# Indigofera suffruticosa Mill as new source of healing agent: Involvement of prostaglandin and mucus and heat shock proteins

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## ARTICLE INFO

## ABSTRACT

*Ethnopharmacological relevance:* Indigofera suffruticosa is specie typical of the "Cerrado" or Brazilian savannah; it is a member of the Fabaceae family – in folkmedicine is used for gastric disorders, infection and inflammation.

*Aim of the study:* Ethyl acetate fraction (AcF) and aqueous fraction (AqF) of the methanolic extract of *I. suffruticosa* leaves were evaluated against acute gastric ulcer. The AcF fraction was selected to assess its activity in ulcer healing and its gastroprotective effects via mucus and gastric secretion.

*Materials and methods:* The gastroprotective action of AcF and AqF fractions were evaluated in a rodent experimental model. The action mechanisms, involvements of the antisecretory action, mucus and prostaglandin production, toxicological and healing activity of the AcF (100 mg/kg, p.o.) were evaluated. We also used histological analysis (HE and PAS) and immunohistochemical (PCNA and HSP-70) assays to evaluate the effects of *I. suffruticosa.*

*Results:* AcF significantly inhibited the gastric mucosal damage caused by ethanol. This effect was statistically significant in 100 mg/kg group compared vehicle. AcF did not interfered with gastric secretion, significantly increased the $PGE_2$ and mucus production (validated in PAS technique). The gastroprotection was attenuated by pretreatment with N-ethylmaleimide, but not L-NAME. In acid-acetic-induced ulcer model AcF accelerated ulcer healing. Immunohistochemistry analysis showed induction of proliferating cell (PCNA) and heat shock protein (HSP 70).

*Conclusions:* These results showed that AcF acted as gastroprotective agent stimulating prostaglandin, mucus and HSP70.

## 1. Introduction

Peptic ulcers are a common disorder of the entire gastrointestinal tract that occurs mainly in the stomach and the proximal duodenum. This disease is multifactorial and its treatment faces great difficulties due to the limited effectiveness and severe side effects of the currently available drugs (Mota et al., 2009). Numerous plants and herbs are used to treat gastrointestinal disorders in traditional medicine (Schmeda-Hirschmann and Yesilada, 2005).

There has been renewed interest in identifying new antiulcer drugs from natural sources (Mota et al., 2009). The biodiversity of Brazilian flora, especially the Cerrado, a savannah like region is very rich in medicinal plant species which present antiulcer activities. Among the medicinal plants present in this region, we find the *Indigofera suffruticosa* Mill (Fabaceae). The specie is known to be a rich source of glycoside flavonoids derived from quercetin and alkaloids (Calvo et al., 2009). In popular Brazilian medicine, it has been used as an infusion or decoct to treat infection and inflammation (Matos, 1999; Vieira et al., 2007). In Cuba, this specie is used to treat gastrointestinal disorders, such as gastrointestinal pain (Roig, 1988). Recent pharmacological studies shown that *I. truxillensis* Kunth present antiulcerogenic and antioxidant activity (Cola-Miranda et al., 2006; Farias-Silva et al., 2007).

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The present work was carried out to investigate the possible antiulcerogenic effect of the ethyl acetate fraction (AcF) of leaves of *I. sulffruticosa* Mil against acute and chronic experimental models of gastric ulcer in rodents. The possible mechanism of action of AcF was also studied. Finally, we also performed an immunohistochemistry analysis for proliferating cell nuclear antigen (PCNA) and heat shock protein (HSP 70).

## 2. Materials and methods

### 2.1. Animals

Male Unib: WH rats (180–250 g) obtained from the breeding of the State University of Campinas (CEMIB/UNICAMP) Brazil, were used. The colony rats were fed a certified Nuvilab CR-diet, with free access to tap water, and were housed on a 12 h light/dark cycle at $60 \pm 1\%$ humidity and $21.5 \pm 2\,^{\circ}C$. The experimental protocols were approved by the institutional Committee for Ethics in Animal Experimentation (CEEA/UNICAMP) and were done in accordance with the Canadian Council Guide lines for Animal Care.

### 2.2. Plant material

The aerial parts of *I. suffruticosa* were collected along the Domingos Sartori highway at Rubião Junior, Botucatu, São Paulo State, Brazil, in June 2003. The plants were identified by Dr. Jorge Tamashiro of the Institute of Biology at UNICAMP and a voucher specimen (UEC: 131.827) was deposited in the Herbarium at UNICAMP.

