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Invivo gastric antiulcer activity of syringin (phenyl propanoid glucoside) studied in different ulcer induced experimental rat models

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ABSTRACT

Most of the currently available oral antiulcer drugs for the treatment of peptic ulcer provoke detrimental adversative effects. Hence, the exploration for plant-derived products for the treatment of ulcer continues. Syringin, a phenylpropanoid glucoside found in the tepals of Musa Paradisiaca, has many biological properties, including as an antioxidant, immunomodulatory and antidiabetic agent. The preventive and curative effects of syringin for ulcers were evaluated using models of acute gastric lesions induced by ethanol and indomethacin in rats. Moreover, the effects of ethanolic extract of syringin on gastric content volume, total acidity and pH, using the pylorus ligated model were also evaluated. Animals pretreated with syringin extract showed a significant reduction in lesion index in both ethanol and indomethacin induced ulcer models in a dose dependent manner when compared to the control group. Similarly, post-treatment with syringin (50 mg/kg body weight) for a period of 15 days revealed a statistically significant improvement in the ulcer healing process ($p < 0.05$). In the pylorus ligated model, it was observed that the syringin displayed an antisecretory activity, which led to a significant reduction in the gastric juice volume, total acidity and pH. These findings indicate that syringin displays both ulcer preventive and ulcer curative properties and provides a scientific rationale for the use of syringin in the traditional medicinal system.

Keywords: Peptic ulcer, syringin, antiulcer activity, antisecretory activity.

INTRODUCTION

Gastrointestinal disorders are one of the most significant causes of morbidity for the human population. Though diseases in general can affect any part of the body and give rise to undesirable changes that alters the metabolic processes of the system [1], diseases of the gastrointestinal system are more important because they are liable to send the whole system out of gear. A peptic ulcer is a benign lesion in the lining of the stomach or duodenum, where acid and pepsin bathes the surface [2]. Factors such as stress, smoking, alcohol usage, nutritional deficiencies and frequent ingestion of non-steroidal-antlinflammatory drugs (NSAlDs) have been shown to contribute to gastric ulcer Incidence [3]. Although there is evidence to implicate Helicobacter pylori in the development of peptic ulcers, the proportion of ulcers not related to either H. pylori or NSAIDs has increased, and this affects the management of peptic ulcers [4]. Five to ten percent of populations experience a peptic ulcer at some point in their lives. Ulcers can also irritate or inflame pancreas leading to pancreatitis. The most prominent symptom of a peptic ulcer is pain. Risk of ulcerogenesis is now greatly enhanced due to socio-economic problems and exposure of man to many noxious agents and chemicals [5].

Some suggested risk factors such as diet, and spice consumption, were hypothesized as ulcerogens until late in the 20th century, but have been shown to be of relatively minor importance in the development of peptic ulcers [6]. Similarly, while studies have found that alcohol consumption increases risk when associated with H. pylori infection,

it does not seem to independently increase risk, and even when coupled with H. pylori infection, the increase is modest in comparison to the primary risk factor [7].

The currently used antiulcer drugs like H_2 – receptors blockers, proton pump inhibitors, antimuscuranics produce adverse reactions such as hypersensitivity,arrhythmia, impotence and haemopoietic changes with is a possibility of increased rate of ulcer recurrence within one year after cessation of the treatment. Because of the above mentioned demerits reported with the current antiulcer therapy there is a need for the search of newer therapeutic antiulcer agents preferentially from plant sources continues. In plants some of the most attractive sources of new drugs show to produce promising and favourable reasons in the treatment of ulcer.

In traditional medicine, *Musa paradisiaca* raw flowers are treated as remedy for ulcers. The juices of tepals have been used for fevers, hemorrhages, hysteria. Dysentery, digestive disorders, and diarrhea can also be cured by tepal extract. Anemia, blood pressure, constipation, depression can be controlled by the flower extract. Sanyal *et al*, have reported the anti-ulcerogenic activity of dried powder of banana pulp against ulcers induced by histamine in guinea pigs and, phenylbutazone, restraint stress and prednisolone in rats [8 - 11]. The flower extract has been reported to possess antioxidant as well as antiulcer activity [12 – 13].

