



ANTI-ULCEROGENIC ACTIVITY OF FEW PROBIOTICS

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ABSTRACT

Over the past few years, the probiotic field has exploded with a number of new cultures, each purported to elicit a variety of benefits. Probiotics are nonpathogenic microbe used to confer health benefits on the recipient. The derangement of normal body flora has been held responsible for causation of various disorders. Oral administration of probiotics has been proved beneficial in various gastrointestinal disorders such as ulcers, diarrhea and colonic cancers. In the present study probiotics containing bacillus coagulans and saccharomyces cerevisiae were evaluated for their antiulcer activity using Restraint stress + Aspirin induced ulcers model of gastric ulceration in rats. Probiotic were administered in the dose of 1g/animal. Mean ulcer number, size and index were determined. Ranitidine was used as a reference drug. The probiotics showed significant antiulcer activity in Restraint stress + Aspirin induced ulcers model. However, the antiulcer activity was less than that of ranitidine.

Keywords: Probiotics, *Bacillus coagulans*, *Saccharomyces cerevisiae*, Antiulcer activity.

INTRODUCTION

The main aim of this study is to evaluate the antiulcer activities of some probiotics. Conventionally, food healthiness has been associated with nutritional factors such as fat, fiber, salts and vitamins. In addition to this traditional healthiness, food may contain single components that may have a positive impact on our well-being. Food processing and biotechnology has enabled the food industry to make food with special characteristics. Probiotics, prebiotics, synbiotics and functional foods have been created to describe food products with special characteristics. Functional Foods are generally described as foods that promote health beyond providing basic nutrition. Probiotics are been used for the fermentation of food and alcoholic materials. These are the micro organisms which are used as the food supplements of bacteria. The word 'PROBIOTIC' can be described as, the food supplements which are designed to improve the human health¹. According WHO –USA, probiotics are "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host." In general, ulcer is defined as "the development of a lesion on a membrane". The formation of lesion is due to a break in skin or mucus membrane with loss of surface tissue, disintegration and death of epithelial tissue and often with pus formation² Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors³. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defenses (mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide⁴. The goals of treating peptic ulcer

disease are to relieve pain, heal the ulcer and prevent ulcer recurrence.

MATERIALS AND METHODS

Selection of probiotics

Procurement of Probiotics: Baker's yeast was procured from Bio Ethicals Pharma Limited, Hubli, and Karnataka. *Bacillus coagulans* was obtained from local market.

Procurement of materials: All the media were obtained from Himedia Laboratories Ltd., Mumbai. Albino rats were procured from Mahaveer Enterprises, Hyderabad, Andra Pradesh.

Cultivation of probiotic:

I. *Bacillus coagulans*

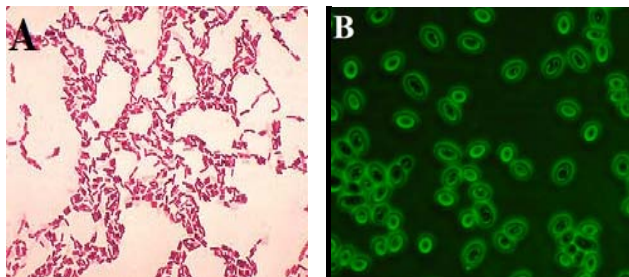
1 gm of sporolac powder containing *Bacillus coagulans* was suspended in 10 ml of sterile saline solution. The suspension was heated to 70°C on water bath for 5 minutes and cooled. During this process the organism in sporulated form breaks its dormancy. The activated microorganism was then transferred aseptically in laminar air flow chamber to a sterile conical flask containing nutrient broth (pH 7.2). And the organism in nutrient broth was incubated in the incubator at 37°C for 3 days. After incubation period this nutrient broth was centrifuged at 1800 rpm for 10 minutes to form a pellet. The supernatant was decanted and the pellet of organism was collected using normal saline solution (Photograph: A).