### 2.3. Preparation of fractions

The aerial parts (1500 g) of *I. suffruticosa* were air dried (7 days at $40\,^{\circ}C$), powdered, and then exhaustively extracted with chloroform ($CHCl_3$) and methanol (MeOH) successively, at room temperature (three chloroform-methanol cycles, with 72 h for each solvent). The solvents were evaporated in vacuum to provide a $CHCl_3$ extract and a MeOH extract. A portion (3.0 g) of the MeOH extract was partitioned in ethyl acetate and water (1:1, v/v) to yield 2.3 g and 0.5 g of the AqF and AcF respectively.

### 2.4. Determination of the total flavonoid content

The flavonoid content of the AcF was determined as follows: 0.1 ml of each fraction was diluted with 80% aqueous ethanol (0.9 ml) and an aliquot of 0.5 ml was added to test tubes containing 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M aqueous potassium acetate and 4.3 ml of 80% ethanol. After 40 min at room temperature, the absorbance was determined at 415 nm. The total flavonoid content was calculated using quercetin as a standard (Moreno et al., 2000).

### 2.5. Drugs

The following drugs were used: cimetidine, lansoprazole, carbenoxolone, indomethacin, and N-ethyl-maleimide (NEM) were all obtained from Sigma Chemical Co. (St. Louis, MO, USA). The chemicals used and other solutions were all of analytical grade. All drugs and reagents were prepared immediately before use.

### 2.6. Antiulcerogenic acitivity

#### 2.6.1. Ethanol-induced gastric lesions

Ethanol-induced ulcers were produced in rats according to the method of Morimoto et al. (1991). Rats were randomly separated into ten groups and fasted for 24 h before the experiment. One hour after the oral administration of AcF from *I. suffruticosa* (25, 50, and 100 mg/kg), lansoprazole (30 mg/kg), 12% Tween 80 (10 mL/kg), 1 ml of 99.5% ethanol was given orally to the rats. Animals were killed by cervical dislocation 1 h after ethanol administration. Their stomachs were removed, opened along the greater curvature, and fixed between two glass plates. The inner surface of the stomach was examined with a dissecting microscope (Nikon SMZ800) and the number of gastric lesions was counted. The ulcer index was calculated according to the method of Szelenyi and Thiemer (1978).

#### 2.6.2. Gastric secretion in lesions induced by pylorus ligature

For this assay, the method of Shay et al. (1945) was used with some modifications. Rats were fasted for 36 h and immediately after pylorus ligature, AcF from *I. suffruticosa* (100 mg/kg), cimetidine (100 mg/kg), 12% tween 80 (10 mL/kg) was administered intraduodenally. The rats were killed 4 h later, and their abdomens were opened and the stomachs removed. The gastric juice was collected and weighed (g) and its pH were determined using a pH meter (Quimis Aparelhos Científico Ltda, model Q400A, Brazil).

#### 2.6.3. Prostaglandin synthesis determination

Male rats were divided into 5 groups ($n = 6$). After a 24 h fast, the animals received a pretreatment of saline, s.c. (groups 1 and 2), or indomethacin (dissolved in 5% sodium bicarbonate solution) 30 mg/kg, s.c. (groups 3 and 4). The fifth group consisted of the sham control animals. Thirty minutes after pretreatment, saline (groups 1 and 3) or AcF 100 mg/kg (groups 2 and 4) was administered orally. Thirty minutes after treatments, all the animals were sacrificed and their abdomens opened. A sample of the corpus (full thickness) was excised, weighed and suspended in 1 mL of 1 mM sodium phosphate buffer, pH 7.4. The tissue was finely minced with scissors and then incubated at $37\,^{\circ}C$ for 20 min. PGE2 in the buffer was measured by the enzyme immunoassay (R&D systems) and the absorbance was read at 450 nm (Curtis et al., 1995).

#### 2.6.4. Determination of the gastric mucus content

This assay was done as described by Rafatullah et al. (1990) with some modifications. After a 36 h fast, rats received AcF from *I. suffruticosa* (100 mg/kg), carbenoxolone (200 mg/kg), 12% Tween 80 (10 mL/kg) orally. Thirty minutes after treatment, the pylorus was ligated. The animals were killed by cervical dislocation 4 h after pylorus ligation and the glandular portion of the stomachs were removed and weighed. Each segment was immediately immersed in 10 ml of 0.1% Alcian blue solution (0.16 M sucrose/0.05 M sodium acetate, pH 5.8) for 2 h, after which the excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 min and then for 45 min. Each stomach was than transferred to 0.5 M magnesium chloride solution for 2 h. Four milliliters of the dye solution was then vigorously shaken with an equal volume of ether and the resulting emulsion was centrifuged at $2000 \times g$ and the absorbance of the aqueous layer was measured at 580 nm. The amount of blue dye extracted per gram of wet glandular tissue was then calculated from a standard curve of dye prepared in sucrose-acetate solution.

