Antiulcerogenic and ulcer healing effects of Indian propolis in experimental rat ulcer models

S. Iyyam Pillai¹, M. Kandaswamy¹, and S. Subramanian²*

¹Department of Inorganic chemistry, University of Madras, Guindy Campus, Chennai-600 025, Tamilnadu, India
²Department of Biochemistry, University of Madras, Guindy Campus, Chennai-600 025, Tamilnadu, India

Received 16 September 2009, accepted subject to revision 17 December 2009, accepted for publication 23 December 2009.
*Corresponding author: Email: subbus2020@yahoo.co.in

Summary

Propolis is a resinous hive product collected by worker bees from various parts of the plants. It is widely used in Indian folk medicine for the treatment of stomach ulcers. The preventive and curative effects of Indian propolis for ulcers were evaluated using models of acute gastric lesions induced by ethanol and indomethacin in rats. Moreover, the effects of ethanolic extract of propolis on gastric content volume, total acidity and pH, using the pylorus ligated model were also evaluated. Animals pretreated with propolis 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 propolis (300 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 Indian propolis extract displayed an antisecretory activity, which led to a significant reduction in the gastric juice volume, total acidity and pH. These findings indicate that Indian propolis displays both ulcer preventive and ulcer curative properties and provides a scientific rationale for the use of propolis in the traditional medicinal system.

Keywords: Indian propolis, ulcer index, ulcer preventive, ulcer curative, antisecretory

Introduction

The term ulcer was first coined by Quike in 1882 (Clinch, 1989) and it is now regarded as the new “plague” of the 21st century (O’Melley 2003). A peptic ulcer is a benign lesion in the lining of the stomach or duodenum, where acid and pepsin bathes the surface. Gastric mucus is a highly hydrated viscoelastic gel that protects the epithelium from mechanical stress, as well as from erosion by acid and pepsin (Allen et al., 1993). Peptic ulcers are caused when the balance between aggressive factors (such as acid and pepsin) and defence mechanisms (such as mucus, bicarbonate, blood flow and mucosal turnover) are shifted in favour of the former (Lima et al., 2006). Factors such as stress, smoking, alcohol usage, nutritional deficiencies and frequent ingestion of non-steroidal-anti-inflammatory drugs (NSAIDs) have been shown to contribute to gastric ulcer incidence (Belaiche et al., 2002). 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 (Chan and Leung, 2002). On rare occasions, a gastric ulcer may become malignant.

The first drug effective against gastric ulcer was carbenoxolone, which was discovered as a result of research into a commonly used indigenous plant, Glycyrrhiza glabra (Leguminosce) (Hossenbocus and Colin-Jones, 1974). Studies on cabbage, previously employed as an antulcer agent in folk medicine, has led to the development of gefarnate (Barbara et al., 1974). Although histamine H₂-receptor blockers (for example, ranitidine and famotidine), proton-pump inhibitors (for example omeprazole and lansoprazole), antibiotics (for example metronidazole, amoxicillin, clarithromycin, and tetracycline,) and other drugs are extensively used in the management of peptic ulcers, there are reports of adverse effects and relapse within one year (Wolfe and Sachs, 2000). A rational therapy still remains elusive and the search for safer potential drugs continues.
Traditionally plants have not only provided food and shelter for mankind, but have also been used to cure many different ailments (Gilani and Rahman, 2005). Propolis is the generic name for a strongly adhesive resinous material gathered by honey bees (*Apis mellifera* L.) from various plant sources (Moreno et al., 2000). Etymologically, the Greek word propolis is comprised of *pro*, (for before) and *polis*, (for the city), which means “defence of the hive”. Bees use propolis to seal the holes in their honey combs, smooth out internal walls and to cover carcasses of intruders that died inside the hive, in order to avoid their decomposition. Propolis also protects the colony from diseases because of its antiseptic efficacy and antimicrobial properties (Salatino et al., 2005).

