ABSTRACT

The purpose of the study was to elucidate the antiulcer activity of stem of Saccharum Officinarum (Poaceae) in albino rats against aspirin induced gastric ulcer, ethanol induced gastric ulcer and pylorus ligation induced gastric ulcer models. Groups of rats were fasted overnight, received ranitidine (20 mg/kg) as a standard and plant stem juice extract at dose of 0.75 mL/100 gm and 1.5 mL/100 gm as a treatment against aspirin induced gastric ulcer, ethanol induced gastric ulcer and pylorus ligation induced gastric ulcer. The treatment produced significant protection of ulcer induced by aspirin, ethanol and pylorus ligation induced ulcer. The extract also reduced ulcer index, volume of gastric content, free and total acidity, suggesting that extract possesses significant anti-ulcer activity.

INTRODUCTION

An “ulcer” may be defined as an open sore (Johnston T.B. et al, 1958). Peptic ulcer disease (PUD), which may be gastric or duodenal ulcer, is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries (Theodore W. Schafer, 2007). The main mechanism behind peptic ulcer involves an imbalance between offensive (acid, pepsin, and H. pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). It is estimated that around 15,000 deaths occur each year due to peptic ulcer. Peptic ulcer is very common in developing countries like India, accounting around 57658 deaths till 2017 in India alone. Today, there are two main approaches available for treatment of peptic ulcer; the first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection (Diprio). Natural drugs of plant origin are very much gaining popularity and are being investigated for a number of disorders, including peptic ulcer. (Misra Vimlesh et al, 2012). Peptic ulcer is the most common disorder in clinical practice. Considering the several side effects (arrhythmia’s, impotence, funaeocmastia and haematopoeitic changes) of modern medicine, natural drugs possessing less side effects should be looked for as a better alternative for the treatment of peptic ulcer (Sakat & Sachin 2009).

Saccharum Officinarum linn is a medicinally important plant of the family Poaceae. Saccharum Officinarum is coarse growing member of the grass family with juice or sap high in sugar content. Sugarcane is the second major cash crop in Pakistan (Ghulam raza et al.). Sugarcane (Saccharum spp.) is one of the most important agro-industrial crops in the world, cultivated on more than 20 million hectares (Vikas Y et al 2009). It is the only means by which one obtains food sugar in several countries. (Asseng Charles et al 2009). Sugarcane (Saccharum Officinarum L.) is widely cultivated in tropical and subtropical countries of the world for sugar and bioethanol production. It accounts for approximately 80% of the world’s sugar production.

MATERIALS AND METHODS

Plant Material

Fresh Saccharum Officinarum stem was collected from local garden area of Lucknow and its identification and authentification was done by National Botanical Research Institute, Lucknow (Ref No. NBRI/CIF/265/2011).

Animals

The Antiulcer activity was done on Wistar albino rats (150-200g), purchased from animal house of Central
Drug Research Institute, Lucknow. The animals were maintained 12 h light/dark cycle at 25 ± 20°C, and were allowed free access to standard pellet diet and water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) according to the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (1087/07/CPCSEA). All studies were performed in accordance with the CPCSEA guidelines.

Preparation of extract
Before the extraction of juice, the canes were cleaned and washed to remove dirt and foreign particles from the surfaces. A three roller power crusher was used to extract juice. The juice was then filtered through a four-layer muslin cloth which was chilled immediately at 40°C.

Models for anti-ulcer activity

Aspirin Induced Gastric Ulcer
The animals (albino rats, 150–200g) were divided into four groups each group consisting four animals. The first group was given normal distilled water (2 mL/kg), served as control group, the second group was treated with Ranitidine (20 mg/kg), served as positive control and the third and fourth group were treated with Saccharum Officinarum orally at the dose of 0.75 mL/100g and 1.5 mL/100g respectively, served as the test group for 8 days. After 8 days of treatment, animals were fasted for 24 h followed by administration of aqueous suspension of aspirin (a dose of 200 mg/kg orally) on the day of sacrifice. The animals were sacrificed 4 h later and the stomach was opened to calculate the ulcer index and other parameters (Vinothapooshang and Sundar, 2011; Vogel H. Gerhard, 2008).