II. *Saccharomyces cerevisiae*

1 gm of Dried Baker's Yeast powder containing *Saccharomyces cerevisiae* was suspended in 10 ml of sterile saline solution. The suspension was heated to 70°C on water bath for 5 minutes and cooled. During this



process the organism in sporulated form breaks its dormancy. The activated microorganism was then transferred aseptically in laminar air flow chamber to a sterile conical flask containing yeast extract of peptone dextrose (YPD) media (pH 5.5). And the organism in (YPD) media was incubated in the incubator at 37°C for 3 days. After incubation period this YPD media was centrifuged at 1800 rpm for 10 minutes to form a pellet. The supernatant is decanted and the pellet of organism was collected using normal saline solution (Photograph: B).



Photograph (A-B): Microscopic vision (40X) of *B.coagulans* (A), *S. cerevisiae* (B).

Preparation of Nutrient Broth

A 1.3 g of Nutrient broth (Himedia) was added to 100 ml of sterile distilled water in a sterile conical flask and closed with cotton plug and autoclaved for 20 minutes. This broth is used for cultivation of *Bacillus coagulans*.

Preparation of Yeast extract of Peptone Dextrose

A 1 gm of yeast extract, 2 gm of peptone and 2 gm of dextrose were added to 100ml of sterile distilled water in a sterile conical flask, closed with cotton plug and autoclaved for 20 minutes. This broth is used for cultivation of *Saccharomyces cerevisiae*.

Preparation of test drugs

Carboxymethyl cellulose (CMC) was used as a general vehicle for dispersing *Bacillus coagulans* and *Saccharomyces cerevisiae* and standard.

Route of administration of drugs

The drugs were prepared in accurate dose and administered orally slowly by an oral feeding needle inserted into the posterior part of the pharynx.

Maintenance of animals

The animals were acclimatized for about one week prior to dosing. Cage number and individual markings were made to identify the animals. The animals were housed three per cage of same sex in polypropylene cages provided with a bedding of paddy. Pellet chow feed standard diet under good management conditions and water *ad libitum* was provided to the animals. A temperature of 20-25°C and 12 hr each of dark and light cycle was maintained.

Acute Toxicity Studies

In the present investigation, acute toxicity and gross behavioral studies were carried out in rats after

administration of *Bacillus coagulans* and *Saccharomyces cerevisiae*. The test doses were given orally in the form of normal saline. The rats were divided into four groups and received the doses of probiotics at 500, 1000, 1500, 2000 mg/kg. Then rats were continuously and carefully observed for 2 hrs followed by occasionally for further 4 hrs. The behavior and mortality of rats was observed up to 24 hrs.

Anti-ulcerogenic evaluation studies in rats:

Restraint stress + Aspirin induced ulcers:^{5, 6}

Adult male wistar rats (140-180 gm) were divided into five groups (n=6).

- Group I (Control) – Vehicle treated (5ml/kg)
- Group II (Standard) – Ranitidine (30 mg/kg)
- Group III (Positive) – Aspirin (50 mg/kg)
- Group IV – *Bacillus coagulans* (1000 mg/kg)
- Group V – *Saccharomyces cerevisiae* (1000 mg/kg)

The rats were deprived of food for 24 hours with free access to water. After 24 hours aspirin (50 mg/kg p.o.) in 1% carboxymethyl cellulose (CMC) is administered 30 minutes before restraint. The standard drug Ranitidine (30 mg/kg) and the probiotics (1000 mg/kg) were prepared and suspended in 0.9% normal saline were given p.o. 1 hour before the restraint. The rats were subjected to restraint by placing them individually in a piece of galvanized steel window screen that is molded tightly around and held with adhesive tapes so that the animals cannot move. After 6 hours, the animals were killed by an over dose of ether, the stomachs were cut opened through the greater curvature tissues were rinsed with saline and examined by 5-fold binocular magnifier to assess the ulcer formation.

RESULTS

Acute Toxicity Studies

Acute toxicity testing was performed with *Bacillus coagulans* and *Saccharomyces cerevisiae* with increased dose levels of 500, 1000, 1500, 2000 mg/kg body weight and LD50 values indicate that *Bacillus coagulans* and *Saccharomyces cerevisiae* to be safe up to the dose of 2000mg/kg.