Propolis contains more than 300 components, including phenolic aldehydes, polyphenols, sequiterpene quinines, coumarins, steroids, amino acids and inorganic compounds (Khall, 2006). Plant origin and the region of collection bear great significance with respect to the composition of propolis (Christov et al., 2006). Despite the chemical differences, it is well known that samples of different geographical origin and chemical composition usually demonstrate similar pharmacological properties (Marquele et al., 2005). The chemical properties of propolis are not only beneficial to bees but have general pharmacological value as a natural mixture (Teixeira et al., 2005). Administration of propolis to mice or humans does not seem to have side effects (Jasprica et al., 2007). Propolis is still a frequently used remedy for internal and external complaints in many parts of India. Recently, propolis has been extensively used in food and beverages to improve health and prevent disorders such as heart disease, diabetes, inflammation and cancer. (Hirota et al., 2000; Na et al., 2000).

The ethnopharmacological approach to propolis combined with chemical and biological methods may provide useful pharmacological leads. In view of recent reports of therapeutic properties, the present study was designed to investigate the preventive (pretreatment) and curative (post-treatment) potential of an ethanolic extract of Indian propolis using experimental models of gastric ulcer in rats, to suggest a mechanism for its pharmacological action. Both antisecretory and cytoprotection effects were evaluated.

### Methods and materials

#### Drugs and chemicals

Indomethacin, omeprazole and cimetidine were purchased from Sigma Aldrich, (St Louis, MD, USA). All other used reagents and solvents were of analytical grade. All the drugs and reagents were prepared freshly before use.

#### Preparation of Propolis extract

Propolis samples were collected in Mudivaithanendal, Tamil Nadu, India from *Apis mellifera* hives and kept desiccated in the dark until processing. Samples were air-dried at 40°C for 48 h. 100g of propolis powder was extracted in 500 ml of ethanol by stirring overnight at room temperature (Gekker et al., 2005). After filtration, the extract was concentrated in a rotary evaporator under reduced pressure at 60°C and the residue was stored in the dark at room temperature until further use. The yield of the ethanolic extract was 8.5% (w/w). A known amount of the solvent free extract was suspended in water to obtain the desired concentration of the propolis.

#### Experimental animals

Healthy, male rats of Wistar strain (160–180 g) were selected for the present study. The animals were provided by the Central Animal House of the Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were housed in cages with raised floors of wide mesh to prevent coprophagy and maintained on sterile, standard pellet diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The light cycle was automatically controlled and room temperature regulated to 22 ± 1°C. The experiments were designed and conducted to meet the ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No. 01/032/04) on animal care. Before initiating the experiments, the animals were allowed to acclimatize to animal house conditions for a period of one week.

#### Acute oral toxicity studies

The acute oral toxicity studies were conducted in the rat model (both sexes), 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, propolis extract 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. The signs and symptoms associated with a single dose of 7000 mg/kg body weight (bw) 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. Observations included changes in body mass, food and water consumption, hair loss, eyes and mucus membrane and autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and the central nervous system (optosis, drowsiness, gait, tremors and convulsion). On the 14th day both control and propolis treated animals were sacrificed, blood was collected and analysed for haematological parameters such as red and white blood cell counts, haemoglobin, haematocrit (HCT) and
mean corpuscular volume (MCV). Biochemical parameters, such as blood glucose, blood urea, protein, creatine, cholesterol and lipid profile were also assessed. The activity of pathophysiological enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were also assayed. Vital organs such as liver, kidney, heart and lungs were observed macroscopically.

The effect of propolis pre-treatment using the ethanol induced ulcer model
The study was performed according to the method of Büyükokuroğlu et al., (2002). After 12 h of fasting, the rats were randomly divided into six groups of eight 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 (Süleyman et al., 2002). Groups 3, 4 and 5 received 100, 200 and 300 mg/kg bw of propolis extract respectively and group 6 animals were treated with omeprazole (30 mg/kg bw). All pretreatments were administered orally. One hour later all of the animals in groups 3, 4, 5 and 6 were given 1 ml of 99.5% ethanol by oral gavages to induce gastric ulcers (Süleyman et al., 2002). After a lapse of 1 h the animals were sacrificed by cervical dislocation and stomachs were removed and opened along the greater curvature. The stomach was gently rinsed with water to remove the gastric contents and blood clots for subsequent ulcer scoring.

