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Anti-ulcerogenic Screening of *Cichorium intybus* L. Leaf in Indomethacin Treated Rats

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Abstract: Although introduction of modern drugs to the classic anti-ulcer therapy has been revolutionized the treatment of peptic ulcer but the complete cure of the disease has yet to be discovered. In an effort to explore indigenous medicinal plants the powdered leaves of *Cichorium intybus* (CI) and its extracts i.e., aqueous, ethanol, were investigated for their anti-secretory and anti-ulcer activities in the albino rats (Sprague-Dawley, weighing 180-220 g) with gastric ulcer induced by indomethacin (25 mg kg⁻¹, subcutaneous). The treatment of CI attenuated the indomethacin-induced changes in gastric juice volume, pH, acid-output and ulcer index in a dose-dependent manner. The aqueous (CI-Aq) and ethanol (CI-E) extracts (equivalent to 2.0 g kg⁻¹ of body weight) of the powder also protected the rats against gastric effects induced by indomethacin. CI and its extracts showed significant acid buffering *in vitro* activities. The findings indicate that CI and its extracts possess active principle(s) that protect the gastric mucosa against indomethacin-induced gastric changes, possibly due to its antioxidant effect that inhibits lipid peroxidation.

Key words: *Cichorium intybus*, anti-ulcer, anti-secretory, ulcer index, acid buffering, indomethacin

INTRODUCTION

Cichorium intybus, L. (Syn: Cichory, Kasni) (Compositae) has long been used for the treatment of different diseases in the traditional medicine. It has been prescribed in various forms for the treatment of Gastrointestinal (GIT) disorders including gastric ulcers. The plant is abundantly found in Pakistan (Kirtikar and Basu, 1987). Its young leaves have long been used as salad. All parts of the plant contain a bitter tasting milky white juice that can stimulate the appetite (Limami *et al.*, 1988; Kalantari and Chang, 1997). Plant has been found to be useful in thirst, headache, inflammation of eye and throat, enlargement of spleen, fever and vomiting (Aktay *et al.*, 2000; Amirghofran *et al.*, 2000). It has been reported to possess stomachic, diuretic, antimicrobial actions and its seeds are considered tonic for brain. (Lee *et al.*, 2000; Kim and Shin, 1998). The alcoholic extract of seeds has been found to possess the anti-hepatotoxic activity [Ahmed *et al.*, 2003] The alcoholic extract of roots has been shown anti-tumour effect against Ehrlich ascites carcinoma in mice (Hazra *et al.*, 2002). Alkaloids; adenine and zeatin, carbohydrates like fructose and glucose, sesquiterpenes including cichoriolid A, B, C, crepidiaside B, sonchusid A, C and traces of Zn⁺², Si⁺³, K⁺, Na⁺, Mg⁺² and Fe⁺² have been reported to be present in the roots (Roberfroid, 2000; Rees and Harborne, 1984; Molan *et al.*, 2003). Astragalin, kaempferol-3-O-β-D-glucuronoids (flavonoids), aesculetin and aesculin (coumarins) have also been isolated from leaves while apigenin, apigenin-7-O-α-1-arabinoside and cyanaroside (flavonoids) in aerial

parts of the plant (Kisiel and Zielinska, 2001; Bais *et al.*, 1999; Hebette *et al.*, 1998). The presence of alpha-amyrin, taraxerone, baurenyl acetate and beta-sitosterol has also been reported to be isolated from roots (Du *et al.*, 1998).

MATERIALS AND METHODS

Plant drug and extracts: *Cichorium intybus*, leaf (CI) was purchased locally from herbal dealer in Bahawalpur-Pakistan. The leaves were authenticated and compared with standard in the herbarium maintained by Department of Botany, University of Agriculture, Faisalabad and a sample has been preserved in the Pharmacognosy laboratory, Department of Pharmacy, Islamia University Bahawalpur during 2002-2003. The shade-dried leaves were finely powdered to the mesh size 200 before the experimental use (Rifat-uz-Zaman *et al.*, 2004). The aqueous extract was prepared by maceration; 1.0 kg of the powder was soaked in about 1.5 L of distilled water for 24 h with occasional shaking. The extract was decanted; remaining material was re-extracted in the distilled water twice similarly. The collective extract was dried completely by Rotavapour at 37°C. The ethanol extract was also prepared by the same method (Rifat-uz-Zaman *et al.*, 2004).

Chemicals: The analytical grade chemicals were used in this study, which obtained from E. Merck (Darmstadt, FRG), BDH Poole (England) and Sigma Chemical Co. (USA).