The flowers of *Musa paradisiaca* was reported to contain various biologically active phytochemicals such as pectin, leucocyanidin, quercetin, syringin, β sitosterol and terpenoid glucosides [14 – 15]. Preliminary studies conducted by us using different solvents revealed that the ethanolic extract of *M. Paradisiaca* tepals contain relatively increased amount of syringin.

Syringin, a phenylpropanoid glucoside is a found to be distributed in the tepals of *Musa pardisiaca*. Various pharmaceutical actions of syringin have been reported. Syringin is effective for the treatment of psychogenic behavior disorder.

Several pharmacological actions of syringin include plasma glucose reduction, antioxidation, anti-cancer activity, antidepressant effect, immunomodulation, etc [16 - 20]. Syringin was found to possess immunomodulatory activity and anti-allergic effects [21] and additionally it exhibit potent cytotoxic effect on several tumour cell lines [22 – 23] and anti-inflammatory activity [24].

In the absence of systemic studies in the literature regarding the antiulcerogenic properties of syringin, in the present study an attempt has been made to isolate and characterize syringin from the tepals of *Musa paradisiaca* extract. It is also aimed to evaluate its antiulcerogenic potential in experimentally induced different ulcer models.

MATERIALS AND METHODS

Preparation of plant extract

The Male flowers from the tip of the peduncle at the time of harvesting of unripe plantain were collected freshly from Thirukazhukundram, Kanchipuram District, Tamil Nadu, India during the months of January and February and authenticated by a Taxonomist in the Centre for Advanced Studies in Botany, Universityof Madras. The tepals were selectively removed from the bracts; shadow dried and powdered using a pulverizer. The powdered flower material was defatted with petroleumether (60–80°C) and then extracted with 95% ethanol in a Soxhlet apparatus. The solvent was selectively removed under reduced pressure, which yields a black sticky residue (26.5% w/w) withrespect to dried flowers. The dried flower extract was stored in a desiccator till further investigation.

Isolation and characterization of compounds from the ethanol extract

1 Kg of shadow dried, powdered tepals of M. paradisiaca were repeatedly extracted with ethanol and evaporated to dryness under reduced pressure. The resultant residue (260g) was suspended in 100 ml of distilled water and was fractionated successively by partitioning first with chloroform (3 × 100 ml) and then with a 3:1 mixture of chloroform and ethanol (3 × 100 ml). The remaining solution was labeled as water fraction. After being dried under reduced pressure at 40 °C, each fraction was analyzed by thin layer chromatography (TLC). The most active ethanol fraction was subjected to silica gel preparative thin layer chromatography (PTLC) using butanol: acetic acid: water (4:1:5, upper phase) as a mobile phase. The purity of the whitish semi-crystalline substance obtained was checked by analytical TLC using an authentic sample of syringin. The isolated compound was investigated using various analytical spectral studies such as UV-Visible and NMR.

Experimental Animals

Healthy, male albino rats of wistar strain (150-170 g) were selected for the present study. The rats were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The rats were housed in well ventilated; colony cages in the departmental animal house. Coprophagy was prevented by keeping the animals in cages with gratings as the floors. The animals were maintained on sterile, standard pellet diet and water ad libitum. The experiments were designed and conducted according to the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines for the investigation of experimental pain in conscious animals (IAEC No. 21/02/2010). Before beginning the experiments, the animals were allowed to acclimatize to animal house condition for a week period.

Acute toxicity studies and dosage fixation studies

The acute oral toxicity studies were conducted in the rat model, according to the method of Litchfield and Wilcoxon (1949) and as described in "Guidelines for Testing of Chemicals-Acute Oral Toxicity-Fixed Dose Procedure" (OECD 420, 2001). In this study, syringin was orally administered to a group of at least 10 rats after a 12 h fast. The control group received equal amount of water by gavage with the aid of a metal gastric cannula. Dosage fixation studies were carried out by administering graded doses of syringin (10, 20, and 30, 50, 100 mg/kg bodyweight [b.w]) to determine the dose-dependent effect. The signs and symptoms were observed carefully after 0, 30, 60, 120, 180 and 240 min and then once a day for the subsequent 14 days to record toxic manifestations. Body weight, food intake, morphological and behavioral changes were monitored periodically to assess the signs of toxicity of syringin, if any. At the end of the experimental period, the animals were sacrificed and the blood was collected with and without anti-coagulant.