#### 2.6.5. Determination of the role of nitric oxide (NO) and sulfhydryl compounds (SH) in gastric protection

Male rats ($n = 5$) were divided into 6 groups and pretreated (i.p.) with saline, L-NAME (N-nitro-L-arginine methyl 70 ester mg/kg) an inhibitor of the NO synthesis or NEM (N-ethylmaleimide, 10 mg/kg) a blocker of SH compounds (Arrieta et al., 2003). Thirty minutes after the pretreatment the animals were administered (p.o.) vehicle, carbenoxolone (100 mg/kg) or AcF (100 mg/kg). After 60 min all the groups received 1 mL absolute ethanol to induce gastric

ulcers. One hour after receiving ethanol the rats were killed for determination of gastric lesions.

### 2.6.6. Healing in acetic-induced gastric lesion

The experiment was done according to the method described by Takagi et al. (1969) with some modifications Okabe and Amagase (2005). Three groups of male Unib: WH rats fasted for 24 h were used in this experiment ($n = 5$). Under anesthesia, a laparotomy was done in all animals through a midline epigastric incision. After exposing the stomach, 0.05 ml (v/v) of a 30% acid acetic solution was injected into the subserosal layer in the glandular part of the anterior wall. The stomach was bathed with saline (20 °C) to avoid adherence to the external surface of the ulcerated region. The abdomen was then closed and all the animals were fed normally. We selected the lower effective dose of AcF (100 mg/kg) of *I. suffruticosa*; cimetidine (100 mg/kg) or vehicle (10 mL/kg) for the determination of the healing effects by the subacute treatment. All treatments were done orally once a day during 14 consecutive days beginning one day after surgery.

One day after the last drug administration, the rats were killed and the stomachs were removed. The gastric lesions were evaluated by examining the inner gastric surface with a dissecting magnifying glass. The macroscopic ulcer area (mm$^2$) and curative ratio (%) were subsequently determined as described by Takagi et al. (1969).

### 2.6.7. Histology methods

The stomach of the rats submitted by different treatment of gastric ulcers in the acid acetic model with different treatments were pushed off and opened by the large curves and the lesion was localized. The lesion was sectioned, and fixed in ALFAC solution (alcohol, chloroform and acetic acid) for 24 h in 4 °C. Then the samples were routine processed for embedding in paraplast, and cut into 7 μm thick section. Hematoxylin and eosin and PAS (Behmer et al., 1976; Vacca, 1985). The samples were analysed with a Leica microscope associated with Leica Qwin Software (Leica-England).

### 2.6.8. Immunohistochemical methods

The histological cuts were deparaffinized, rehydrated and immunostained by the ABC method. Nonspecific reaction was blocked with $H_2O_2$ and goat serum prior to the incubation with the specific antiserum. After rinsing in phosphatase buffer saline (PBS 0.01 mol/L, pH 7.4), the sections were incubated in secondary antiserum (ABC kit). They were washed in PBS buffer, the ABC complex was applied, and the reaction was finally carried out in a DAB solution (3,3′-diaminobenzidine-tetrahydrochloride) containing 0.01% $H_2O_2$ in PBS buffer. After immunostaining, the sections were lightly counterstained with hematoxylin and the immunoreactive cells were observed under a Leica microscope associated with Leica Qwin Software (Leica-England). For the control reaction, some slides were processed omitting the primary antibody and other slides omitting the primary and secondary antibodies. These procedures were done for heat-shock protein 70 (HSP-70-Santa Cruz Biotechnology) and Proliferating cell nuclear antigen (PCNA-Novo Castra).

### 2.7. Statistical analysis

The results were expressed as the mean ± standard derivation. Statistical comparisons were done by one-way analysis of variance (ANOVA) followed by the Dunnett's or Tukey's post hoc test, with the level of significance set at $p < 0.05$.

**Table 1**
Effects of lansoprazole (30 mg/kg), AqF and AcF from *I. suffruticosa* (25, 50 and 100 mg/kg) on ethanol-induced gastric mucosal ulcers in rats. The results are reported as the mean ± S.D. ANOVA followed by Tukey's test.

| Treatments (p.o.) | Dose (mg/kg) | Ulcer index | Inhibition (%) |
|---|---|---|---|
| 12% Tween | 10 mL/kg | 34.4 ± 6.3 | – |
| Lansoprazole | 30 | 8.4 ± 3.6** | 75 |
| AcF – *I. suffruticosa* | 25 | 33.8 ± 4.9 | 1.7 |
| | 50 | 33.8 ± 3.5 | 1.7 |
| | 100 | 9.8 ± 3.7** | 71 |
| Saline | 10 mL/kg | 51.2 ± 16.8 | – |
| Lansoprazole | 30 | 9.2 ± 3.5** | 82 |
| AqF – *I. suffruticosa* | 25 | 35.4 ± 8.0 | – |
| | 50 | 41.0 ± 2.4 | – |
| | 100 | 40.8 ± 10.5 | – |

