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Research Article

EVALUATION OF ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF *SECHIUM EDULE* FRUITS IN EXPERIMENTAL RATS

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ABSTRACT

The antiulcer activity of ethanolic extract of *Sechium edule* fruits was investigated in pylorus ligation and ethanol induced ulcer models in wistar rats. The ethanolic extract of *Sechium edule* fruits at doses of 200,100 mg/kg p.o produced significant ($p<0.01$) inhibition of the gastric fluid volume, free acidity, total acidity and gastric induced by pylorus ligation induced ulcer. In ethanol induced gastric ulcer, the ethanolic extract of *Sechium edule* fruits at doses of 200,100 mg/kg p.o produce significant ($p<0.01$) decrease in ulcer index and also protected the mucosal epithelial of stomach from the damage caused by ethanol. These results may further suggest that ethanolic extract was found to possess antiulcerogenic property.

Keywords: *Sechium edule,* Pylorus ligation, Ethanol, Ulcer index, Free acidity, Total acidity, Mucosal epithelial.

INTRODUCTION

Peptic ulcers are a deep gastrointestinal erosion disorder that involves the entire mucosal thickness, penetrating the muscular mucosa. For decades it was believed that the excessive secretion of gastric acid caused gastrointestinal ulcerations, but many patients presenting such ulcerations had normal acid secretion rates. Then, researchers reported that peptic ulcers were been caused by an imbalance between the aggressive factors and a number of known defense mechanisms. Exogenous aggressive factors such as smoke, anti-inflammatory drugs, alcohol, stress, fatty foods and *Helicobacter pylori* infections triggered tissue necrosis through mucosal ischemia, free radical generation and cessation of nutrient delivery, hydrochloric acid together with pepsin, pancreatic enzymes and bile decreased the defense mechanisms of gastrointestinal mucosa such as the intercellular junctions, local blood flow, mucus/bicarbonate secretion and cellular growth [1].

Peptic ulcer therapy has undergone many strides over the past few years and a number of drugs are available for treatment. These drugs are broadly classified into two groups, those that decrease or counter acid pepsin secretion and those that afford cytoprotection by virtue of their effects on mucosal defence factors.These drugs act by different mechanisms, most of the commonly used drugs are H_2 blockers (ranitidine, famotidine etc.,), M_1 blockers (pirenzepine, telenzepine etc.,).proton pump inhibitors (omeprazole, lansaprazole etc.,) decrease secretion of acid while drugs like sucralfate and carbanoxolone promote mucosal defence. Recently the role of these drugs on the defensive factors gaining importance [2].

It is now assumed that these drugs ultimately balance the aggressive factors (acid, pepsin, *H.Pylori*, bile salt) and defensive factors (mucin secretion, cellular mucus, bicarbonate secretion). Although these drugs have brought about remarkable changes in ulcer therapy, the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects (arrythmiasis, impotence, gynaecomastia) and danger of drug interactions during ulcer therapy .hence search for an ideal anti ulcer drug continuous and has also been extended to herbal drug in search for new and novel molecules, which afford better protection and decrease the incidence of relapse [3].

Sechium edule is an edible plant that belongs to the family cucurbitaceae also known as chayote, choko, chocho, chow-chow, and vegetable pear. The chayote is a herbaceous, perennial, monoecious, vigorous creeper or climbing plant. The fruits grow either individually or in pairs on a shared peduncle. They are fleshy or fleshy-fibrous, may have longitudinal ridges or furrows, and come in many different shapes (globose, ovoid, subovoid, pyriform) and colours (dark or light green) [4]. The fruits and the seed especially, are rich in several important amino acids. A lectin from the exudate of *Sechium edule* was purified [5]. Eight flavonoids, including three *C*-glycosyl and five *O*-glycosyl flavones, were detected [6]. Twenty known Gibberellins' have been identified in extracts of the seeds of *Sechium edule* [7]. The leaves and fruits have diuretic, cardiovascular and anti-inflammatory properties, the leaves has been used in the treatment of arteriosclerosis and hypertension, and to dissolve kidney stones [8, 9]. It has been reported that the ethanolic extracts of dried leaves and water extracts of seeds were found to possess higher radical-scavenging, reducing power and antioxidant activities by the mechanism of inhibition of lipid peroxidation, free radical scavenging activity [10]. Literature reviews indicated antiulcer activity of this plant has not been evaluated so far. In view of this, the present study was aimed to evaluating the antiulcer activity of ethanolic extract of *Sechium edule* fruits.