The effect of syringin pre-treatment using the ethanol induced ulcer model

The study was performed according to the method of Büyükokuroğlu et al., (2002) [25]. After 12 h of fasting, the Male Wistar rats weighing about 160-180 g were randomly divided into four groups of six rats each. Group 1 represented the control group which received water only and the group 2 was given 1 ml of 99.5% ethanol by oral gavages to induce gastric ulcer [26]. Group 3 received 50 mg/kg body weight of syringin respectively and group 4 animals were treated with omeprazole (30 mg/kg bw). All pretreatments were administered orally. One hour later all of the animals in group 3 and 4 were given 1 ml of 99.5% ethanol by oral gavages to induce gastric ulcers. After a lapse of 1 h the animals were sacrificed by cervical dislocation and stomachs were removed and opened along the greater curvature.

The effect of syringin post-treatment using the Indomethacin induced ulcer model

Four groups of at least six rats each were set-up. Group I was the control which received 1 ml water only. Ulcers were induced in all rats in groups II, III and IV by indomethacin (100 mg/kg bw) by oral gavage. Rats in group III were treated with 50 mg/kg bw syringin daily for 15 days and rats in group IV were treated with cimetidine (100 mg/kg bw) daily for 15 days. After 15 days, all animals were sacrificed and ulcers assessed as before. The sum of length of lesions (mm) was calculated and expressed as lesion index.

Shay ulcer model

In this model, the rats were divided into three groups each comprising of a minimum of ten rats. The rats were fasted for 24 h with free access to water. Thirty minutes after oral administration of single dose of syringin (50 mg/kg b.w), cimetidine (100 mg/kg b.w) as a positive control or water (10 ml/kg bw) as a negative control, the pylorus ligature was performed under phenobarbital anesthesia at a dose of 35 mg/kg bw [27]. Animals were allowed to recover and stabilize in individual cages and were deprived of water during the postoperative period. Four hours later, the animals were sacrificed by cervical dislocation and the abdomen was opened to place another ligature at the oesophageal end. The stomachs were removed and gastric content was carefully collected and centrifuged at 3000 rpm for 10 min. The amount of gastric juice and pH was determined by titration with 0.01N NaOH solution and phenolphthalein as an indicator. Gastric lesions were evaluated by examining the inner gastric surface as described separately. Ulcer index was calculated from percentage ulcerated surface as described by Tan et al. (1996.) [28].

Histological Studies

A portion of the ulcer region in the stomach tissue was dissected out and fixed in 10% buffered neutral formalin solution for histological observations. After fixation, tissues were embedded in paraffin, solid sections were cut at 5 mm and stained with hematoxylin and eosin [29]. The sections were examined with the help of a qualified pathologist under light microscope and photomicrographs were taken.

Determination of degree of ulceration

The surface area (A) mm^2 covered by each lesion was measured (Murakami et al., 1990) and the sum of erosion areas per rat stomach was calculated. Percentage ulcerated surface (US) was calculated as

$$\% \text{ US} = \frac{\text{Total area covered by ulcers}}{\text{Total corpus mucosal surface}} \times 100$$

Ulcer index was calculated from percentage ulcerated surface as described by Tan et al. (1996) [32].

Statistical analysis

The values were expressed as mean ± S.E.M for six rats in each group. All data were analyzed with SPSS/16.0 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD) test. Values of @P < 0.05, #P < 0.01, *P < 0.001, and were considered as significant.

RESULTS

The acute oral toxicity evaluated after a single oral administration of 50 mg/kg bw syringin revealed non-toxicity (results not shown). All of the animals survived for 14 days. There were no significant alterations in food and water consumption or body weight gain during the experimental period. Analysis of both haematological and biochemical parameters indicated no significant changes in the syringin treated group of rats when compared to animals in the control (untreated) group. Macroscopic observation on vital organs also confirmed the non-toxic nature of syringin. These preliminary studies indicate the absence of acute toxic effects of syringin.