After 15 days, all animals were sacrificed by cervical dislocation, the stomachs was dissected out and opened along the greater curvature. Stomachs were gently rinsed with water to remove the gastric contents and blood clots for subsequent ulcer scoring.

The effect of propolis pre-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 300 mg/kg bw propolis extract 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 each. The rats were fasted for 24 h with free access to water. Thirty minutes after oral administration of single dose of propolis extract (300 mg/kg bw), cimetidine (100 mg/kg bw) 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 (Shay et al., 1945). 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.

Determination of degree of ulceration (ulcer index)
The surface area (A) mm² covered by each lesion was measured (Murakami et al., 1992) and the sum of erosion areas per rat stomach was calculated. Percentage ulcerated surface (US) was calculated as

\[
\% \text{ US} = \frac{\text{Total area covered by ulcer}}{\text{Total corpus mucosal surface}} \times 100
\]

Ulcer index was calculated from percentage ulcerated surface as

The effect of propolis post-treatment using the ethanol induced ulcer model
Male Wistar rats weighing about 160-180 g were divided into four groups of at least 6 rats each. Group I was the control group which received 1 ml water only. Ulcers were induced in all rats in groups II, III and IV by 1 ml 99.5% ethanol by oral gavages (Süleyman et al., 2002). Rats in group III were treated with 300 mg /kg bw propolis extract for each of 15 successive days. Rats in group IV were treated with 30 mg / kg bw omeprazole for each of 15 successive days.
described by Tan et al., (1996). The following score was used in order to calculate ulcer index:

| 0. No ulcer | 6. 15 - 20 |
| 1. US <0.5 | 7. 20 - 25 |
| 2. 0.5 - 2.5 | 8. 25 - 30 |
| 3. 2.5 - 5 | 9. 30 - 35 |
| 4. 5-10 | 10. US >35 |
| 5. 11-15 |

Statistical analysis
The values are expressed as mean ± SEM for six rats in each group. All the data were analyzed with SPSS/13 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc performed with Least Significant Difference (LSD) test. A p value of less than 0.05 was considered to indicate statistical significance.

Results
The acute oral toxicity evaluated after a single oral administration of 7000 mg/kg bw of propolis 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 propolis treated group of rats when compared to animals in the control (untreated) group. Oral administration of propolis extract showed no effect on serum pathophysiological enzyme activities. Macroscopic observation on vital organs also confirmed the non-toxic nature of propolis. These preliminary studies indicate the absence of acute toxic effects of Indian propolis.

Proplis Pretreatment studies
The ulcer preventive effect of the propolis extract on both ethanol and indomethacin induced gastric lesions in experimental rats is summarized in Tables 1 and 2. Pretreatment with propolis 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 ulcer preventive effects of propolis in both models were comparable to standard antilucerogenic drugs omeprazole and cimetidine, respectively. The results obtained suggest that the crude extract of propolis has a significant antilucer effect in each of these ulcer induced models.

Table 1. Effect of propolis pretreatment on ethanol induced ulcerated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcerated surface (%)</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Ethanol induced ulcer</td>
<td>27.18 ± 1.09 a*</td>
<td>8</td>
</tr>
<tr>
<td>Ethanol + Propolis extract (100 mg/kg bw)</td>
<td>11.27 ± 0.83 b*</td>
<td>5</td>
</tr>
<tr>
<td>Ethanol + Propolis extract (200 mg/kg bw)</td>
<td>4.88 ± 0.51 b*</td>
<td>3</td>
</tr>
<tr>
<td>Ethanol + Propolis extract (300 mg/kg bw)</td>
<td>1.93 ± 0.27 b*</td>
<td>2</td>
</tr>
<tr>
<td>Ethanol + Omeprazole (30 mg/kg bw)</td>
<td>1.66 ± 0.31 b*</td>
<td>2</td>
</tr>
</tbody>
</table>

Values of ulcerated surfaces (%) are expressed as mean ± SEM of eight rats in each group. * p<0.05. Using one way ANOVA followed by post hoc test LSD. Comparisons are made between Control a, Ethanol induced ulcer b.

