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Gastroprotective activity of *Spirulina platensis* in acetic acid and ethanol induced ulcers in rats.

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Abstract

Objective: The effects of gastroprotective properties of *Spirulina platensis* was investigated in acetic acid and ethanol induced ulcers in rats. Methods: Administration of 2 and 4mg/kg *Spirulina platensis* extract for 7 days. After day 7, oral administration of either 80% (v/v) ethanol or 6% (v/v) acetic acid. Control rats received saline or anti-ulcer drug omeprazole (20 mg/kg) prior to ulcer induction. Results: The extract inhibited the mean lesion score of acetic acid, 4.333 to 3.000. Whereas, for ethanol induced ulcers, the extract reduced the lesion scoring from 2.833 to 1.677. However, this activity was statistically less potent than the anti-ulcer drug, omeprazole. *Spirulina platensis* alone did not induce any ulcers in rats. Conclusions: These results suggested that *Spirulina platensis* has gastroprotective activity against ulcers induced by acetic acid and ethanol.

Key words: gastroprotective, *Spirulina platensis*, ethanol, acetic acid, gastric ulcers

1. Introduction

Spirulina platensis (SP) is an unbranced, helicoidal, filamentous blue-green alga found as an almost unialgal culture in many alkaline lakes with a very high pH, some reaching pH 11. It contains approximately 70% easily digestible protein where 18 out of 22 amino acids and all of the essential amino acids are available, making it a unique vegetarian source of complete protein (Dillon *et. al.,* 1995) [1]. Carotenoids, vitamins, minerals, and essential fatty acids are available in SP. It is an excellent source of B vitamins, particularly vitamin B12, which is important for vegetarians. This nutritious food also contains vitamin E, a highly bioavailable source of iron, 14 naturally chelated minerals and numerous trace elements (Dillon *et. al.,* 1995) [1].

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Pre-clinical and clinical studies suggest it has various therapeutic effects, such as reduction in blood cholesterol, protection against some cancers (Fox, 1996) [2], enhancement of the immune system, increase of intestinal lactobacilli, reduction of nephrotoxicity by heavy metals and drugs, radiation protection, reduction of hyperlipidemia and obesity (Belay *et. al.*, 1993) [3]. Ayehunie *et. al.*, (1998) [4] reported that an aqueous extracts of *Spirulina platensis* partially inhibited HIV-1 replication in human T-cell lines.

SP is claimed in folk medicine to be a potent inducing wound healing inducer of external and gastrointestinal wounds. Therefore, in this present study, the anti-ulcer activity of water extract of SP has been investigated using acute gastric ulceration induced by ethanol and acetic acid in rats.

2. Materials and Methods

2.1 Materials

SP was cultivated by Universiti Industri Selangor, was dried and extracted using distilled water as solvent in a Soxlet apparatus as described previously (Somchit *et. al.*, 2003b) [5]. Male Sprague Dawley rats (180 to 200 g) were obtained from Institute of Medical Research, Kuala Lumpur, Malaysia. They were kept in polypropylene cages with wood shavings as bedding in 12 h light/dark cycle at 27.0 ± 2.0°C. The animals were adapted to laboratory conditions for 7 days prior to the experiments and were given feed and tap water *ad libitum*. The experimental procedures were carried out in strict compliance with the Animal Ethics Committee's rules and regulation followed in this institute.

2.2 Induction of gastric ulcers

Acute gastric ulcers were induced by oral administration of 80% ethanol at a dose of 10 mL/kg in mice. Detailed method as published previously (Somchit *et. al.*, 2002 [6]; Somchit *et. al.*, 2003a). Briefly, rats were deprived of food but were allowed free access to water 12 h before the ethanol or acetic acid administration. Rats in Group 1 and 2 (n=6/group) were pretreated with 2 and 4 mg/kg/day orally for 7 days *SP* extracts before oral 80% ethanol administration respectively. Rats in Group 3 and 4 (n = 6/group) were pretreated with 2 and 4 mg/kg/day orally for 7 days *SP* extracts before oral 6% acetic acid administration respectively. The Group 5 rats received pretreatment only (4 mg/kg/day orally for 7 days *SP*). Group 6 rats were given only 80% ethanol and group 7 received 6% acetic acid orally. Control animals (Group 8) received equivalent amount of normal saline. The other groups of rats received 20 mg/kg omeprazole (Sigma-Aldrich, UK) orally for 7 days before (Group 9) 80% ethanol or (Group 10) 6% acetic acid administration.

2.3 Assessment of gastric lesions

Rats were sacrificed by cervical dislocation and their stomach and duodenum were dissected out, cut open along the greater curvature, rinsed with normal saline and examined for ulcers. The lesion size in mm was determined by measuring each lesion along its greatest length/diameter using a transparent grid. The severity score assigned according to Minano *et al.* (1987) [7]. Score 0, no pathological changes; Score 1, mucosal oedema and petechial haemorrhages; Score 2, 1-5 small ulcers (1-2 mm); Score 3, more than 5 small ulcers or 1 medium ulcer (3-4 mm); Score 4, 2 medium ulcers or 1 large ulcer (more 4 mm) and Score 5, perforated ulcers. The sum of the total activity score in each group divided by the number of rats in the group was expressed as mean ulcer index.

2.4 Other methods

The results are shown as mean ± SD for body weight. Results were analysed by one-way

analysis of variance (ANOVA). Sequential differences among means by ANOVA were calculated at the level of $P < 0.05$ using Tukey analysis post-test.

3. Results

Figure 1 illustrates that 6% v/v acetic acid induced ulcers in all rats and 80% v/v ethanol produced 83.33% incidence of gastric ulcers. Pretreatment with 2 and 4 mg/kg SP prior to ethanol administration reduced the incidence of ulcers in rats dose-dependently. SP also reduced the ulcer incidence induced by acetic acid. There was no ulcer lesion in oral administration of normal saline and pretreatment (S*pirulina platensis* only) groups.