Syringin pretreatment studies

The ulcer preventive effect of the syringin on both ethanol and indomethacin induced gastric lesions in experimental rats is summarized in Tables 1 and 2. Pretreatment with syringin resulted in a significant reduction of the gastric lesions induced by two damaging agents (ethanol and indomethacin) in a dose dependent manner and the efficacy was found to be similar in both cases. The results obtained suggest that the syringin has a significant antiulcer effect in each of these ulcer induced models.

Syringin Post-treatment studies

The rats receiving water only showed no lesions in their gastric mucosa. Treatment of rats with ethanol as well as indomethacin produced typical acute mucosal lesions with ulcer index scores of 10. Oral administration of syringin at a concentration of 50 mg/ kg bw for 15 days significantly ($p < 0.05$) reduced the ulcer index in both ethanol as well as indomethacin induced experimental ulcer in rats and the results were comparable with standard drugs (Tables 3 and 4). In the gastric secretion determination model, using ligated pylorus, the treatment with syringin, as well as cimetidine, reduced the volume of the gastric juice, total acidity and raised gastric pH significantly in comparison with control groups (Table 5). The ulcer preventive effects of syringin in both models were comparable to standard antiulcerogenic drugs omeprazole and cimetidine, respectively (Tables 6 and 7).

Table 1. Effect of pretreatment with syringin on indomethacin- induced ulcer in rats

Groups	Ulcerated surface (%)	Ulcer index
Control	0.00 ± 0.00	0
Indomethacin	37.46 ± 2.69 [a*]	10
Indomethacin + syringin (100 mg)	12.83 ± 1.19 [a*b*]	5

Values are expressed as Mean ±S.E.M for six animals in each group. One way ANOVA followed by post hoc test (LSD). The results were compared with [a] Control; [b] Indomethacin.

Table 2. Effect of pretreatment with syringin on ethanol induced ulcer in rats

Groups	Ulcerated surface (%)	Ulcer index
Control	0.00 ± 0.00	0
Ethanol	22.85 ± 1.65 [a*]	7
Ethanol + syringin (50 mg)	6.06 ± 0.48 [a*b*]	4

Values are expressed as Mean ±S.E.M for six animals in each group. One way ANOVA followed by post hoc test (LSD). The results were compared with [a] Control; [b] Ethanol.

Table 3. Effect of syringin treatment for 5, 10 & 15 days on indomethacin-induced ulcer in rats

Groups	Ulcerated surface (%)	Ulcer index
Control	0.00 ± 0.00	0
Indomethacin	70.99 ± 5.02 ^{a*}	10
Indomethacin + syringin (5 days)	21.86 ± 1.72 ^{a*b*}	7
Indomethacin + syringin (10 days)	3.91 ± 0.31 ^{ab*}	3
Indomethacin + syringin (15 days)	1.08 ± 0.14 ^{ab*}	2

Values are expressed as Mean ±S.E.M for six animals in each group. One way ANOVA followed by post hoc test (LSD).. The results were compared with ^{a} Control; ^{b} Indomethacin.

Table 4. Effect of syringin treatment for 5, 10 & 15 days on ethanol-induced ulcer in rats

Groups	Ulcerated surface (%)	Ulcer index
Control	0.00 ± 0.00	0
Ethanol	53.48 ± 3.78 ^{a*}	10
Ethanol + syringin (5 days)	14.74 ± 1.09 ^{a*b*}	5
Ethanol + syringin (10 days)	3.11 ± 0.22 ^{ab*}	3
Ethanol + syringin (15 days)	0.91 ± 0.10 ^{ab*}	2

Values are expressed as Mean ±S.E.M for six animals in each group. One way ANOVA followed by post hoc test (LSD). The results were compared with ^{a} Control; ^{b} Ethanol.