Table 1: Effects of *C. intybus* leaf powder and its aqueous and ethanol extracts on gastric secretion volume, pH, acid-output and ulcer index in rats

| Group No. | Treatments | Gastric secretions | | | |
|-----------|--|-------------------------|----------------|---|------------------------------|
| | | Volume (mL) Mean±SEM | pH Mean±SEM | Acid Output (µEq/100 g/4 h) Mean±SEM | Ulcer Index (mm) Mean±SEM |
| 1 | Normal (Untreated control) | 2.20±0.06 | 2.82±0.01 | 2.82±0.15 | 2.62±0.26 |
| 2 | Indomethacin (25 mg kg ⁻¹ s.c.) (Treated Control) | 8.6±0.45** | 1.08±0.20** | 340.23±14.21** | 49.83±2.47** |
| 3 | <i>C. intybus</i> leaf (1.0 g kg ⁻¹ orally) +Indomethacin (25 mg kg ⁻¹ s.c.) | 3.47±0.09** | 1.70±0.07* | 49.45±2.24** | 20.04±1.41** |
| 4 | <i>C. intybus</i> leaf (1.5 g kg ⁻¹ orally) +Indomethacin (25 mg kg ⁻¹ s.c.) | 3.1±0.10** | 1.79±0.05* | 33.52±2.33** | 18.50±1.26** |
| 5 | <i>C. intybus</i> leaf (2.0 g kg ⁻¹ orally) +Indomethacin (25mg kg ⁻¹ s.c.) | 2.80±0.05** | 2.15±0.08** | 14.16±1.28** | 8.57± 0.15** |
| 6 | Aqueous extract of <i>C. intybus</i> leaf (2.0 g kg ⁻¹ orally) + Indomethacin (25 mg kg ⁻¹ s.c.) | 3.43±0.06** | 2.70±0.06** | 6.33±0.66** | 12.14±0.85** |
| 7 | Ethanol extract of <i>C. intybus</i> leaf (2.0 g kg ⁻¹ orally) + Indomethacin (25 mg kg ⁻¹ s.c.) | 5.43±0.15** | 1.51±0.05* | 120.89±2.45** | 21.87±1.76** |

Indomethacin: significant from untreated control (Normal) *p<0.05; ** p<0.001, Test drugs: significant from treated control (Indomethacin) * p<0.05; **p<0.001, All the other values are NS (p>0.05) from treated control (Indomethacin). Mean±SEM = Mean values±Standard Error of Means of six experiments

Test animals: Healthy adult albino male rats (Sprague-Dawley) weighing 180-220 g each were used in this study. The animals were housed under standard conditions of temperature (23±12°C), humidity (55±15%) and 12 h light/dark (7.00 am-7.00 pm). The rats were allowed a free access to a standard feed (M/S Lever Brothers, Rahim Yar Khan-Pakistan) and water *ad libitum*. The animals under trials were fasted for 24 h prior to their experimental use. (Rifat-uz-Zaman *et al.*, 2004). The use of animals in this study was in accord to the principles and guidelines of the Canadian Council on Animal Care.

Acid buffering activity: Five hundred milligram of CI powder and CI-Aq, CI-E (Equivalent to 500 mg of powder) dissolved in 2.0 mL of distilled water each treated with 10 mL of HCl solution, pH 1.0 separately. 2.0 mL of distilled water was added in the controlled test tubes in addition to above 10 mL of HCl solution. The final pH values were recorded after 1 and 30 min (Rifat-uz-Zaman *et al.*, 2005).

Pepsin binding capacity: The 500 milligram of CI powder and its equivalent CI-Aq and CI-E extracts were added into separate test tubes to 1 mL of pepsin solution, 2 mg mL⁻¹ each. 4 mL of 0.2 N HCl buffered with 1 mL of 0.2 N sodium citrate solutions to ensure the pH 1.6 was allowed to react. The excess pepsin was treated with protein (bovine serum albumin; 5 mg mL⁻¹) Biuret reagent was used to recover the excess protein (Rifat-uz-Zaman *et al.*, 2005).

Induction of gastric ulcer: The experimental gastric ulcers in rats were produced by the modified method of Yoshikawa *et al.* (1993). The pylorus of 24 h fasted rats

were ligated under the light ether anesthesia. The test substances were given orally, immediately following pylorus-ligation and indomethacin was injected subcutaneously (*s.c.*) in the treated groups of rats. All the test drugs were given to the animals by oral route t.d.s (8.00 am, 3.00 and 10.00 pm) for 14 consecutive days while pylorus was ligated on the 15th day (Yoshikawa *et al.*, 1993; Rifat-uz-Zaman *et al.*, 2004).