** $P < 0.01$ compared to the corresponding vehicle group.

## 3. Results

### 3.1. Ethanol-induced gastric lesions

The oral doses (25, 50 and 100 mg/kg) were initially used to establish a general profile of the antiulcerogenic activity of the both AcF and AqF (Table 1). These data suggest that AcF (100 mg/kg) produce a gastroprotective effect since they significantly reduced ethanol-induced ulcers. However, the AcF fraction failed to reduce the gastric lesions that had been induced by absolute ethanol. The dose of 100 mg/kg of AcF demonstrated 71% protection against gastric ulcer induced by ethanol absolute when compared with respective control. Therefore, with the purpose of investigating the probable gastroprotective mechanisms involved in the action promoted by AcF, we continued our studies using only a single dose 100 mg/kg in subsequent assays, since it had produced the best results in this model.

### 3.2. Gastric secretion in lesions induced by pylorus ligature

The antiulcerogenic activity of AcF (100 mg/kg) was unrelated to the mechanisms that control gastric acid secretion. In the model of pyloric ligature, pretreatment with AcF did not significant modify the pH of gastric juice (Table 2).

### 3.3. Prostaglandin synthesis determination

Fig. 1 shows that AcF (100 mg/kg) was able to maintain a high PGE$_2$ level despite administration of indomethacin. AcF was able to promote a sustained increase of PGE$_2$ levels, which is vital to the integrity of gastric mucosa. Furthermore, the association of AcF and NSAID was not capable of reducing levels of PGE$_2$ release in a significant manner.

### 3.4. Determination of the gastric mucus content

Still looking for a possible mechanism for the increase in the gastric mucosa protective factors, we investigated the effect of AcF on mucus production. Fig. 2, shows that pretreatment with carbenox-

**Table 2**
Effects of intraduodenal adminstration of cimetidine (100 mg/kg) and AcF from *I. suffruticosa* (100 mg/kg) on the biochemical parameters of gastric juice in pylorus-ligated rats. The results are the mean ± SD of five rats per group. ANOVA followed by Dunnett's test.

| Treatments (p.o.) | Dose (mg/kg) | pH (units) | Gastric juice (mg) |
|---|---|---|---|
| 12% Tween | 10 mL/kg | 3 ± 0 | 0.8 ± 0.4 |
| Cimetidine | 100 | 4 ± 0** | 0.8 ± 0.4 |
| AcF – *I. suffruticosa* | 100 | 3.4 ± 0.5 | 1 ± 0 |

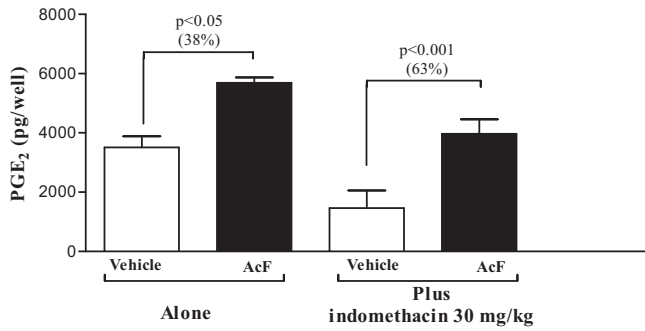** $P < 0.01$ compared to the corresponding vehicle group.

**Fig. 1.** Effect of orally administered AcF (100 mg/kg) from *I. suffruticosa* and indomethacin on gastric prostaglandin $E_2$ ($PGE_2$) production in rats. The results are reported as the mean ± S.E.M. ANOVA followed by Tukey's test. $p < 0.05$ compared to the corresponding vehicle group.

olone (200 mg/kg) and AcF (100 mg/kg) significantly increased the amount of adherent mucus in the gastric mucus mucosa when compared to the control group.

### 3.5. Determination of the role of nitric oxide (NO) and sulfhydryl compounds (SH) in gastric protection

Fig. 3, shows that N-ethylmaleimide (NEM) an SH blocker, significant attenuated the gastroprotective effect of AcF (100 mg/kg), which suggested that part of the protective action was mediated by endogenous SHs. On the other hands, when we administered L-NAME, an NO blocker, the gastroprotection was slightly affected (Fig. 4).

### 3.6. Healing in acetic-induced gastric lesion

In our experiments, the oral administration of AcF (100 mg/kg) for 14 consecutive days accelerated the healing of gastric ulcers (40%) in rats. The cimetidine group, utilized as positive group, accelerated the healing of gastric by 66% (Fig. 5).