Table 5. Effect of syringin on pylorus-ligated ulcer in rats

Groups	Ulcerated surface (%)	Ulcer index	Gastric volume (ml)	Gastric acidity (mEq/L)	pH
Control	9.35 ± 0.86	4	4.57 ± 0.20	47.52 ± 3.06	3.10 ± 0.12
Syringin (50 mg/kg)	4.46 ± 0.29 ^{a*}	3	4.10 ± 0.22 ^{a}	39.72 ± 2.52 ^{a}	3.12 ± 0.20 ^{a}

Values are expressed as Mean ±S.E.M for six animals in each group. One way ANOVA followed by post hoc test (LSD). The results were compared with ^{a} Control.

Table 6. Effect of syringin on the extent of ulceration in indomethacin-induced ulcer in rats

Groups	Ulcerated surface (%)	Ulcer index	Gastric volume (ml)	Gastric acidity (mEq/L)	pH
Control	0.00 ± 0.00	0	1.90 ± 0.09	3.66 ± 0.18	4.10 ± 0.21
Indomethacin	74.46 ± 4.07 ^{a*}	10	4.28 ± 0.21 ^{a*}	6.12 ± 0.34 ^{a*}	2.60 ± 0.19 ^{a*}
Indomethacin + syringin	1.21 ± 0.14 ^{ab*c}	2	2.46 ± 0.17 $^{a@b*c}$	3.99 ± 0.31 ^{ab*c}	3.70 ± 0.18 ^{ab*c}
Indomethacin + Cimetidine	1.04 ± 0.08 ^{ab*}	2	2.92 ± 0.18 ^{a*b*}	4.75 ± 0.30 $^{a@b\#}$	4.06 ± 0.18 ^{ab*}

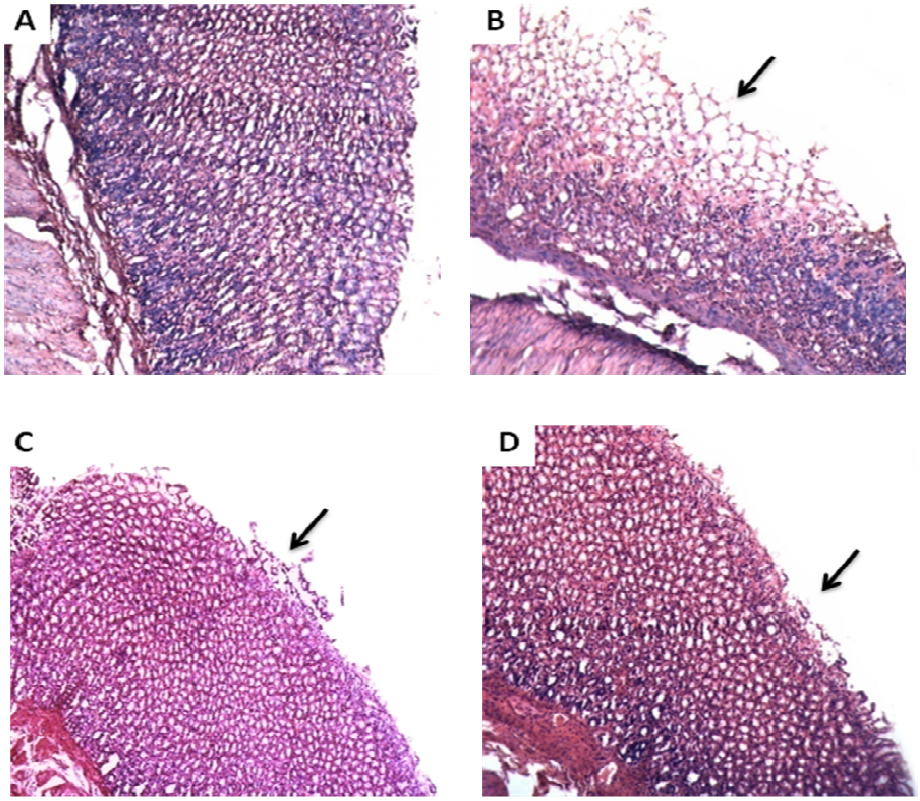
Values are expressed as Mean ±S.E.M for six animals in each group. One way ANOVA followed by post hoc test (LSD). The results were compared with ^{a} Control;^{b} Indomethacin; ^{c} Indomethacin + Cimetidine treated rats.