Calculation of Ulcer Index and Inhibition Percentage:

After opening the stomach through the greater curvature, the number of ulcers were counted and scoring was undertaken according to the reported methods.^{7, 8} The scores were given as

0	Normal stomach
0.5	Pink or red coloration of stomach
1	Superficial ulcer
1.5	Spot ulcer

- 2 Hemorrhage spot
- 2.5 Scattered hemorrhage streak
- 3 Bleeding ulcer
- 4 Perforated ulcer

US – Avg. of severity score

UP – Percentage of animals with ulcers.

Inhibition percentage was calculated by the formula:⁹

$$\% \text{ Inhibition} = \frac{(\text{Control ulcer index}) - (\text{Test ulcer index})}{(\text{Control ulcer index})} \times 100$$

Ulcer index was calculated by the formula

$$UI = UN + US + UP \times 10-1$$

Where UI – Ulcer index

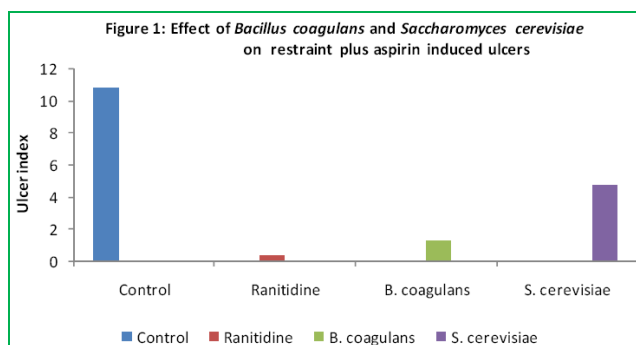
UN – Avg. no. of ulcers/ animal

The anti-ulcerogenic activity of probiotics was evaluated *in vivo* by inducing gastric ulcers in the different groups of rats by restraint plus aspirin induced ulcer method at a dose of 1000 mg/kg body weight. The results pertaining to this evaluation are shown in table 1 and figure 1.

Table 1: Effect of probiotics on restraint plus aspirin induced ulcers

Sl. NO	Treatment	Dose p.o (mg/kg)	Ulcer Index	% inhibition
1.	Control	-	10.75±0.06	-
2.	Ranitidine	30	0.32±0.18***	97
3.	<i>Bacillus coagulans</i>	1000	1.23±0.78***	88
4.	<i>Saccharomyces cerevisiae</i>	1000	4.69±1.46**	56

*P<0.05-statistically significant relative to control; **P<0.01- statistically highly significant relative to control; ***P<0.001- statistically very highly significant relative to control; Number of rats=6.



The results obtained in this investigation of probiotics indicate that the ulcer index and percentage protection against ulcer formation with *Bacillus coagulans* was very highly significant with 88% inhibition of ulcer formation followed by highly significant *Saccharomyces cerevisiae* 56% inhibition of ulcer formation and reducing ulcer index in relative to control.

Histopathology examination

Observations of histopathology was presented in figures 2A to 2D.

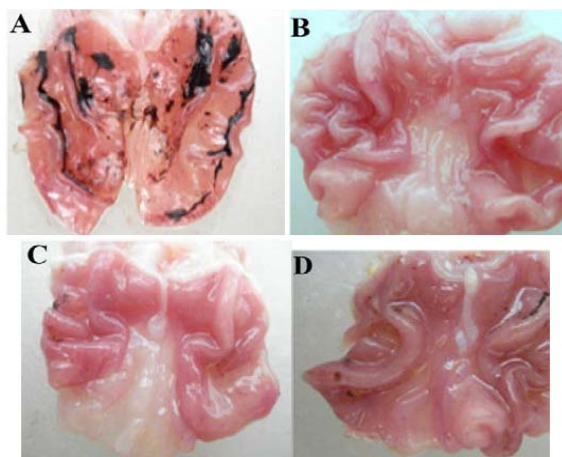


Figure 2A-D: Histopathology study of Rat stomach; (A) Control (Aspirin, 50mg/kg), (B) Standard (Ranitidine, 30mg/kg), (C) *Bacillus coagulans*

(1000mg/kg), (D) *Saccharomyces cerevisiae* (1000mg/kg) treated rat stomach sections.