### 3.7. Immunohistochemistry

In intention to evaluate the mechanism of healing of the gastric ulcer promoted by the AcF (100 mg/kg) histological analysis in the stomachs of the animals with acid acetic-induced gastric ulcer. We
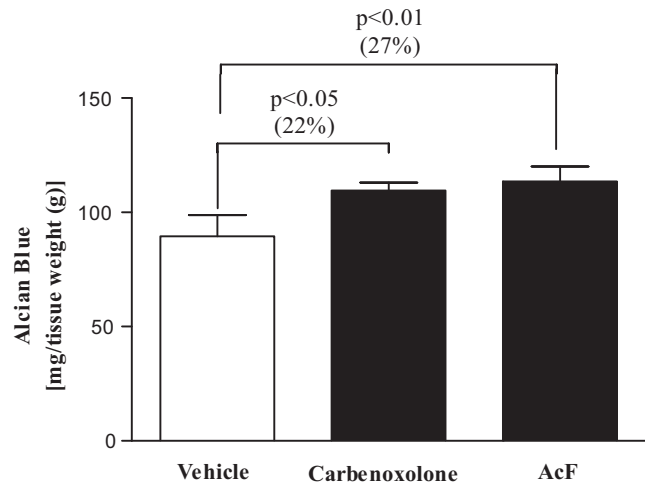


**Fig. 2.** Effect of orally administered AcF (100 mg/kg) from *I. suffruticosa* and carbenoxolone (200 mg/kg) on the production of adherent gastric mucus (measured as the amount of bound alcian blue) in pylorus-ligated rats. The results are reported as the mean ± S.D. ANOVA followed by Dunnet's test. $p < 0.01$ compared to the corresponding vehicle group.
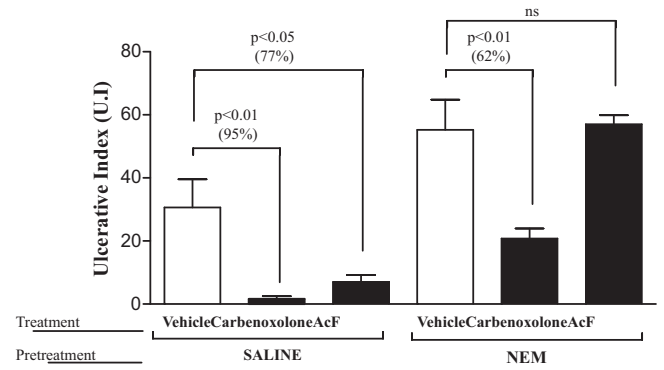


**Fig. 3.** The ulcer index for gastric ulcers induced by ethanol in rats pretreated with NEM (10 mg kg$^{-1}$) alone or together with carbenoxolone (100 mg/kg) and AcF (100 mg/kg) of *I. suffruticosa*. The results are reported as the mean ± S.D. ANOVA followed by Tukey's test. $p < 0.05$ compared to the corresponding vehicle group.
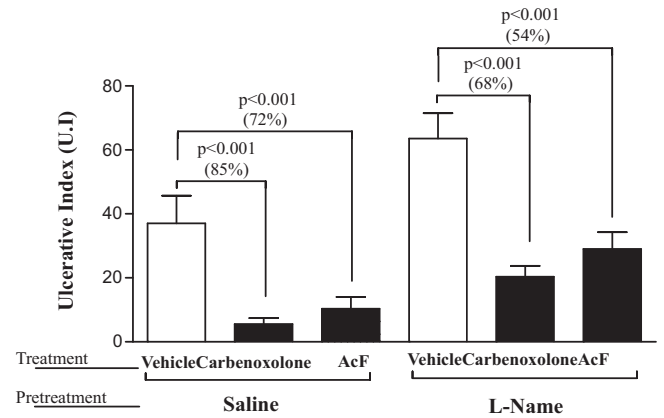


**Fig. 4.** The ulcer index for gastric ulcers induced by ethanol in rats pretreated with L-NAME (10 mg/kg) alone or together with carbenoxolone (100 mg/kg) and AcF (100 mg/kg) of *I. suffruticosa*. The results are reported as the mean ± S.D. ANOVA followed by Tukey's test. $p < 0.001$ compared to the corresponding vehicle group.
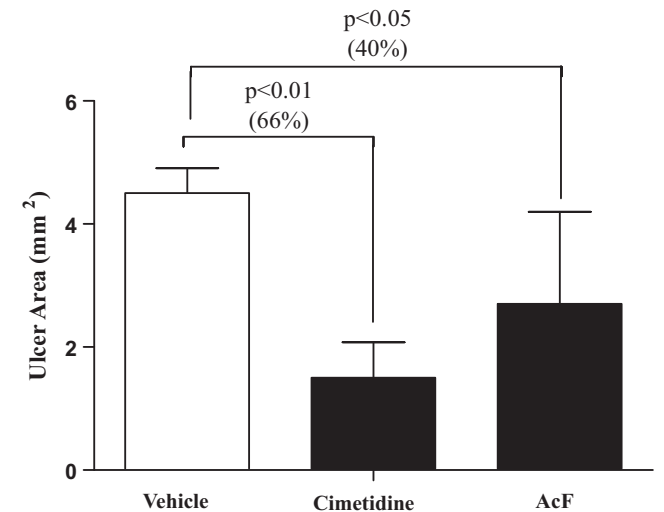