The normal (untreated) and treated control groups received 3 mL kg⁻¹ of 2.5% gum tragacanth vehicle orally. (Rifat-uz-Zaman, *et al.*, 2004). Rats of different treated groups received CI powder 1.0, 1.5 and 2.0 g kg⁻¹ of body weight while CI-Aq and CI-E (equivalent to 2.0 g kg⁻¹ of body weight of powder) orally, separately. The treated control and treated animals were administered a single dose of indomethacin 25 mg kg⁻¹ (*s.c.*) on 15th day of treatment. (Rifat-uz-Zaman *et al.*, 2004). Takeuchi *et al.* (2001) operative procedure was adopted. After ligation of pylorus, drinking water was withheld and gastric juices were allowed to collect for a period of 4 h. The gastric contents were collected through the esophagus. The gastric mucosa was washed with 3 mL of distilled water. The gastric contents and washings were homogenized and centrifuged at 4000 rpm for 10 min. The stomachs were inflated with 10 mL of 1% formalin and the esophagus ligated. The stomachs were then immersed in 10% formalin for 10 min to fix the inner and outer walls. The glandular portions of stomachs were examined for gastric ulcers following the incisions along the greater curvatures of stomachs (Hirohashi *et al.*, 1993; Takeuchi *et al.*, 2001).

Determination of gastric juice volumes, pH and acid-outputs: The volume and pH of centrifuged gastric

Table 2: Acid buffering activities of *C. intybus* leaf and its extracts (*in vitro*)

| Drug used | pH |
|--|--------------|
| Standard | 1.000±0.013 |
| Plant drug (500 mg) | 1.618±0.009* |
| Aqueous extract plant drug (equivalent to 500 mg) | 1.589±0.057* |
| Ethanol extract of plant drug (equivalent to 500 mg) | 1.12±0.0115* |

Test drugs: significant from standard * p<0.05

Mean±SEM = Mean values±Standard Error of Means of ten experiments

Table 3: Pepsin binding capacities (% age) of *A. graveolus* and its extracts (*in vitro*)

| Drug used | Pepsin binding % age |
|--|----------------------|
| Standard | 100 |
| Plant drug (500 mg) | 70.40±1.13** |
| Aqueous extract plant drug (equivalent to 500 mg) | 0.61±0.053 |
| Ethanol extract of plant drug (equivalent to 500 mg) | 0.35±0.032% |

Test drugs: significant from standard ** p<0.001

Mean±SEM = Mean values±Standard Error of Means of ten experiments

secretions were measured by pipette and pH meter, respectively (Hirohashi *et al.*, 1993). The acid-outputs were calculated by using the following equation according to the method of Ishizuka *et al.* (1996).

$EqH^+/100\text{ g/4 h} = 1/\text{antilog pH} \times 1000 \times \text{Volume of gastric juice (mL)} \times 100/\text{body weight of animal (g)}$

Calculation of gastric ulcer index: Glandular portions of stomachs were examined under a simple microscope to locate the gastric lesions in the gastric mucosa as elongated black-red lines parallel to the long axis. The length (mm) of each lesion was measured and lesion index was calculated by adding the length of all the lesions in the glandular region (Hirohashi *et al.*, 1993; Rifat-uz-Zaman *et al.*, 2004).

Statistical analysis: The data was expressed as means±SEM (Standard Error of Means) and analyzed statistically by the application of SPSS (Statistical Package for Social Sciences) for Windows version 7.5. The Student's t test was applied and P values were determined. Differences were considered non-significant at p>0.05, significant at p<0.05 and highly significant at p<0.001 (Sanders, 1990).

RESULTS

Acid-buffering and pepsin-binding effects: CI powder increased significantly (p<0.05) the pH of acidic solution from 1.0 upto 1.618±0.009. CI-Aq and CI-E were also significantly increased the pH levels of acidic solution. CI-Aq raised the pH from 1.0 up to 1.589±0.057 and CI-E from 1.0 upto 1.12±0.0115 (Table 2). However, CI exhibited highly significantly pepsin binding activity (70.40±1.13%) while CI-Aq and CI-E 0.61±0.053% and 0.35±0.032%, pepsin binding capacities, respectively (Table 3).

