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Sonali Joshi, Sagar Mandawgade, Vinam Mehta & Sadhana Sathaye

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Antiulcer Effect of Mammalian Lignan Precursors from Flaxseed

Sonali Joshi, Sagar Mandawgade, Vinam Mehta, and Sadhana Sathaye

Department of Pharmaceutical Sciences and Technology, University Institute of Chemical Technology, Mumbai, India

Abstract

The antiulcer potential of the crude extract of lignans from flaxseed (*Linum usitatissimum* Linn, Linaceae) was evaluated and compared against β-carotene in NSAID-induced gastric ulcers in the Wistar rat model. The isolated stomach tissue was studied for percentage ulcer index and reduced glutathione (GSH) content. The crude extract of lignans exhibited significant protection and better recovery ($p < 0.05$) against ulcer formation as well as in GSH content maintenance. Further, the antiulcer effect of the crude extract of lignans was comparable with that of standard β-carotene.

Keywords: Antiulcer activity, flaxseed lignans, NSAID, reduced glutathione, ulcer index.

Introduction

In ancient times, flaxseed (*Linum usitatissimum* Linn.) (Linaceae) was a crop used in many aspects of life. In addition to the use of its fibers for weaving linen, flaxseed was also used for various medicinal purposes such as treatment of gastric disorders, as a soothing balm for inflammation, and as a laxative (Judd, 1995).Currently, flaxseed is largely grown for the use of its oil in paints and varnishes. However, the use of flaxseed as a potential nutraceutical is being considered from evidence of its beneficial effects in cancer (Serraino & Thompson, 1991; Thompson et al., 1996), risk factors for cardiovascular diseases and diabetes (Cunnane et al., 1993, 1995), inflammatory diseases such a lupus nephritis (Clark et al., 1995), renal function (Ingram et al., 1995), and even antimalarial activity (Levander & Ager, 1995). Flaxseed is the richest source of mammalian lignan precursors, the major one identified as secoisolariciresinol diglucoside (SDG) (Axelson et al., 1982; Thompson et al., 1991). Apart from SDG, the other lignans present in flaxseed are matairesinol, lariciresinol, and pinoresinol, which are present in minor quantities. In mammals, they exist as enterolactone and enterodiol as the metabolic products of lignans.

Lignans exhibit both antiestrogenic and estrogenic activity depending on circulatory levels of estrogens. Lignans also have exhibited antioxidant activity (Hua & Geng-Tao, 1991), antiangiogenic activity, cytotoxic effects on non–estrogen dependent human breast cancer and promyelocytic leukemic cell lines, and antimitotic activity (Thompson, 1994). Lignans did not exhibit any toxicity (Obermeyer et al., 1993). The intake of antioxidants present in food is now considered as important as vitamins for health promotion and protection against damage due to oxidative stress (Tarwadi & Agte, 2003). It is also known that antioxidative imbalance and disruption in defensive mucosal factors are the underlying causes of peptic ulcer. Among various factors, the major causative ones that initiate peptic ulcer are alcohol abuse, acute and chronic stress, infection with *Helicobacter . pylori,* and the prolonged use of NSAIDs (Vogel & Vogel, 1997). Considering the fact that NSAID-induced gastric ulcers are caused by the depletion of glutathione due to generation of free radicals, flaxseed may protect against peptic ulcers due to the high content of mammalian lignan precursors that have good antioxidant potential. The various undesirable side effects of conventional drugs for ulcer treatment are GI discomfort, diarrhea, CNS disturbances, nephritis, hepatitis, granulocytopenia, dry mouth, headache, and gynacomastia. It potentiates the action of anticoagulants and may lead to bleeding ulcers. This further contributes to the investigation of the antiulcer drugs from natural sources. Hence, the current study is aimed at evaluation and comparison of the protective potential of

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Address correspondence to: Dr. Sadhana Sathaye, Department of Pharmaceutical Sciences and Technology, University Institute of Chemical Technology, N. P. Marg, Matunga, Mumbai 400 019, India. Tel.: +91-022-24145616 ext. 432; Fax: +91-022-24145614; E-mail: ssathaye@udct.org

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lignans in flaxseed meal against standard β-carotene in the NSAID-induced ulcerative rat model.