**Fig. 5.** Effect of orally administered AcF from *I. suffruticosa* on healing of ulcers produced by the injection of a 30% acetic acid solution into the stomachs of rats. The ulceration was scored on the 15th day after surgery. The results are reported as the mean ± S.D. ANOVA followed by Tukey's test. $p < 0.05$ compared to the corresponding vehicle group.
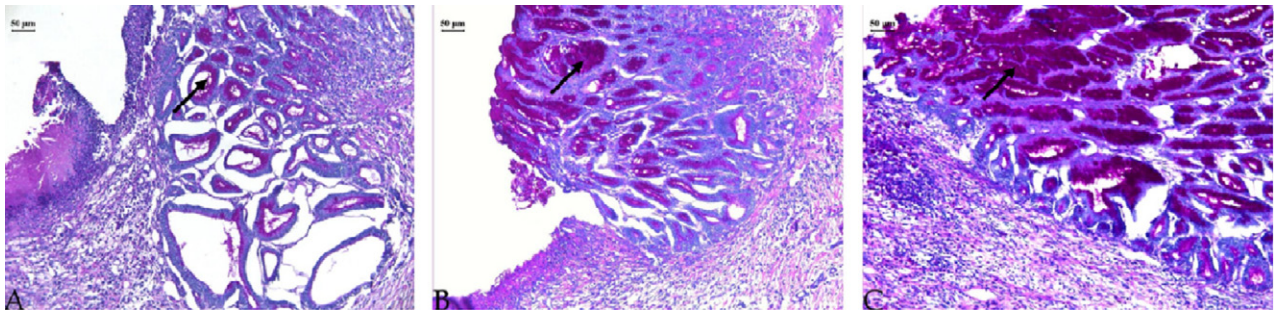
**Fig. 6.** Acetic-acid-induced gastric ulcer on day 14 after ulcer induction. Histological analyses of stomach of rat (A) vehicle (10 mL/kg), (B) cimetidine (100 mg/kg) and (C) AcF of *I. suffruticosa* (100 mg/kg), PAS method.
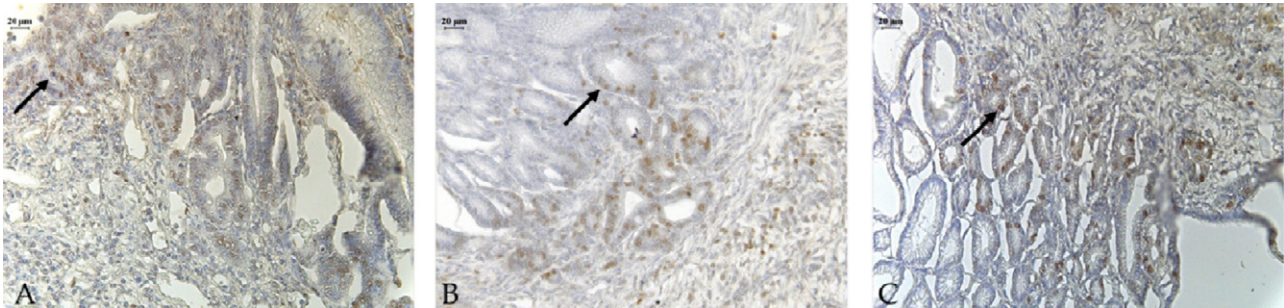


**Fig. 7.** Acetic-acid-induced gastric ulcer on day 14 after ulcer induction. Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) in the margin ulcer of animals treated with Vehicle (10 mL/kg), (B) cimetidine (100 mg/kg) and (C) AcF of *I. suffruticosa* (100 mg/kg).

investigated the gastric mucus production (PAS) and involvement of proliferating cell nuclear antigen (PCNA) and heat shock protein (HSP70).

The increased gastric mucus production using AcF was verified via an increase in PAS-positive mucus (Fig. 6) and it is an indicative of a gastroprotection a number of factors appear to influence ulcer healing; but mucus and bicarbonate secretion may be important in the ulcer healing process. In the morphologic analysis of the submitted slides with PCNA (Fig. 7), in all groups were observed cell proliferation, in AcF (100 mg/kg) there are cell proliferation in base of mucosa glands in lesion region. When we evaluated the immunostained area for HSP-70 was larger in cimetidine and AcF (100 mg/kg) groups, which indicates that these proteins participated in the gastroprotective effect of the AcF (Fig. 8).