MATERIALS AND METHODS

Plant

The fresh fruits of *Sechium edule* were collected from Bangalore. The fruit material was taxonomically identified and authenticated at Regional Research Institute (Ay.), Bangalore, where the voucher specimen is conserved under the reference number (RRCBI/MCW/7/2008) for future reference.

Preparation of Extract

The fruits of *Sechium edule* were first thoroughly washed under running tap water and then washed with distilled water. Then the fruits were chopped and air dried under shade and milled to a coarse powder. The powder was used for the preparation of ethanolic extract. The powder was then subjected to maceration with sufficient volume of ethanol (99.9%) for 72hrs with intermittent shaking. Then the extract was filtered and subjected to distillation to remove the solvent. The extract was then rotary evaporated at 40°C to dryness. The extract was stored at 4°C for further use.

Animals

Studies were carried out using Wistar albino rats (120–150 gm) of either sex were used. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. All the animals were housed in polypropylene cages maintained in controlled temperature (27 ± 2°C) and light cycle (12 h light and 12 h dark). They were provided with standard rat pellet diet and water ad libitum. All the animals were given a week time to get acclimatized with the laboratory conditions. The experiments were carried out according to guidelines of Committee for Prevention and Control of Scientific Experimentation on Animals (CPCSEA).

Drugs and Chemicals

Omeprazole (Dr.Reddy's Lab, India) and Topfers reagent (Nice Chemicals, India) were used in this study. All other chemicals used in present study were of analytical grade.

Pylorus ligation ulcer model [11-12]

The animals were divided into following groups of six animals each.

Group 1: Served as control and was Animals were treated vehicle only

Group 2: Animals were treated with standard drug Omeprazole (20mg/kg, p.o)

Group 3: Animals were treated with ethanolic extract of *Sechium edule* (200mg/kg, p.o)

Group 4: Animals were treated with ethanolic extract of *Sechium edule* (100mg/kg, p.o)

Overnight fasted rats were anaesthetized with anaesthetic ether. Then an incision of 1cm long was given in the abdomen just below the sternum. The stomach was exposed and a thread was passed around the pyloric sphincter and a tight knot was applied. Abdomen wall was closed by putting the sutures.

After 45 minutes of Sechium *edule* and Omeprazole treatment pyloric ligation was performed. After 4 hr of pyloric ligation animals were sacrificed by decapitation. Abdomen was opened and the oesophagus was tied at the end of the stomach. A small cut to the pyloric region just above the knot was given and contents of the stomach were collected in a centrifuge tube.

The following parameters were analyzed:

1. Volume of gastric juice (in ml): Gastric content was centrifuged at 1000 rpm for 10min and measured the volume.

2. Determination of free and total acidity: Pipetted out 1ml of supernatant liquid and diluted it to 10ml with distilled water. The P^H of this solution was noted with the help of P^H meter. The solution was titrated against 0.01N NaOH using topfers reagent (Dimethyl-amino-azo-benzene with phenolptheline) as indicator. The end point was noted when the solution turns to orange color; this corresponds to the free acidity. Titration was continued further till the solution regained pink color. This volume corresponds total acidity.

Acidity was calculated by using the formula:

$$\text{Acidity} = \frac{\text{volume of NaOH x Normality x 100}}{0.1} \quad \text{mEq/lt/100g}$$

Ulcer Scoring & Ulcer Index Determination

0 - Normal Mucosa

0.5 - Red coloration

1.0 - Spot ulcers

1.5 - Hemorrhagic streaks

2.0 - Ulcers >3 but <5

2.5 - Ulcer >5.

Mean ulcer score of each group were calculated, which was designated as the ulcer index and percentage of protection was calculated as

$$\frac{C - T}{C} \times 100$$

(C = ulcer index in control group; T = ulcer index in test group)

Ethanol – induced gastric mucosal damage [11]

Twenty four male wistar rats were divided into following groups of six animals each.

Group 1: Animals were treated with vhicle

Group 2: Animals were treated with standard drug Omeprazole (20mg/kg, p.o)

Group 3: Animals were treated with ethanolic extract of *Sechium edule* (200mg/kg, p.o)

Group 4: Animals were treated with ethanolic extract of *Sechium edule* (100mg/kg, p.o)

Animals are fasted for 36hrs before an experiment but allowed free access to water.1ml of 96% ethanol was administered orally to rats. In treatment groups (groups 2-4), drugs were administered orally 1/2hr before the administration of ethanol. After 1hr of ethanol treatment, animals were sacrificed; stomachs were removed, opened along the greater curvature, and examined for lesions. Lesion severity was determined by measuring ulcer index.