Ethanol-induced gastric ulcer
The experimental rats were fasted for 18h with access to water ad libitum. The animals were randomly divided into four groups of four animal each. The first group was given distilled water (2ml/kg), while the second group was treated with Ranitidine (20mg/kg). The remaining groups received plant juice of Saccharum Officinarum orally. One hour later, ulceration was induced by oral administration of 1ml of 90% ethanol to each rat. After one hour of ethanol administration, the animals were sacrificed under ether anesthesia and the stomach were surgically removed and opened along the greater curvature to examine the lesions macroscopically. The number and gravity of erosions were scored (Mohammed Safwan, 2011; Vogel H. Gerhard, 2008).

Pylorus ligation induced gastric ulcer
Albino rats weighing around 150-200g were selected for pyloric ligation ulcer model. Rats were divided into four groups, each group consisting of four animals. Animals were fasted for 24 h. One group received distilled water 2 mL/kg (control), the second group received Ranitidine 20 mg/kg orally (positive control) and the third and fourth group received plant juice of Saccharum Officinarum by oral route, 30 min prior to pyloric ligation. The animals were anesthetized with ketamine. The abdomen was opened by a small midline incision below the xiphoid process, pylorus was secured and ligated by a fine thread. The stomach was placed carefully in the abdomen and wound was sutured by interrupted sutures. Animals were sacrificed 4 h later and the stomach was opened to collect the gastric contents (Vinodhapooshang and Sundar, 2011; Mohammed Safwan, 2011; Vogel H. Gerhard, 2008).

Collection and measurement of gastric juice
The stomachs were excised carefully by keeping the oesophagus closed and opened along the greater curvature, luminal contents were removed. The gastric contents were collected and centrifuged at 1000 rpm for 10 min. The centrifuged samples were decanted and volume of gastric juice was noted (Mohammed Safwan, 2011).

Determination of pH of gastric juice
One mL of the supernatant liquid was diluted to 10 mL by distilled water. The pH of the solution was recorded by digital pH meter (Mohammed Safwan, 2011).

Estimation of free and total acidities
The above solution was titrated against 0.01 N NaOH using Topfer’s reagent as indicator. As the solution turned orange in colour, volume of NaOH was noted that corresponds to free acidity. Further, the titration was continued till the solution regained pink colour. The total volume of NaOH was noted, that corresponds to the total acidity (Mohammed Safwan, 2011).

Acidity was expressed as
Acidity = volume of NaOH X Normality X 100 mEq/Lt / 0.1

Assessment of ulcer index
Mean ulcer score for each animal is expressed as Ulcer Index. The stomach was washed in running water to detect ulcers in the glandular portion of the stomach. The number of ulcers per stomach was noted and severity scoring was done microscopically with the help of hand
lens (10X) and scoring was done (Mohammed Safwan, 2011).

- 0 = Normal color Stomach
- 0.5 = Red Colouration
- 1 = Spot Ulcers
- 1.5 = Haemorrhagic Streaks
- 2 = Deep ulcer

Calculation of Percentage Protection

The percentage protection was calculated by the following formula:

\[
\% \text{ Protection} = \frac{\text{CMUI} - \text{TMUI}}{\text{CMUI}} \times 100
\]

\[
\text{CMUI/TMUI} = \text{control/toxic mean ulcer index}
\]

Calculation of Titratable Acidity

The volume of NaOH required was noted and was taken as corresponding to the total acidity.

RESULTS

Effect of *Saccharum Officinarum* juice in aspirin treated rats

- **Effect of gastric ulcer index**: Oral administration of *Saccharum Officinarum* stem juice in 0.75 mL/100 gm and 1.5 mL/100 gm dose, inhibited gastric ulcer formation induced by aspirin in a dose dependent manner. (Table 1.1) The decrease in gastric ulcer index (anti-ulcerogenic effect) was highly significantly higher \((p < 0.001)\) with 1.5 mL/100 gm dose.

- **Juice of gastric secretion volume**: Oral administration of juice of *Saccharum Officinarum* stem in 0.75 mL/100 gm and 1.5 mL/100 gm doses inhibited the increase in gastric secretion volume induced by aspirin in a dose dependent manner. (Table 1.1) The effect was highly significant \((p < 0.001)\) with 1.5 mL/100 gm dose.

- **Effect on gastric pH**: Oral administration of juice of *Saccharum Officinarum* stem in 0.75 mL/100 gm and 1.5 mL/100 gm doses inhibited decrease in gastric pH induced by aspirin in a dose dependent manner. (Table 1.1) The inhibitory effect was highly significantly \((p < 0.001)\) with 1.5 mL/100 gm dose.

