

Effect of solvent, temperature and time on extraction process of anti ulcer compound present in *Amaranthus spinosus* Linnaeus

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Abstract

Effect of solvent, temperature and time on extraction process of anti ulcer compound present in the leaves of *Amaranthus spinosus* Linnaeus was studied. Results showed that extraction of leaves of *Amaranthus spinosus* with ethanol (50 %, v/v) at 40° C for one hour had maximum anti ulcer effect against indomethacin induced gastric ulceration in albino rats.

Key words: Extraction process, Anti ulcer effect, *Amaranthus spinosus*, Indomethacin

INTRODUCTION

Amaranthus spinosus Linnaeus, a medicinal plant of the family of Amaranthaceae, is distributed in hot tropical to middle hills (upto 1500 m amsl) of entire country including Eastern Himalaya. The plant grows in cultivated areas as well as in waste places. Leaves of *Amaranthus spinosus* Linnaeus are stacked and alternate. The plant is known as “prickly amaranthus” in English and “*ban lure*” or “*dhuti ghans*” in Nepali and “*Kanta Note*” in Bengali. Medicinal uses of *Amaranthus spinosus* as mentioned in Ayurveda (Chopra & Chopra 1958; Das *et al* 2010) are: (i) leaf infusion is diuretic and used in anemia; (ii) root paste is used in gonorrhoea, eczema, menorrhoea etc. Ethnic uses of *Amaranthus spinosus* is mainly with the villagers of Sikkim who use leaf infusion of the plant in stomach disorder specially in case of indigestion and peptic ulcer (Gurung 2002).

Recently we observed anti-ulcer activity of the leaves of *Amaranthus spinosus* against indomethacin induced gastric ulcer in albino rats. Tempted with this observation we undertook studies for isolation of the active compound present in *Amaranthus spinosus* responsible for anti gastric ulcer activity. In this communication we report effects of solvent, temperature and time on extraction process of anti ulcer compound from the leaves of *Amaranthus spinosus* Linnaeus.

METHODOLOGY

Collection of plant material

Leaves of *Amaranthus spinosus* Linnaeus were collected from the medicinal plant garden of the University of North Bengal and its identity has been confirmed in the Taxonomy and Environmental Biology Laboratory of the Department of Botany, North Bengal University. A voucher specimen of the plant has been preserved in the Biochemistry department of the North Bengal Medical College for future references.

Preparation of leaves for anti-ulcer screening

Leaves of *Amaranthus spinosus* were sundried and powdered. 10 grams of this powder was separately extracted with 100 ml of different solvents at different temperatures and time on a rotary shaker. The extract was filtered and the solvent was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 degree Celsius. A brownish mass was obtained. This mass was kept for anti-ulcer screening.

Effect of solvents in extraction process

Distilled water as well as 50 % (v/v) of acetone, ethanol, methanol and petroleum ether were used separately in extraction process.

Effect of time in extraction process

Extraction processes were done separately for 30, 60, 90 and 120 minutes after adding the solvent.

Effect of temperature in extraction process

In separate experiments extraction processes were done at 30, 40 and 50 degree Celsius.

Reagents

All reagents required for the experiment were procured from Merck, USA.

Experimental animals

Wistar strain albino rats of both sexes were used for the study. The animals were housed in colony cages (5 rats/ cage) and were kept for at least one week in the experimental wing of the animal house (room temperature 25 – 28° C and humidity 60 – 65 % with 12 hours light and dark cycle) before experimentation. Animals were fed on laboratory diet with water *ad libitum*. For each set of experiment 10 animals were used. The animal experiment was approved by the ethics committee of the Institute.

Indomethacin

Indomethacin was collected from Torrent Research Centre, Gandhinagar, India.

Test drug

Brownish mass obtained from extraction process was used as the test drug.

Production of Indomethacin induced gastric ulcer (Parmar & Desai 1993)

Indomethacin (10 mg/kg) was given orally through feeding tube to rats in two doses at an interval of 15 hours. One hour after the last dose of indomethacin feeding, animals were sacrificed by cervical dislocation and the stomach was taken out and incised along the greater curvature. Stomach was then examined for the presence of ulcers.