Materials and Methods

Extraction of lignans from *Linum usitatissimum*

Seeds of *Linum usitatissimum* were obtained from the local market and were authenticated by Dr. Harshad Pandit of the Department of Botany, Guru Nanak Khalsa College (Mumbai, India). The herbarium specimen has been deposited in the pharmaceutical division of UICT, Mumbai (specimen no. 020601). The seeds washed, dried, and ground to a fine powder in a grinder to get flaxseed meal. The meal was defatted twice using *n*-hexane in the ratio 1:10 in an ice bath for 1 h, and the solvent was removed by centrifugation at 5000 rpm. The defatted meal was extracted with 70% methanol (1:10) by magnetic stirring for 5 h. The solution was centrifuged at 3000 rpm for 10 min to obtain the methanol extract. Base hydrolysis was carried out on this extract using 5% of 1 M NaOH while stirring overnight. The pH of the extract was adjusted to 5 with 1% HCl, and it was then evaporated to dryness. The residue (crude extract of lignans) thus obtained was dissolved in distilled water and used as a crude extract.

Evaluation of antiulcer potential

Male Wistar rats weighing 180–220 g were allowed to acclimate 1 week in the UICT animal house before experimentation. The animals were maintained under standardized environmental conditions (25–30°C, 12-h dark/light cycle) and fed with standard rat feed and water *ad libitum*. All the procedures and study protocols were approved by the institutional animal ethical committee (study approval no. UICT/PH/IAEC/0206121). The antiulcer activity was evaluated using two ulcer models: (1) recovery/healing from NSAID-induced gastric ulcers; (2) Protection from NSAID-induced gastric ulcers.

Recovery/healing from NSAID-induced gastric ulcers

The animals were divided into four groups: group I, control (no NSAID, no treatment, n = 18); group II, negative control (NSAID administered, n = 18); group III, test (NSAID and treatment with flaxseed lignans 2.5 mg/200 g body weight, n = 18); and group IV, positive control (NSAID and treatment with β-carotene 5 mg/200 g body weight, n = 18). Animals in Groups I and II were fasted for 24 h. NSAID was orally administered (diclofenac sodium, 20 mg/kg body weight) to group II. The oral feeding of molar solution of NSAID was continued for 3 days. No feed was provided during this period except water *ad libitum*. On day 4, six animals from groups I and II were sacrificed. The stomach was isolated from each animal. The total stomach area was measured along with the area of ulcers produced, and the percent ulcer index was calculated. The stomach tissue was weighed (200 mg) and then analyzed for the content of reduced glutathione (GSH). The aforementioned step was repeated for both groups on days 7 and 10. Groups III and IV were fasted for 24 h and then administered NSAID orally (diclofenac sodium, 20 mg/kg body weight), followed by oral administration of respective test substances (after 3 h). The oral administration of NSAID and flaxseed lignans/β-carotene was continued for 3 days. On day 4, six animals from groups III and IV were sacrificed and the percent ulcer index was calculated after isolating the stomach tissue. The stomach tissue was weighed (200 mg) and then analyzed for the content of GSH. The aforementioned step was repeated for both groups on days 7 and 10.

Protection from NSAID-induced gastric ulcers

The animals were divided into two groups: group V, test (treatment with flaxseed lignans and NSAID, n = 6); group VI, positive control (treatment with β-carotene and NSAID, n = 6). Values for the control and negative control groups for this model were kept the same as for groups I and II, for comparison on day 4. Groups V and VI were treated with

Table 1. Recovery/healing effect of flaxseed lignan extract on gastric ulcers.

Groups	I	II	III	IV
Day 4				
GSH (μg/mL)	3.78 ± 0.06	1.25 ± 0.05	1.42 ± 0.03[#]	1.88 ± 0.06*
U.I.(%)	26.97 ± 1.17	69.16 ± 1.23	19.69 ± 1.52*	47.97 ± 1.77*
Day 7				
GSH (μg/mL)	3.85 ± 0.11	1.65 ± 0.06	2.54 ± 0.06*	2.07 ± 0.07*
UI(%)	12.2 ± 1.17	54.68 ± 1.24	16.7 ± 1.35*	35.99 ± 1.41*
Day 10				
GSH (μg/mL)	3.95 ± 0.11	2.18 ± 0.09	3.33 ± 0.08*	2.78 ± 0.07*
UI (%)	2.16 ± 0.98	35.34 ± 1.04	8.4 ± 1.16*	12.11 ± 1.77*

Values are mean ± SEM. GSH, reduced glutathione, UI, ulcer index.
*Significant ($p < 0.01$) when compared with group II, n = 6.
[#]Significant ($p < 0.05$) when compared with group II, n = 6.

Table 2. Protective effect of flaxseed extract on gastric ulcers.