Ulcer index was scored by,

10 = shedding of epithelium

20 = haemorrhage

30 = one or two ulcer

40 = many ulcer

50 = perforated ulcer.

Mean ulcer score of each group were calculated, which was designated as the ulcer index and percentage of protection was calculated as

$$\frac{C - T}{C} \times 100$$

(C = ulcer index in control group; T = ulcer index in test group)

Histopathology Study

At the end of the study, all the rats were sacrificed by cervical decapitation and the stomach were isolated, washed in ice cold saline. Then the tissue was immediately fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin and serial sections were taken and each section is stained with hematoxylin and eosin. The slides were then examined under light microscope and photographs were taken[13].

Statistical Analysis

Statistical analysis was carried out using Graphpad Prism software. All data were expressed as Mean ± SE .Groups of data were compared with an one way analysis of variance followed by Dunnett 't' test .Values were considered statistically significant at $p < 0.05$.

RESULTS

In pyrolic ligation model, the ethanolic extract of *Sechium edule* (200mg/kg, p.o. and 100mg/kg, p.o.) showed significant ($p<0.01$) rise in pH as compared to control. The free acidity gastric content is increased in control animals. The ethnolic extract of *Sechium edule* (200mg/kg, p.o.) showed significant ($p<0.01$) decrease in free acidity as compared to control. The extract (200mg/kg, p.o. and 100mg/kg, p.o.) significantly reduced the total acidity and ulcer index ($p<0.001$) as compared to control (Table 1).

The percentage protection of *Sechium edule* (200mg/kg, p.o and 100mg/kg, p.o) was found to be 68% and 60% whereas the percentage protection of omeprazole was found to be 75% (Table 1).

In ethanol induced gastric ulcer, on induction of gastric ulceration by using ethanol (96%, v/v), the pretreatment with ethanolic extract of *Sechium edule* showed a dose dependent reduction in the severity of the lesions.

The results of anti ulcer activity of the *Sechium edule* are shown in the Table 2. The control group has shown increased ulcer index whereas the group treated with omeprazole (20mg/kg, p.o.) the ethanolic extract of *Sechium edule* (200mg/kg, p.o. and 100mg/kg, p.o.) has shown significant ($p<0.01$) reduction in ulcer index. The *Sechium edule* (200mg/kg, p.o. and 100mg/kg, p.o.) has shown 78% and 75% of percentage protection respectively.

Table 1: Effect of ethanolic extract of *Sechium edule* on various parameters in pyloric ligation induced gastric ulcers

Groups	Ulcer index	Protection (%)	PH of gastric juice	Gastric juice in (ml)	Free acidity mEq/l	Total acidity mEq/l
Normal control	4.5± 0.10	0.00	2.42± 0.08	2.2± 0.10	27.25± 0.85	47.5± 0.64
Omeprazole (20mg/kg)	1.2± 0.09	75	3.35± 0.06**	3.42± 0.04**	16.5± 0.64**	34.5± 0.64**
Sechium edule (200mg/kg)	1.5± 0.08	68	3.15± 0.06**	3.05± 0.06**	19± 0.91**	37.25± 0.85**
Sechium edule (100mg/kg)	1.9± 0.09	60	2.80± 0.10**	2.82± 0.08*	23.5± 0.64*	40.25± 0.47**

Values are mean + SEM for six animals in each group.

*P< 0.05 considered statistically significant as compared with control group.

**P< 0.01 considered statistically significant as compared with control group.

Table 2: Effect of ethanolic extract of *Sechium edule* on ethanol induced gastric ulcers

Groups	Ulcer index	Protection%
Normal control	78±1.08	0.00
Omeprazole (20mg/kg)	22.75±1.109**	71
Sechium edule (200mg/Kg)	15.75±0.4787**	78
Sechium edule (100mg/kg)	19.5±0.6455**	75

Values are mean + SEM for six animals in each group.

**P< 0.01 considered statistically significant as compared with control group.

Histopathology of stomach of various groups in ethanol induced ulcer model (Hematoxin&Eosinx100)

Photomicrographs (original magnification 100×) of histopathological studies of stomach various groups stained with haematoxylin and eosin of histopathological studies of stomach of various groups stained with haematoxylin and eosin. Ethanol treated group shows the ulcerated mucosa with haemorrhage and discontinuity of lining of epithelium (Figure 1). Pre-treatment with *Sechium edule* (200mg/kg, p.o. and 100mg/kg, p.o.) protected the mucosal epithelial from the damage caused by ethanol (Figure 3). Omeprazole (20mg/kg) also had shown protection against ethanol induced gastric damage.