Table 2. Effect of propolis pretreatment on indomethacin induced ulcerated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcerated surface (%)</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Indomethacin induced ulcer</td>
<td>51.24 ± 5.49 a*</td>
<td>10</td>
</tr>
<tr>
<td>Indomethacin + Propolis extract (100 mg/kg bw)</td>
<td>24.18 ± 2.07 b*</td>
<td>7</td>
</tr>
<tr>
<td>Indomethacin + Propolis extract (200 mg/kg bw)</td>
<td>12.97 ± 1.21 b*</td>
<td>5</td>
</tr>
<tr>
<td>Indomethacin + Propolis extract (300 mg/kg bw)</td>
<td>2.44 ± 0.68 b*</td>
<td>2</td>
</tr>
<tr>
<td>Indomethacin + Cimetidine (100 mg/kg bw)</td>
<td>2.09 ± 0.71 b*</td>
<td>2</td>
</tr>
</tbody>
</table>

Values of ulcerated surfaces (%) are expressed as mean ± SEM of eight rats in each group. * p<0.05. Using one way ANOVA followed by post hoc test LSD. Comparisons are made between Control a, Ethanol induced ulcer b.
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 propolis extract at a concentration of 300 mg/kg b.w 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 propolis extract, 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).

### Table 3. Effect of propolis on the extent of ulceration in ethanol induced ulcerated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcerated surface (%)</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Ethanol induced ulcer</td>
<td>49.68 ± 5.17 a*</td>
<td>10</td>
</tr>
<tr>
<td>Ethanol + Propolis extract (300 mg/kg b.w)</td>
<td>4.18 ± 0.56 b*</td>
<td>3</td>
</tr>
<tr>
<td>Ethanol + Omeprazole (30 mg/kg b.w)</td>
<td>3.95 ± 0.31 b*</td>
<td>3</td>
</tr>
</tbody>
</table>

Values of ulcerated surfaces (%) are expressed as mean ± SEM of eight rats in each group. * p<0.05. Using one way ANOVA followed by post hoc test LSD. Comparisons are made between Control a, Ethanol induced ulcer b.

### Table 4. Effect of propolis on the extent of ulceration in indomethacin induced ulcerated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcerated surface (%)</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Indomethacin induced ulcer</td>
<td>78.83 ± 6.41 a*</td>
<td>10</td>
</tr>
<tr>
<td>Indomethacin + Propolis extract (300 mg/kg bw)</td>
<td>4.59 ± 0.73 b*</td>
<td>3</td>
</tr>
<tr>
<td>Indomethacin + Cimetidine (100 mg/kg bw)</td>
<td>3.19 ± 0.54 b*</td>
<td>3</td>
</tr>
</tbody>
</table>

Values of ulcerated surfaces (%) are expressed as mean ± SEM of eight rats in each group. * p<0.05. Using one way ANOVA followed by post hoc test LSD. Comparisons are made between Control a, Ethanol induced ulcer b.

### Table 5. Effect of propolis on the extent of ulceration in pylorus-ligated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg bw)</th>
<th>Total gastric volume (ml)</th>
<th>Gastric acidity (mEq/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>2.61 ± 0.73</td>
<td>5.69 ± 0.83</td>
<td>2.17 ± 0.41</td>
</tr>
<tr>
<td>Propolis</td>
<td>300</td>
<td>1.63 ± 0.08 a*</td>
<td>3.49 ± 0.21 a*</td>
<td>4.23 ± 0.34 a*</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100</td>
<td>1.48 ± 0.06 a*</td>
<td>3.27 ± 0.25 a*</td>
<td>4.08 ± 0.22 a*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of ten rats in each group. One way ANOVA followed by post hoc test LSD. * p<0.05. Comparisons are made between Control a.