Evaluation of ulcer index (Szelenyi & Thiemer 1978)

Gastric lesions were counted and the mean ulcerative index was calculated as follows:

- I - Presence of edema, hyperemia and single sub mucosal punctiform hemorrhage
- II – Presence of sub mucosal hemorrhagic lesions with small erosions
- III – Presence of deep ulcer with erosions and invasive lesions

$$\text{Ulcer index} = (\text{number of lesion I}) \times 1 + (\text{number of lesion II}) \times 2 + (\text{number of lesion III}) \times 3.$$

Anti gastric ulcer study

Test drug in the dose of 1g/ kg body weight was given orally through feeding tube 30 minutes prior to administration of each dose of indomethacin.

Statistical analysis

The values were expressed as mean \pm SEM and was analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th versions. Differences between means were tested employing Duncan's multiple comparison tests and significance was set at $p < 0.05$.

RESULTS

Table – 1 shows the effect of solvents on extraction process for isolation of anti gastric ulcer compound from the leaves of *Amaranthus spinosus* Linnaeus. It was found that ethanol (50 %, v/v) extract of the leaves of *Amaranthus spinosus* had maximum anti ulcer activity against indomethacin induced gastric ulcers in albino rats. Ulcer index was calculated to only 10.8 ± 0.7 when indomethacin induced gastric ulcer index in rats was 26.6 ± 1.3 . The result was statistically significant up to the level of $p < 0.001$. Acetone as well as water extracts had also good anti ulcer activity. Anti ulcer activities came 15.5 ± 0.9 for acetone extract and 18.3 ± 1.0 for water extract. Methanol and petroleum ether extracts had, however, no significant anti gastric ulcer activity against indomethacin induced gastric ulcers in albino rats.

Table 1. Anti ulcer activity of extract of leaves of *Amaranthus spinosus* Linnaeus against indomethacin induced gastric ulcers in albino rats : Effect of solvents on extraction process.

Solvent	Dose of extract	Ulcer index
Water	1 g/ kg	18.3 ± 1.0
Acetone (50 %, v/v)	1 g/ kg	15.5 ± 0.9
Ethanol (50 %, v/v)	1 g/ kg	$10.8 \pm 0.7^*$
Methanol (50 %, v/v)	1 g/ kg	23.1 ± 1.1
Petroleum ether (50 %, v/v)	1 g/ kg	25.5 ± 1.3

Data shown in mean \pm SEM (n = 10). Indomethacin induced ulcer index was 26.6 ± 1.3 * $p < 0.001$

Effect of time on extraction process for isolation of anti gastric ulcer compound from the leaves of *Amaranthus spinosus* is shown in Table – 2. It appears from the table that anti ulcer activity increased with time of extraction. This was up to one hour. Extraction with ethanol (50 % v/v) for thirty minutes as well as for one hour gave ulcer index 17.3 ± 1.1 and

10.9 ± 1.3 respectively. There was, however, no significant change in anti ulcer activity even when extraction time was extended up to two hours. Anti ulcer activity after one and half hours of extraction came 10.8 ± 1.5 and after two hours period of extraction it was 10.9 ± 1.2 .

Table 2. Anti ulcer activity of extract of leaves of *Amaranthus spinosus* Linnaeus against indomethacin induced gastric ulcers in albino rats : Effect of time on extraction process.

Solvent	Time (minutes)	Ulcer index
Ethanol (50 %, v/v)	30	17.3 ± 1.1
	60	10.9 ± 1.3*
	90	10.8 ± 1.5
	120	10.9 ± 1.2

Data shown in mean ± SEM (n = 10). Indomethacin induced ulcer index was 26.6 ± 1.3 *p<0.001

Table – 3 shows the effect of temperature on extraction process for isolation of anti gastric ulcer compound from the of leaves of *Amaranthus spinosus*. Increase in temperature during extraction elevated anti gastric ulcer activity. When extraction was done at 30° C ulcer index came 18.3 ± 1.3, at 40° C it was 10.7 ± 1.0. After that, increase in temperature during extraction had no effect on anti ulcer activity. Ulcer index came 10.7 ± 1.3 when temperature was increased to 50° C.

Table 3. Anti ulcer activity of extract of leaves of *Amaranthus spinosus* Linnaeus against indomethacin induced gastric ulcers in albino rats : Effect of temperature on extraction process.