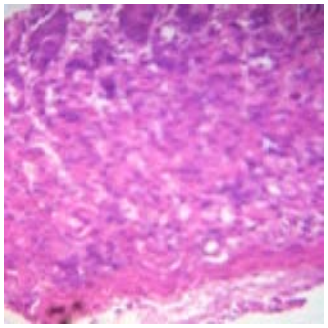

Fig. 1: Treated with 1ml Ethanol

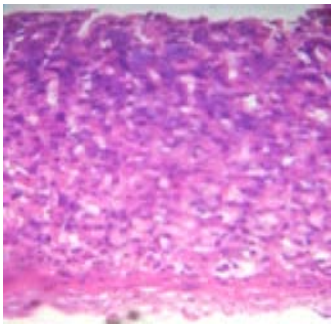

Fig. 2: Treated with Omeprazole (20mg/kg) and 1ml Ethanol

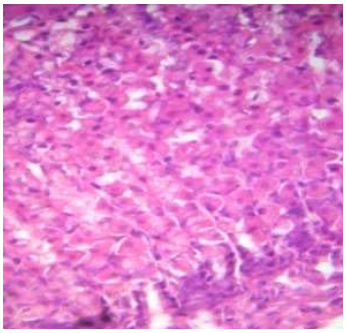

Fig. 3: Treated with *Sechium edule* (200 mg/kg) and 1ml Ethanol

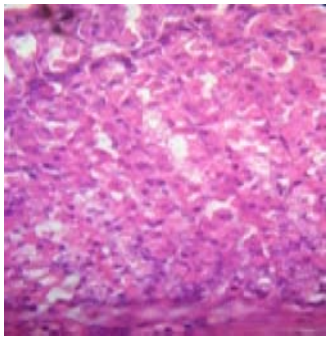

Fig. 4: Treated with *Sechium edule* (100mg/kg) and 1ml Ethanol

DISCUSSION

In the present study, the activity of *Sechium edule* extract on secretion in pyloric ligation model was evaluated. Pretreatment with ethanolic extract of *Sechium edule* at the dose of 200mg/kg, p.o and 100mg/kg, p.o significantly ($p<0.01$) reduced the gastric fluid volume, free acidity, total acidity and gastric p^H when compared with the control, indicating that it posses antisecretory potency.

The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid [14]. Oxidative damage is also considered to be a common factor in the pathogeneses of ulcers.

Ethanol is also known to produce free radicals and induce peptic ulcers. The free radicals produced cause lipid peroxidation, leading to membrane fluidity which in turn increases the influx of Ca^{2+} ions and results in the reduced membrane integrity of surface epithelial cells, thereby generating gastric ulcer. Free radicals have been demonstrated as contributing factor in tissue injury and in the modulation of pain [15]. The incidence of ethanol induced ulcers predominant in the glandular part of the stomach has been reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa [16, 17].

In the present study, the control group treated orally with ethanol produced the expected ulceration. Pretreatment at the dose of 200mg/kg, p.o and 100mg/kg, p.o with ethanolic extract of *Sechium edule* significantly ($p<0.01$) decreased the ulcer index when compared with control rats. These results indicate that *Sechium edule* extract displays an antiulcerogenic effect related to cytoprotective activity, since it significantly reduced the ethanol induced ulcer.

In histopathological examination of stomach mucosa ethanol treated group shows the ulcerated mucosa with haemorrhage and discontinuity of lining of epithelium (Figure 1). Pretreatment with *Sechium edule* (200mg/kg, p.o. and 100mg/kg, p.o.) protected the mucosal epithelial from the damage caused by ethanol (Figure 3 & 4).

The antioxidant activity of flavonoids has been well documented in the literature. Moreover, flavonoids have been reported for their antiulcerogenic activity and gastro protection already . It has been also reported that flavonoids like Quercetin seem to play a very important role in the prevention and treatment of peptic ulcer. It acts by promoting mucus secretion, thereby serves as gastroprotective agent. Among other flavonoids such as methyl-3-(+)-catechin interferes with the formation of histamine in gastric mucosa and hence produces the protective effect [15, 18]. The antioxidant activity of *Sechium edule* was already reported [10] and also eight flavonoids from *Sechium edule* were detected [6]. So, further investigation of flavonoidal fraction is required to establish the antiulcer mechanism *Sechium edule* fruits.

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