### Propolis 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 propolis extract at a concentration of 300 mg/kg b.w 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 propolis extract, 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).

### Discussion

In this propolis antiulcerogenic study, acute toxicity in rats was investigated and a single oral administration of propolis at a concentration of 7000 mg/kg indicated the non-toxic nature of Indian propolis. After treatment of rats with different concentrations of propolis (up to 6 mg/kg/day) different extracts (water or ethanol) and varying time of administration (30, 90 and 150 days) has been demonstrated not to induce significant alterations in haematological or biochemical parameters (Mani et al., 2006). Administration of propolis to mice or humans does not seem to have side effects (Cuesta et al., 2005; Jas Prica et al., 2007). Burdock (1998) reported that the safe dose of propolis for humans could be 70 mg/day. Here dosage fixation studies indicate that the Indian propolis extract at a concentration of 300mg/kg bw/p.o showed the maximum antiulcerogenic activity in both alcohol and indomethacin induced experimental ulcer models in rats.
Characteristically propolis is a lipophilic material that is hard and brittle when cold, but soft, pliable and sticky when warm. Hence the name bee glue (Hausen et al., 1987). The usual manner to extract the fraction soluble in alcohol yields ‘propolis balsam’, leaving the alcohol insoluble or wax fraction (Ghisalberti, 1979). Since ethanol has a low viscosity and readily solubilizes propolis, ethanolic extract is found to be more effective than the aqueous extract in eliciting biological properties (Kawabe et al., 2000). Therefore, the present study was carried out with an ethanol extract of Indian propolis.

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. The ulcer preventive and ulcer curative activities of Indian propolis were evaluated using ethanol, indomethacin and pylorus ligated ulcer models; the most commonly used experimental models for the evaluation of antulcer activity is in rats. Based on the results obtained, it is suggested that Indian propolis extract could prevent and cure gastric lesions caused by necrotizing agents such as alcohol, ulcerogenic agents such as indomethacin and the pylorus ligated model. Gastric mucosal damages induced by different experimental ulcer models have different mechanisms (Samonina et al., 2004). However, the net imbalance in offensive and defensive factors brought about by them is thought to be the cause of ulcerogenesis (Goel and Bhattacharya, 1991).

Mucus serves as the first line of the defence against ulcerogens. The mucus is secreted by mucus neck cells and covers the entire gastrointestinal mucosa thereby preventing physical damage and back diffusion of hydrogen ions (Williams and Turnberg, 1980). Gastric mucus consists of a viscous, elastic adherent and transparent gel formed by water and glycoproteins. The protective effects of mucus barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface. The ability of the gastric mucosa to resist injury caused by endogenous secretions (acid, pepsin and bile) and by ingested irritants such as alcohol, aspirin and NSAIDs can be attributed to a number of factors that have been generally referred as mucosal defence (Wallace, 2001).

Ulcers caused by ethanol are due to superficial damage to the mucosal cells (Miller and Henagan, 1984). Exposure to ethanol increases the extension of cellular damage in a dose dependent way (Mutoh et al., 1990). Ethanol induced gastric damage may be due to stasis in gastric blood flow, which contributes to the development of haemorrhage and necrotic aspects of tissue injury (Guth et al., 1984). This action is direct on the gastric epithelium also causing perturbation of mast cells and release of a vasoactive mediator such as histamine (Oates and Hakkinen, 1988). Some reports show that changes in gastric circulation after ethanol administration remains unknown, but it has been reported that microcirculation damage can be prevented by prostaglandin administration (Guth et al., 1984). 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 (Pihan et al., 1987) and hyperoxidation of lipids (Puurunen et al., 1980). These data suggest that ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. Pihan et al., (1987) reported that oxygen-derived free radicals are involved in the mechanism of acute and chronic ulceration in the gastric mucosa. Furthermore, disturbances in gastric secretions, damage of gastric mucosa, alterations in permeability, gastric mucus depletion and free radical production are observed after the administration of ethanol (Salim, 1990). These data suggest that antioxidant compounds could be active in this experimental model, producing antiulcerogenic effects. This effect is known as cytoprotection.