## 4. Discussion

Peptic ulcer disease embraces both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality (Malfertheiner et al., 2009). It is estimated that peptic ulcer occurs in at least 10% of the world population (Grob, 2004). The complex and multifactorial pathogenesis of peptic ulcer has been studied over several decades, and results from an imbalance of aggressive gastric luminal factors acid and pepsin and defensive mucosal barrier function (Mota et al., 2009).

The present study was designed to assess the antiulcerogenic activity and action mechanisms of an AcF of *I. suffruticosa*. The phytochemical screening indicated that the main constituents of the AcF were flavonoid glycosides derived from quercetin and alkaloids.

Flavonoids and alkaloids protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species (de Sousa Falcão et al., 2008; Mota et al., 2009). Several mechanisms have been proposed to explain the gastroprotective effect of flavonoids; these include anti-secretory, cytoprotective and antioxidant activity (Mota et al., 2009). Recent studies showed that the alkaloid indigo obtained from *Indigofera truxilensis* significantly inhibits gastric lesions in rats (Farias-Silva et al., 2007).

Studies focusing on the pathogenesis of ethanol-induced gastric mucosal injury suggest that ethanol acts by exerting a direct toxic effect on the epithelium, leading to the formation of characteristic
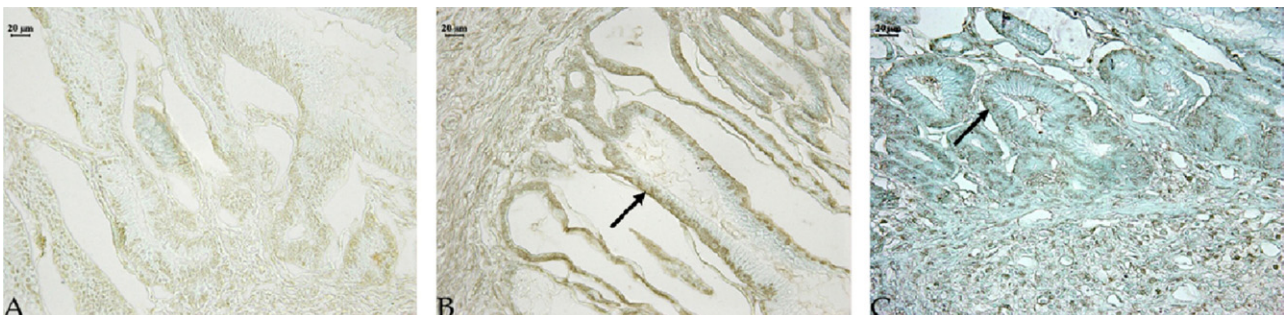


**Fig. 8.** Acetic-acid-induced gastric ulcer on day 14 after ulcer induction. Immunohistochemical staining of Heat Shock Protein 70 (HSP70) in the margin ulcer of animals treated with vehicle (10 mL kg$^{-1}$), (B) cimetidine (100 mg/kg) and (C) AcF of *I. suffruticosa* (100 mg/kg).

necrotic lesions due to a reduction in the mucus production and bicarbonate secretion (Massignani et al., 2009) that initial event is disruption of the vascular endothelium resulting in increased vascular permeability, edema formation, and epithelial lifting (Kvietys et al., 1990).

It is well known that ethanol-induced gastric lesions are not inhibited by anti-secretory agents like cimetidine, but are inhibited by agents who enhance mucosal defensive factors (Morimoto et al., 1991). The gastroprotetive effect observed in our ethanol-induced ulcer model indicates that AcF (100 mg/kg) enhanced the cytoprotective mechanisms of the gastric mucosa, since it does not alter the secretion acid.

Non-steroidal anti-inflammatory drugs (NSAID) are one of the most widely prescribed medication in the world (Sostres et al., 2010). NSAID injures the upper and lower gut by depleting COX-1 derived prostaglandins and causing topical injury to the mucosa (Sostres et al., 2010). Prostaglandins play important roles in modulating the mucosal integrity and various functions of the gastrointestinal tract (Malfertheiner et al., 2009). We observed that a single AcF treatment was able to increase $PGE_2$ level of gastric mucosa ($5692 \pm 180.4$ pg/well) when compared to vehicle group ($3512 \pm 368.4$ pg/well). AcF significantly ($p < 0.05$) increased $PGE_2$ to levels approximately 38% those in the vehicle group. But when AcF was administered jointly with indomethacin, a cyclooxygenase inhibitor, this fraction still maintained higher $PGE_2$ levels ($3968 \pm 489.5$ pg/well). The same results did not occur under treatments with vehicle and NSAID, since NSAID administration significantly diminished the $PGE_2$ levels ($1466 \pm 593$ pg/well).