Solvent	Degree Celsius	Ulcer index
Ethanol (50 %, v/v)	30	18.3 ± 1.3
	40	10.7 ± 1.0*
	50	10.7 ± 1.3

Data shown in mean ± SEM (n=10).Indomethacin induced ulcer index was 26.6 ± 1.3, *p<0.001

DISCUSSION

Due of its frequency and worldwide distribution peptic ulcer continues to be a subject of numerous investigations, both experimental and clinico pathological. In this respect peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology.

There are medicines to treat peptic ulcer (Tierrey *et al.* 1978). These drugs are broadly classified into two categories:

1. Those can decrease or counter acid – pepsin secretion viz. ranitidine, famotidine etc. (H₂ - blockers), pirenzepine, telenzepine etc. (M1– blockers), omeprazole, lansaprazole, pantoprazole etc. (proton pump inhibitors)
2. Those can affect cytoprotection by virtue of their effects in mucosal defense factors like sucralfate, carbenoxolone etc. (Yeomans *et al.*1998)

No doubt the above mentioned drugs have brought about remarkable changes in peptic 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 and danger of drug interactions during ulcer therapy. Hence, the search for an ideal anti – ulcer drug continues and has also been extended to medicinal plants / herbs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.

Numerous medicinal plants, fruits etc. showed anti gastric ulcer activity. Sanyal *et al.* (1961) found that vegetable banana is efficacious not only for experimentally induced gastric ulcers in albino rats, guinea pigs etc. but also for human being suffering from peptic ulcers. Akah & Nwafor (1999) demonstrated anti gastric ulcer activity of the herb *Cissampelos mucronata* A. Rich. Likewise Shetty *et al.* (2000), Sairam *et al.* (2001), Maity *et al.* (1995, 2003), Dharmani & Palit (2006) and Malairajan *et al.* (2008) confirmed anti gastric ulcer activities of *Ginkgo biloba* Linnaeus, *Convolvulus pluricaulis* Choisy, tea root extract, *Vernonia lasiopus* O. Hoffmann and *Polyalthia longifolia* (Sonnerat) Thwaites respectively. We also reported anti gastric ulcer activities of few medicinal plants in different experimental ulcer models (Mitra 1980, 1981, 1982, 1985, 2001; Mitra & Mitra 2005, 2008; Mitra *et al.* 2008, 2010, 2011).

Recently we observed anti ulcer activity of the leaves of *Amaranthus spinosus* against indomethacin induced gastric ulcer in albino rats [unpublished observation]. We undertook isolation studies to get the active compound present in the leaves of *Amaranthus spinosus*. Extraction process is a part of isolation work. Different solvents yield different extracts variable compositions (Zarnowski & Suzuki 2004). Therefore, a suitable extracting solvent should be selected for extraction of the active compound for its maximum activity (Wang & Weller 2006). We thus used distilled water, acetone, ethanol, methanol and petroleum ether separately as solvents for the extraction of the active compound from leaves of *Amaranthus spinosus*. Results showed that ethanol (50 %, v/v) extract had maximum anti ulcer activity against indomethacin induced gastric ulceration in albino rats. This was followed by acetone extract. Water extract had also some anti gastric ulcer activity but methanol and petroleum ether extracts had no anti ulcer activities.

As extraction time is important to extract active compound in maximum amount, we allowed extraction time as half an hour, one hour, one and half hours as well as two hours in separate experiments. It was found that one hour extraction time produced the extract from leaves of *Amaranthus spinosus* had maximum anti gastric ulcer activity against indomethacin induced gastric ulcers in albino rats.

The extraction temperature is another important factor influencing the recovery of the bio reactive compound from the source (Wang & Weller 2006). In separate experiments we thus conducted extraction at temperature stages like 30, 40 and 50 degree Celsius. Results revealed that extraction of the leaves of *Amaranthus spinosus* at 40° C had more anti gastric ulcer activity against indomethacin induced gastric ulcers in albino rats than the extraction temperature at 30° C. Further increase of extraction-temperature, however, had no significant effect on the anti gastric ulcer activity against indomethacin induced gastric ulcers in albino rats.

We are now interested to see the effect of mixture of solvents on the process of extraction. Works in this direction are in progress.

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