the present study, LPS from *H. pylori* 26695 also stimulated acid secretion, confirming its role in gastric mucosal damage.

Pepsin, a protease present in the gastric lumen, appears to play a crucial role in ulceration of the stomach and duodenum; gastric acid does not cause ulceration in the absence of pepsin. Pepsin is secreted by the chief cells of the gastric mucosa as an inactive precursor, pepsinogen, which is activated by acid in the gastric lumen. Increased pepsinogen activity has been reported in association with gastric mucosal damage induced by *H. pylori* LPS (Moran 1996) and the results of the present study concur with these findings. Major benefits of antacid therapy in the treatment of ulcer disease may be inhibition of the conversion of pepsinogen to pepsin, and maintenance of the gastric luminal pH above the optimum for the enzyme. The anti-secretory effect of TA was evident from the attenuation of acid secretory parameters – acidity, acid output and pepsin activity. Increases in these factors have been reported to be essential for many experimental and clinical gastric ulcers (Piper & Stiel 1986); TA significantly decreased acid and pepsin secretion.

Exposure of gastric mucosal cells to *H. pylori* LPS has been reported to lead to a dose-dependent decrease in mucin synthesis (Slomiany & Slomiany 2002). The decrease in the components of protein-bound carbohydrate complexes (hexose, hexosamine, fucose and sialic acid) observed in the present study in rats with HP-LPS-induced ulcers is in agreement with studies that reported decreases in these defensive factors. However, TA-treated rats with HP-LPS-induced ulcers showed an increase in these protein-bound carbohydrate complexes and maintenance of protein levels, which clearly demonstrate the efficacy of TA in enhancing defensive factors; this effect was comparable to that of sucralfate.

Mucus serves as the first line of defence against ulcerogens. Mucus is secreted by the mucus neck cells and covers the gastric mucosa, thereby preventing physical damage and back diffusion of hydrogen ions (Williams & Turnberg 1980). An increase in the TC:P ratio is taken as a reliable marker of mucin secretion (Goel et al 1985a), which results primarily from increases in individual mucopolysaccharides. Further, strengthening of the gastric mucosa is evident from a decrease in leakage of protein into the gastric juice (Goel et al 1986). An increase in the glycoprotein content of gastric mucosa is evidenced from an increase in the TC:P ratio of the mucosal cells, which is taken as a marker for cellular mucus (Goel et al 1994). This increase was due to an increase in mucopolysaccharides, the major constituent of mucus which are also responsible for the viscous nature and gel-forming properties of mucus. The gel is reported to be resistant to a number of ulcerogens, including acid, ethanol and non-steroidal anti-inflammatory agents such as indometacin (Bell et al 1985). Hence, an increase in mucus synthesis may be an important factor contributing to the anti-ulcer action of TA. These results are consistent with the activity of TA in various gastric ulcer models, in which the protective effect is evident from a significant decrease in offensive factors such as acid and pepsin secretion, and increases in mucosal protective factors like mucus secretion and adherent mucus content (Devi et al 2007a,b).

The inflammatory responses and pathological condition of the mucosa resulting from *H. pylori* LPS of ATCC 43504 is characterized by the infiltration of the lamina propria with lymphocytes and plasma cells, oedema, hyperaemia and epithelial haemorrhage extending from the lamina propria to the surface of the mucosa (Slomiany et al 2000). In the present study, LPS from *H. pylori* 26695 caused changes that mimic

ulcer pathology, as evident from histopathology of the gastric mucosa. Macroscopic examination of the mucosa from TA-treated rats with HP-LPS-induced ulcers also confirmed the cytoprotective role of TA, as evident from reduced mucosal ulceration, which is probably due to the presence of phyto constituents that inhibit the LPS-induced mucosal damage.

Ulcers are caused by imbalances between offensive and defensive factors in the gastric mucosa. Antiulcerogenic activity is reported to result from an increase in defensive factors, rather than a decrease in offensive factors (Goel et al 1985b). The enhancement of mucosal factors by the methanolic extract of TA was not only evident against HP-LPS-induced ulceration, but has also been shown against diclofenac and dexamethasone-induced gastric ulcers, confirming its potential role as an anti-ulcer agent.

Various compounds from plants, such as terpenes and flavonoids, have been shown to inhibit gastric acid secretion, and possess anti-ulcer activity against several experimental models (Pillai & Santhakumari 1984; Murakami et al 1992). Flavonoids have been reported to have anti-ulcer (Beil et al 1995) and anti-secretory activities (Yamahara et al 1990; Murakami et al 1990; Geissberger & Séquin 1991; Alarcon de la Lastra et al 1993; Alarcon de la Lastra et al 1994; Beil et al 1995). Triterpenoid saponins have anti-ulcer properties (De Pasqquale et al 1995). Tannins prevent gastric lesions (Khennouf et al 2003). The methanolic extract of TA has been shown to contain tannins, glycosides, flavonoids, terpenes, polyphenols, phytosterols and steroids as its constituents, which are well known for their effects in maintaining mucosal integrity and protecting cells from damage. Hence it cam be concluded that the various phyto constituents (triterpenoids, flavonoids, glysosides) of TA antagonize aggressive factors that play a crucial role in the pathogenesis of gastric ulcers (induced by acid, pepsin or HP-LPS) and also augments the defensive mucosal factors that protect the stomach.

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