- **Effect on gastric acid output**: Oral administration of the juice, prevented increase in gastric acid output induced by aspirin in a dose dependent manner. (Table 1.1) The inhibitory effect was highly significantly \((p < 0.001)\) with 1.5 mL/100 gm dose.

Effect of *Saccharum Officinarum* juice in ethanol treated rats

- **Effect of gastric ulcer index**: Oral administration of *Saccharum Officinarum* stem juice in 0.75 mL/100 gm and 1.5 mL/100 gm dose, inhibited gastric ulcer formation induced by ethanol in a dose dependent manner. (Table 1.2) The decrease in gastric ulcer index (anti-ulcerogenic effect) was highly significantly higher \((p < 0.001)\) with higher dose, 1.5 mL/100 gm.

- **Effect of gastric secretion volume**: Oral administration of juice *Saccharum Officinarum* stem in 0.75 mL/100 gm and 1.5 mL/100 gm doses inhibited the increase in gastric secretion volume induced by ethanol in a dose dependent manner. (Table 1.2) The effect was highly significant \((p < 0.001)\) with 1.5 mL/100 gm dose.

- **Effect on gastric pH**: Oral administration of juice of *Saccharum Officinarum* stem in 0.75 mL/100 gm and 1.5 mL/100 gm doses inhibited decrease in gastric pH induced by ethanol in a dose dependent manner. (Table 1.2) The effect was highly significant \((p < 0.001)\) with 1.5 mL/100 gm dose.

- **Effect on gastric acid output**: Oral administration of the juice, prevented increase in gastric acid output induced by ethanol in a dose dependent manner. The inhibitory effect was highly significantly \((p < 0.001)\) with 1.5 mL/100 gm dose.

Effect of *Saccharum Officinarum* juice in Pylorus Ligation Treated Rats

- **Effect of gastric ulcer index**: Oral administration of *Saccharum Officinarum* stem in 0.75 mL/100 gm and 1.5 mL/100 gm dose, inhibited gastric ulcer formation induced by pylorus ligation in a dose dependent manner (Table 1.3) The decrease in gastric ulcer index (anti-ulcerogenic effect) was highly significantly higher \((p < 0.001)\) with 1.5 mL/100 gm dose.

- **Effect of gastric secretion volume**: Oral administration of juice of *Saccharum Officinarum* stem in 0.75 mL/100 gm and 1.5 mL/100 gm doses inhibited the increase in gastric secretion volume induced by pylorus ligation in a dose dependent manner. (Table 1.3) The effect was highly significant \((p < 0.001)\) with 1.5 mL/100 gm dose.

- **Effect on gastric pH**: Oral administration of juice of *Saccharum Officinarum* stem in 0.75 mL/100 gm and 1.5 mL/100 gm doses inhibited decrease in gastric pH induced by pylorus ligation in a dose dependent manner. (Table 1.3) The effect was highly significant \((p < 0.001)\) with 1.5 mL/100 gm dose.

- **Effect on gastric acid output**: Oral administration of the juice, prevented increase in gastric acid output induced by pylorus ligation in a dose dependent manner. (Table 1.3) The inhibitory effect was highly significantly \((p < 0.001)\) with 1.5 mL/100 gm dose.

DISCUSSION AND CONCLUSION

Ulcers are caused due to imbalance between aggressive and defensive factors of the gastric mucosa. Pepsin and gastric acid make up the offensive factors whose pro-
teolytic effect is buffered by mucin secretion, mucosal glycoprotein cell shedding, cell proliferation and prostaglandins (Goyal and Bhattacharya 1999).

Several non-steroidal anti-inflammatory drugs like aspirin are known to induce gastric damage by suppression of prostaglandins. Prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus maintaining mucosal blood flow and regulating mucosal cell turn over repair. Oxy radicals may play important role in the aspirin induced erosive gastritis. After an initial hydrophobic intermolecular interaction, the free carboxyl group present in all NSAIDs forms a strong electrostatic bond with the positively charged head group of zwitterionic phospholipids of mucus layer and, in doing so, increase the phospholipids solubility, neutralize its surface activity. Thus, NSAIDs topically act on tissue to disrupt the hydrophobic protecting lining of the mucus gel layer.

Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosal and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid (Dashputre et al., 2011).

Saccharum Officinarum is one of the common natural source of sugar and has been found to inhibit ulceration induced by aspirin, ethanol, pylorus ligation model very efficiently.

REFERENCES


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