Groups	I	II	V	VI
Day 18				
GSH (μg/mL)	3.78 ± 0.06	1.25 ± 0.05	2.62 ± 0.1*	2.62 ± 0.16*
UI (%)	26.97 ± 1.17	69.16 ± 1.23	18.1 ± 1.1*	15.09 ± 1.96*

Values are mean ± SEM. GSH, reduced glutathione; U.I., ulcer index.
*Significant ($p < 0.01$) when compared with group II, $n = 6$.

flaxseed lignans (2.5 mg/200 g body weight) and β-carotene (5 mg/200 g body weight), respectively, for 14 days. After fasting for 24 h, NSAID (diclofenac sodium, 20 mg/kg body weight) was orally administered for 3 days. During this period, no feed was provided except water *ad libitum*. All animals of groups V and VI were sacrificed on day 18. The percentage ulcer index was calculated after isolating the stomach tissue. The stomach tissue was weighed (200 mg) and then analyzed for the content of GSH.

For the assessment of ulcer index, the total area of stomach tissue and the area of ulceration were measured using graph paper (cm^2). The ulcer index was expressed as percent of total area of glandular stomach (Majumdar et al., 2003). GSH is a protective thiol produced by body tissues to trap any reactive oxygen molecules. The amount of GSH reduces with oxidative damage. Reduction in GSH content is evident after NSAID administration in experimental animals, which probably leads to formation of ulcers. The GSH content of stomach tissue was determined according to the method of Owens and Belcher (1965). The method was modified to estimate the content of GSH from the body tissues. Here, 5-5′-dithiobis-2-nitrobenzoic acid (DTNB; Ellman's reagent) was used as a substrate that is converted by GSH to 5-thio-2-nitrobenzoic acid (TNB), which is a chromophore that can be read at 412 nm. Statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnett's multiple comparison test at a level of significance $p < 0.05$ (Bolton & Bon, 2004).

Results and Discussion

Recovery/healing from NSAID-induced gastric ulcers

When compared on day 4, the GSH content of the stomach tissue was highest in the control group (3.78 ± 0.06 μg/mL); whereas GSH content found to be decreased ($P < 0.01$) upon ulcer induction in the negative control group II (1.25 ± 0.05 μg/mL), test group III (1.42 ± 0.03 μg/mL) and positive control group IV (1.88 ± 0.06 μg/mL). At the end of treatment on day 10, when groups III and IV were compared for GSH content recovery, results revealed that group III (3.33 ± 0.08 μg/mL) showed better recovery compared group IV (2.78 ± 0.07 μg/mL) and negative control group II (2.18 ± 0.09 μg/mL). In the case of percent ulcer index, when groups III and IV were compared with the negative control, significant recovery ($p < 0.01$) from ulcer formation was observed on days 4, 7 and 10 (see Table 1). Further, at the end of day 10, flaxseed lignans heal ulcer formation (8.4 ± 1.16%) better than does β-carotene (12.11 ± 1.77%). Hence, flaxseed lignans show better recovery than that of β-carotene in terms of glutathione content and percentage ulcer index at the end of the study.

Protection from NSAID-induced gastric ulcers

On day 18, the GSH content in the control was 3.78 ± 0.06 μg/mL whereas it was decreased ($p < 0.01$) upon ulcer induction in negative control group II (1.25 ± 0.05 μg/mL). At the end of the study, GSH content of test group V and positive control group VI were 2.62 ± 0.1 μg/mL and 2.62 ± 0.16 μg/mL, respectively, which were compared with negative control and found to be significantly better ($p < 0.01$) in GSH content maintenance. When the results of groups V and VI were compared, the data were comparable in terms of GSH content maintenance. In percent ulcer index measurement, when the results of groups V (18.1 ± 1.1%) and VI (15.09 ± 1.96%) were compared with the negative control (69.16 ± 1.23%), a significant protection ($p < 0.01$) against ulcer formation was observed on day 18. Further, in comparison between groups V and VI, the results were comparable in terms of ulcer protection (see Table 2).

Conclusions

The crude extract of flaxseed lignans exhibited significant antiulcer effect by recovery and protection against ulcer formation in the NSAID-induced gastric ulcer model in Wistar rats. Further, the antiulcer potential of flaxseed lignans was comparable with that of β-carotene. The mechanism of action could be attributed to the maintenance of reduced glutathione (GSH) by free radical scavenging activity of flaxseed lignans. This property of crude extract of flaxseed lignans can be used as a nutritional support during gastric ulcers.

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