Mucus contributes to mucosal defense by providing a physical barrier against bacteria and acts as a lubricant to reduce physical abrasion of the mucosa. Mucus also protects the mucosa from damage induced by acid and luminal toxins (Laine et al., 2008). Pretreatment with AcF-induced significant increase of gastric mucus production (27%), a rise which is correlated with its anti-ulcer effect observed throughout these studies.

A network of endogenous factor is involved in the regulation of mucosal gastric functional homeostasis. We evaluated the role of endogenous nitric oxide (NO) and sulfhydryl compounds (SH) in the gastroprotective action of AcF. When we observed that even with the nitric oxide synthetase inhibitor (L-NAME), the pretreatment with AcF maintained its gastroprotective action against ethanol gastric damage (54%), thereby showing that its activity does not depend on NO. But when the animals had been pretreated with NEM, a sulfhydryl (SH) inhibitor, the gastroprotective effects of AcF stopped acting on gastric mucosa. These results demonstrated that the activity of the AcF is directly related to the presence of SH compounds in the gastric mucosal barrier.

Sulfhydryl groups (SH) have a broad range of roles in the cell, and the redox status of cysteine residues can affect the structure and function of numerous enzymes, receptors and transcription factors (Grant, 2001). SH have been implicated in the maintenance of gastric integrity, particular when reactive oxygen species are involved in the pathophysiology of tissue damage (Kimura et al., 2001). Endogenous sulfhydryl radicals and other antioxidant mechanisms appear to play significant roles in counteracting gastric injury associated with *Helicobacter pylori* infection (Jung et al., 2001).

The ulcer produced by the injection of acetic acid into the rat stomach wall was assumed to be similar to the human chronic ulcer, since it is difficult to be treated and it takes a long time to be healed (Takagi et al., 1969). Studies demonstrated that reepithelialized mucosa of grossly "healed" experimental gastric ulcers has prominent histologic and ultrastructural abnormalities reduced height, marked dilation of gastric glands, increased connective tissue, a disorganized microvascular network and increased capillary permeability (Tarnawski et al., 1991). These prominent abnormalities

may interfere with mucosal defense and cause ulcer recurrence when ulcerogenic factors are present (Tarnawski, 2005).

Postoperative treatment with AcF for 14 consecutive days demonstrated, for the first time, that this plant accelerated ulcer healing. On the day 14 after surgery, the percentage of rats with cicatrised ulcers in AcF was significantly higher than the vehicle group.

Ulcer healing, a genetically programmed repair process, includes inflammation, cell proliferation, reepithelialization, formation of granulation tissue, angiogenesis, interactions between various cells and the matrix and tissue remodeling, all resulting in scar formation (Tarnawski, 2005).

Proliferating cell nuclear antigen (PCNA) is well known as a cell cycle marker, is well known as a DNA sliding clamp for DNA polymerase delta and as an essential component for eukaryotic chromosomal DNA replication and repair (Naryzhny, 2008). In the morphologic analysis of the submitted slides with PCNA, in all groups were observed cell proliferation, in AcF there are cell proliferation in base of mucosa glands in lesion region. Cell proliferating also denotes that AcF induces the expression of the growth factor in the gastric mucosa, thus leading to the gastric ulcer healing activity.

Heat Shock Protein (HSP) is generally induced when cells are subjected to noxious stimuli (Okabe and Amagase, 2005). HSPs may contribute to mucosal protection and ulcer healing by regulating the activity of enzymes such as cyclooxygenase and nitric oxide synthase (Rokutan, 2000), as well as by increasing mucosal blood flow (Shichijo et al., 2003).

We did not determine the histological localization, HSP70 appears to be expressed in growing cells in the ulcer margin. Indeed, a strong relationship has been reported between the expression of HSP70 and a marker for cell proliferation, such as PCNA as observed in human breast cancer biopsy samples. Based on these findings, it was suggested that HSP70 expressed in the proliferating cells and it was involved in the mucosal regeneration in the phase of ulcer healing. In regards to HSP70, the level expressed in normal mucosa was quite low (Tsukimi et al., 2001).

## 5. Conclusion

In conclusion, the promising results found with the AcF obtained from *I. suffruticosa* inhibited the gastric mucosal lesions induced by ethanol and NSAID in rats. Prostaglandin, mucus and SHs are involved in the gastroprotection by AcF. In addition, the expression of PCNA and HSP70 contribute to accelerate the process of healing of the gastric ulcer promoted by the AcF. In view of our results, more precise investigation of their function could provide insight into the mechanism of gastric cytoprotection and the role of active flavonoids and alkaloids presents in AcF.

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