Table 7. Effect of syringin on the extent of ulceration in control and ethanol-induced ulcer groups of rats

Groups	Ulcerated surface (%)	Ulcer index	Gastric volume (ml)	Gastric acidity (mEq/L)	pH
Control	0.00 ± 0.00	0	2.54 ± 0.17	2.94 ± 0.10	4.13 ± 0.21
Ethanol	62.16 ± 4.22 ^{a*}	10	4.79 ± 0.26 ^{a*}	5.92 ± 0.26 ^{a*}	2.32 ± 0.17 ^{a*}
Ethanol + syringin	1.15 ± 0.12 ^{ab*c}	2	2.80 ± 0.25 ^{ab*c}	3.24 ± 0.25 ^{ab*c}	3.74 ± 0.32 ^{ab*c}
Ethanol + Omeprazole	1.01 ± 0.20 ^{ab*}	2	2.65 ± 0.29 ^{ab*}	3.70 ± 0.16 $^{a@b*}$	3.86 ± 0.17 ^{ab*}

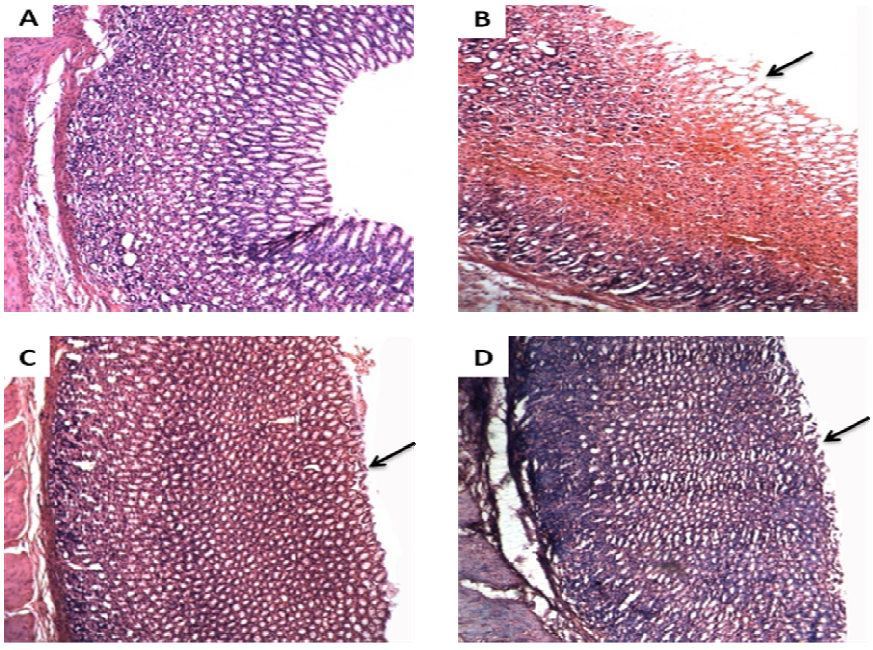
Values are expressed as Mean ±S.E.M for six animals in each group. One way ANOVA followed by post hoc test (LSD). The results were compared with ^{a} Control;^{b} Ethanol; ^{c} Ethanol + Omeprazole treated rats.

Plate 1 : Histological observations on the gastric mucosa of control and experimental groups of rats



Photomicrograph showing of gastric mucosa cells of Control (A); Indomethacin-induced ulcer (B); Indomethacin-induced ulcer + Syringin (C); Indomethacin-induced ulcer + Cimetidine (D) treated rats showed at 200x

Plate 2: Histological observations on the gastric mucosa of control and experimental groups of rats



Photomicrograph of gastric mucosa of Control (A); Ethanol-induced ulcer (B); Ethanol-induced ulcer + Syringin (C); Ethanol-induced ulcer + Omeprazole (D) treated rats showed at 200x

DISCUSSION

In this syringin antiulcerogenic study, acute toxicity in rats was investigated and a single oral adminstration of syringin at a concentration of 50 mg/kg body weight indicated the non-toxic nature of syringin. After treatment of rats with different concentrations of syringin have been demonstrated not inducing significant alterations in haematological or biochemical parameters [30]. Here dosage fixation studies indicate that the syringin at a concentration of 50 mg/kg body weight showed the maximum gastric antiulcerogenic activity in both alcohol and indomethacin induced experimental gastric ulcer models in rats.