DISCUSSION

In order to digest food, absorb nutrients and excrete unabsorbed waste products, the gastrointestinal tract has to perform a number of coordinated activities and the tract has to provide the whole body with a continual supply of water, electrolytes and nutrients. In order to achieve these objects, several organs have to integrate with each other and are regulated by neuronal and hormonal systems, as well as the central nervous system^{10, 11}. Normally human stomach secretes about 2.5 liters of gastric juice in 24 hours.

Mechanism of gastric acid secretion^{12, 13}

Gastric acid secretion follows two types of mechanisms:

A. Central mechanism: The gastric acid secretion is induced by the stimuli from cerebral cortex of brain. These stimuli pass impulse to medulla, which in turn relays parasympathetic (acetylcholine mediated) impulses via vagus nerve to the gastric mucosa. This results in the stimulation of parietal cells, causing gastric acid secretion. Gastrin, a hormone secreted by antrum of stomach enters blood circulation and induces vagal stimulation through activating central mechanisms resulting in gastric acid secretion.

B. Peripheral mechanism: Here gastric acid secretion is mediated through the direct action of various substances on parietal cell wall. Histamine and PG act on H₂ and prostaglandin receptors respectively; whereas acetylcholine and gastrin act on muscarinic and gastrin receptors respectively.

Gastric acid secretion can be influenced by any of the following factors:

Acetylcholine – acts through vagus nerve stimulation.

Gastrin – secreted by gastrin cells present in antrum of stomach.

Histamine – secreted by mast cells.

Prostaglandin E – present in gastric mucosa.

Acetylcholine and gastrin increase the calcium (Ca^{++}) ion concentration in the parietal cell. Ca^{++} ions activate protein kinase, an enzyme responsible for secretion of protons (H^+ ions).

Histamine and prostaglandin E_2 (PGE_2) act through peripheral mechanisms by enhancing cyclic AMP (cAMP) formation inside the parietal cell. Adenylcyclase, an enzyme present on the parietal cell wall is essential for conversion of ATP to cAMP. The cAMP activates protein kinase resulting in proton (H^+ ion) secretion.

The activated protein kinase release H^+ ions (protons) which are pumped from parietal cells into canaliculi with the help of a transport enzyme called H^+/K^+ ATPase (proton pump/ hydrogen-potassium ATPase). The proton pump actively transports H^+ ions from parietal cells into canaliculi while transporting K^+ ions that are present in canaliculi, into the parietal cells. Hydrogen (H^+) ions combine with chloride (Cl^-) ions that actively secreted by parietal cells, to form hydrochloric acid (HCl). The transport of H^+ ions by proton is the final step in gastric acid secretion mechanism.

Mechanism of action of probiotics

Probiotics may protect the host from intestinal disorders, to explain this statement many mechanisms were proposed and were explained. But the benefits of the Mechanisms of Probiotics were incompletely understood.

- Production of inhibitory substances¹⁴.
- Stimulation of immunity^{15, 16}.
- Blocking of adhesion sites^{17, 18}.
- Protection against carcinogenesis^{19, 20}
- Competition for nutrients²¹.
- Degradation of toxin receptors^{22, 23}.
- *Helicobacter pylori*²⁴.

An effective probiotic must:

- Exert a beneficial effect.
- Be non-pathogenic and non-toxic.
- Contain large number of viable cells.
- Be capable of surviving and metabolizing in the gut.

CONCLUSION

Evaluation of anti-ulcerogenic activity was performed by restraint plus aspirin induced ulcers method. *Bacillus coagulans* at a dose of 1000 mg/kg b.wt shows very highly significant effect in reducing ulcer index and good

percentage inhibition in ulcer followed by *Saccharomyces cerevisiae*.

Therefore it can be concluded that "PROBIOTICS" *Bacillus coagulans*, *Saccharomyces cerevisiae* may have wide range of activity. Further study should be directed to evaluate the accurate mechanism of action of PROBIOTICS which is responsible for the Anti-ulcer activity.

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