Anti-secretory and anti-ulcer effects: Oral administration of CI in 1.0-2.0 g kg⁻¹ body weight, doses prevented the changes in gastric juice volume, pH, acid-output and ulcer index (i.e., anti-ulcerogenic effect), induced by indomethacin in a dose-dependent manner. The effects were highly significantly (p<0.001) more with 2.0 g kg⁻¹ dose of the powder. CI-Aq and CI-E equivalent to 2.0 g kg⁻¹ of the powder also caused high significant (p<0.001) inhibition of gastric changes in the treated rats (Table 1).

DISCUSSION

Indomethacin and NSAIDs (non-steroidal anti-inflammatory drugs) have been known to be the potent inhibitor of cyclo-oxygenase enzyme (Levine, 2001). Kapui *et al.* (1993) have been reported a progressive decrease in the mucosal contents of PGI₂, PGE₂ and TxA₂ following indomethacin treatment while increase in Leukotrienes (LTs). The selective inhibition of cyclo-oxygenase pathway increases the level of LTs in gastric mucosa, which has potent action on mucosal vasculature leading to inflammation and pain (Kapui *et al.*, 1993; Reeves and Stables, 1985). It also potentiates further the secretory effects elicited by histamine due to the inhibition of PGE₂ synthesis. Therefore, gastric ulcer and erosion formation is accompanied by progressive decrease in the PGE₂ synthesis (Reeves and Stables, 1985). It has been found that programmed cell death (apoptosis) is an intrinsic part of organismal development and aging (Tabuchi *et al.*, 1994). The recent evidences have been shown that indomethacin and many other NSAIDs can cause apoptosis (Tabuchi *et al.*, 1994). The damage in the stomach is activated further acid secretion by a stimulatory pathway in addition to a PGs, NO and Ca⁺² dependent inhibitory mechanism. PGs may have a dual role in the regulation of acid secretion in the damaged stomach: an inhibitory effect at the parietal cell and an excitatory effect, through enhancing the release of mucosal histamine (Reeves and Stables, 1985; Tabuchi *et al.*, 1994).

Therefore, indomethacin raised gastric secretion volume and acid out-put while decreased pH highly significantly in the treated control group of rats in comparison to control group of animals. Similarly gastric ulcer formation was also caused by indomethacin highly significantly (Table 1) in the treated control animals. The similar gastric changes have already been reported due to its apoptosis activity (Smith and Marnett, 1991; Gurbuz *et al.*, 2002).

C. intybus leaf (CI) and its extracts showed significant acid-buffering activities (*in vitro*). CI bound to

pepsin highly significantly while CI-Aq and CI-E did not show such *in vitro* pepsin binding activity (Table 2 and 3) In the rats having indomethacin-induced ulcerations, CI in 1.0-2.0 g kg⁻¹ doses inhibited the gastric changes induced by indomethacin in a dose-dependent manner and 2.0 g kg⁻¹ dose exhibited maximum preventive effects. CI-Aq and CI-E also showed similar gastric effects (Table 1). The results are in accordance with Gurbuz *et al.* (2002).

Many workers have indicated the pro-oxidant and anti-oxidant activities of CI and its extracts (Papetti *et al.*, 2002; El and Karakaya, 2004). Pieroni *et al.* (2002) have further been reported their strong xanthine oxidase inhibition (*in vitro*) in addition to free radical scavenging activity.

The attenuation of indomethacin gastric effects indicates that the test drugs may interfere with the indomethacin-induced apoptosis activity. Yoshikawa *et al.* (1993) have been reported that the lipid peroxidation induced by oxygen radicals plays an important role in the pathogenesis of indomethacin-induced gastric mucosal changes as well as in gastric injuries. Moon *et al.* (2000) have shown that oxygen free radicals serve as second messengers in pro-inflammatory signal transduction pathways. Both oxygen active species, such as O₂⁻¹, H₂O₂, HO⁻¹ and lipid radicals, such as ROO⁻¹, RO⁻¹ and hydroperoxides, are generated during lipid peroxidation and metabolism (Kwiecien *et al.*, 2001). Therefore, it may further be speculated that the gastric ulcer formation and anti-secretory responses of CI, CI-Aq and CI-E exerted due to the free radical scavenging/antioxidant activity. The data has been suggested that the anti-ulcer active principle(s) of CI is/are both water and ethanol extractable (Table 1).

In conclusion, the reported results have validated the folkloric use of the drug tested for use in the therapy of gastric ulcer disease. In particular, the present studies have pointed out possible gastric anti-secretory and anti-ulcer effects of the CI and its extracts. Nevertheless, detailed chemical studies followed by pharmacological investigations are still required to isolate the pure active principle(s) of the CI and to elucidate the precise mechanism(s) of anti-ulcer actions.

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