Chemically, syringin is the glucoside of sinapyl alcohol. Characteristically syringin is a white crystalline solid, slightly soluble in water [31]. Since syringin has a low viscosity and readily solubilizes aqueous extract is found to be more effective in eliciting biological properties. Therefore, the present study was carried out with an aqueous extract of syringin. In most cases, the stable incidence of ulcer in rat models provides a powerful and convenient tool for the investigation of therapeutic modalities for the disease and for its complications [32]. The gastric ulcer preventive and ulcer curative activities of syringin were evaluated using ethanol, indomethacin and pylorus ligated ulcer models; the most commonly used experimental models for the evaluation of gastric antiulcer activity is in rats [33].

Ulcers caused by ethanol are due to superficial damage to the mucosal cells [34]. On the other hand, ethanol induced gastric lesions are thought to arise as a result of direct damage of gastric mucosal cells, resulting in the production of free radicals [35] and hyperoxidation of lipids [36]. These data suggest that antioxidant compounds could be active in this experimental model, producing antiulcerogenic effects. This effect is known as cytoprotection [32]. The observed decrease in ulcer index in syringin treated groups of rats may be due to its antisecretory or cytoprotective properties or both.

Indomethacin became the first-choice drug to produce an experimental ulcer model as a result of having a higher ulcerogenic potential than other non-steroidal anti-inflammatory drugs. Indomethacin is known to induce gastric ulcer by inhibition of prostaglandins which are cytoprotective to gastric mucosa [37]. The incidence of indomethacin-induced ulceration is mostly on the glandular part of the stomach. Indomethacin administration induced numerous punctiform and filiform gastric ulcers in the control rats. The observed antiulcerogenic property of syringin may be due to increased synthesis of mucous and/or prostaglandins or could possibly be due to its 5-lipoxygenase inhibitory effect [38].

In pyloric ligation-induced ulcer model, increase in gastric acid secretion has been implicated in the severe ulceration of the rat gastric mucosa [39] while the auto-digestion of gastric mucosa has been attributed to the accumulation of pepsin [40]. Hence, indomethacin and pylorus ligation were used to induce ulcer in this research. Though, the mechanism of ulcer formation by indomethacin and ethanol is quite different, the efficacy of the drug was found to be the same in controlling the gastric ulceration [41].

The results of histological observations made on gastric mucosa of control and experimental rats of both models are presented in plates 1 (a-d), and 2 (a-d).

Plate 1a shows the histological observations made on the gastric mucosa of control rats showing normal architecture. Plate 1b represents stomach tissue of indomethacin induced ulcer rats with severe ulcer lesions on the gastric mucosa. Plate 1c illustrates indomethacin induced ulcer rats with prominent lesions and ulcerated surface on the gastric mucosa. Plates 1 c represent syringin (50 mg/kg b.w) treated indomethacin-induced ulcer rats. Plate 1d represents cimetidine treated ulcer rats.

2a shows the histological observations made on the gastric mucosa of control rats showing normal architecture. Plate 2b represents stomach tissue of ethanol induced ulcer rats with severe ulcer lesions on the gastric mucosa. Plates 2 c represent syringin (50 mg/kg b.w) treated ethanol induced ulcer rats. Plate 2 D represents Omeprazole treated ulcer rats.

Administration of syringin was found to protect the gastric surface against ulceration, which is evident, by decreased/absence of lesions in both the ulcer-induced models. The apparently normal architecture of gastric mucosa in syringin treated ulcer rats confirms the gastroprotective effect of syringin. Similar observations were noted in the ulcer rats treated with cimitidine. The histological finding further strengthens the ulcer curative effect of syringin.

In conclusion, the results of the present study show that syringin possesses antiulcer or cytoprotective activity, as evidenced by its significant inhibition in the formation of ulcers by different animal models, as well as ulcer curative properties by decreasing the gastric secretions. Therefore the observed ulcer preventive and ulcer curative activity of syringin extract may be partially due to its relative antioxidant activity.

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