The results of the present study indicate that propolis extract displays an antiulcerogenic effect related to its cytoprotective activity, since it significantly reduced ethanol induced ulcers. Many reports suggest that phytochemicals such as flavonoids and phenolic compounds, which are well known for the antiulcer activity, and other antioxidant compounds could be active in this experimental model producing antiulcerogenic effect (Havsteen, 2002; Repetto and Llesuy, 2002). Therefore the observed ulcer preventive and ulcer curative activity of Indian propolis extract may be partially due to its relative antioxidant activity.

Chronic administration of non-steroidal antiinflammatory drugs (NSAIDs) such as indomethacin, during the course of anti-inflammatory therapy, is often associated with the development of adverse gastrointestinal disorders such as gastric erosions, gastric or duodenal ulceration and other severe complications such as gastrointestinal haemorrhage or perforation that often limited their wide spread clinical use (Villegas et al., 2004). Indomethacin is known to induce gastric ulcer by inhibition of prostaglandins which are cytoprotective to gastric mucosa (Wallace, 2001), particularly due to the inhibition of cyclooxygenase pathway of arachidonic acid metabolism resulting in excessive production of leukotrienes and other products of 5-lipoxygenase pathway (Rainsford, 1987). In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair (Hayllar and Bjarnason, 1995). Thus, the suppression of prostaglandins synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastroduodenal ulceration. Several studies have indicated that gastroduodenal protection by prostaglandins is due to increasing the mucosal resistance as well as the decrease in aggressive factors, mainly acid and pepsin (Aly, 1987). The observed antiulcerogenic property of propolis may be due to increased synthesis of mucous.
and/or prostaglandins or could possibly be due to its 5-lipoxygenase inhibitory effect.

A review of antiulcer drugs of plant origin shows that triterpenes (because their ability to strengthen defensive factors such as stimulation of mucous synthesis or maintenance of the prostaglandins content of gastric mucosa at high levels) are potentially the compounds with antiulcer activity (Lewis and Hanson, 1991).

The ligature of the pyloric sphincter leads to an accumulation of gastric juice in the stomach. The pylorus ligated technique revealed that propolis extract possess anti-secretary inhibitory effect.

In conclusion, the results of the present study show that Indian propolis 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. These results were similar to the results obtained with Brazilian green propolis extract, (de Barros et al., 2007) suggesting that the observed effects of propolis is probably a synergistic effect between reported phytochemicals. Although the active compounds of Indian propolis are not identified quantitatively, the presence of flavonoids, triterpenes and glycosides (Kumar et al., 2009) may be regarded as possible active compounds against gastric lesions by increase of protective factors or antioxidant activity.

**References**


JASPRICA, I; MORNAR, A; DEBELJAK, Z; SMOLCIC-BUBALO, A; MEDIC-SARC, M; MAYER, L; ROMIC, Z; BUCAN, K; BALOG, T; SOBOCANE, S; STEFANO, V (2007) *In vivo* study of propolis supplementation effects on antioxidative status and red blood cells. *Journal of Ethnopharmacology* 110: 548-554. DOI: 10.1016/j.jep.2006.10.023


MURAKAMI, S; ARAI, I; MURAMATSU, M; OTOMO, S; BABA, K; KIDO, T; KOZAWA, M (1992) Inhibition of gastric H⁺,K⁺-ATPase and acid secretion by cassigarol A, a polyphenol from Cassia garrettiana Craib. *Biochemical Pharmacology* 44: 33-37. DOI: 10.1016/0006-2952(92)90034-G

MUTOH, H; HIRAISHI, H; OTA, S; IVEY, K J; TERANO, A; MURAKAMI, S; ARAI, I; MURAMATSU, M; OTOMO, S; BABA, K; KOZAWA, M (1992) Inhibition of gastric H⁺,K⁺-ATPase and acid secretion by cassigarol A, a polyphenol from Cassia garrettiana Craib. *Biochemical Pharmacology* 44: 33-37. DOI: 10.1016/0006-2952(92)90034-G