SP at 2 and 4 mg/kg/day for 7 days also reduced the ulcer length by approximately 19 and 28% respectively for ethanol-induced ulcers (Table 1). This inhibition was bigger in acetic acid induced ulceration in rats with 15 and 36% inhibition respectively. However, these figures were lower than inhibition by a commercial anti-ulcer drug, omeprazole. The drug inhibited ulcer length by approximately 56% for ethanol induced- and 58% for acetic acid induced ulceration.

Table 1: Effect of Spirulina platensis on gastric ulcer in rats

Treatment	Ulcer Length (mm)	Mean Ulcer Index	Percentage Inhibition of Ulcer Length*	Percentage Inhibition of Ulcer Index*
Control (Saline)	0.3 ± 0.1^{a}	0.3333	-	-
Ethanol (70% v/v)	3.2 ± 0.3^{c}	2.8333	-	-
Acetic acid (6% v/v)	5.5 ± 0.9^{a}	4.3333	-	-
SP (4 mg/kg)	0.4 ± 0.2^{a}	0.3333	-	-
SP (2 mg/kg) + Ethanol	2.6 ± 0.9^{c}	2.6667	18.75	5.87
SP (4 mg/kg) + Ethanol	2.3 ± 0.4^{b}	1.6667	28.13	41.17
SP (2 mg/kg) + Acetic acid	4.7 ± 1.4^{b}	3.3333	14.55	23.08
SP (4 mg/kg) + Acetic acid	3.5 ± 0.7^{b}	3.000	36.36	30.78
Omeprazole (20 mg/kg) + Ethanol	1.4 ± 0.2^{a}	1.3333	56.25	52.94
Omeprazole (20 mg/kg) + Acetic acid	2.3 ± 0.3^{a}	1.5000	58.18	65.38

(n=6/group).
[a-c] Mean with different superscript differs significantly in the same column ($p<0.05$).
* Percentage inhibition from respective positive controls ie. Ethanol (80% v/v) or Acetic acid (6% v/v) groups.

Fig. 1
Effects of Spirulina platensis on the incidence of Gastric Ulcers in rats

SP: *Spirulina platensis*

Similar trends were also observed for mean ulcer index. The highest ulcer index was in the acetic acid group with 4.333. SP (2 and 4 mg/kg/day) reduced this index to 3.333 and 3.000 respectively. SP also reduced the ulcer index from 2.833 in the ethanol group to 2.667 and 1.667 respectively. Omeprazole potently reduced this index for both ulcerogens (Table 1). SP alone did not induce ulcers and the mean ulcer index was similar to the controls.

4. Discussion

Many previous studies have used ethanol as an ulcerogen. Alkofahi *et. al.*, (1999) [8], reported that ethanol (50% v/v) induced both long ulcers and petechial lesions within relatively short time in laboratory animals. In this present study, 80% ethanol and 6% acetic acid induce ulceration in rats with different severity. Acetic acid cause more severe gastric ulcers in rats than ethanol as observed by the ulcer length, ulcer index and ulcer incidence. Acetic acid causing loss of the superficial epithelium that extends into gastric pits with associated hemorrhage into the mucosa. *Spirulina platensis* inhibited the development of acute gastric ulcers induced by ethanol and acetic acid in rats.

Baggio *et. al.*, (2003) [9] had indicated that ethanol is a good inducer of gastric ulceration. Induction of ulcers by ethanol may involve the reduction of gastric blood supply and production of leukoterines (Guth *et. al.*, 1984) [10]. However, the mechanism of anti-ulcer activity of SP is unclear. Trepenoids has been reported to strengthen the mucosal lining of stomach, making it more resistant to ulcer development (Cheng and Koo 2000) [11] and this phyto compound has been isolated from SP. Furthermore, certain polysaccharides have been used to treat ulcers. Ulceration index was reduced by 20 times with the treatment of rats with extracts of *Plantago major* which contain high concentration of polysaccharides (Sameulsen 2000) [12]. This mechanism may be applicable in SP induced gastroprotection in this study.

Gastric ulcer is believed to be due to an imbalance between acid and pepsin, and weakness of the mucosal barrier (Baron *et. al.*, 1980) [13]. Several mechanisms have been suggested for the effect of gastroprotective principles, including increasing the gastric hexosamine level and enhancing the strength of the gastric barrier either physically or by blocking the H^+, K^+ - ATPase pump (Akhtar *et. al.*, 1992 [14], Akhtar and Ahmed, 1995) [15], stimulation of membrane stabilization by interference with Ca^+ influx, scavenging oxygen generated free radicals and inhibition of biological membranes

(Koch and Loffler, 1985 [16], Cholbi *et. al.*, 1991) [17]. SP may exert its property by one or more of these proposed mechanisms. However, it should be pointed out that the SP contains tannis and flavonoids to which the gastroprotective effects could be attributed.

C-Phycocyanin is one of the major biliproteins of SP. This water soluble protein pigment is shown to be hepatoprotective (Vadiraja *et. al.*, 1998) [18], antioxidant, radical scavenger (Bhat [19] and Madyastha 2000) [20], anti-arthritic (Remirez *et. al.*, 1999) [21], and anti-inflammatory (Romay *et. al.*, 1998) [22] in both *in vitro* and *in vivo* experimental models. The properties of this phytocompound in SP may enhance the wound/ulcer healing activity and may also protect the gastric mucosal layer from insult by ethanol or acetic acid. Further studies are required to isolate the active properties and to elucidate the exact mechanism of gastroprotective properties of SP.

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