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## Antinociceptive Activity Evaluation of Methanolic Extract of Roots of *Raphanus sativus* L.

Safia Parvin, Md. Masta Hasenur Reza Chawdhury, Nargis Ara, Prashanta Kumer Paul, A.K.M. Mahmudul Haque, Shahnaz Rahman, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh

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### ABSTRACT

The antinociceptive potential of methanolic extract of roots of *Raphanus sativus* were evaluated in intraperitoneally administered acetic acid-induced pain model in mice. Administration of methanolic extract to mice at doses of 50, 100, 200 and 400 mg per kg body weight lead to dose-dependent reductions in the number of abdominal constrictions (writhings) induced by acetic acid. At the afore-mentioned four doses, the percent reductions in the number of constrictions were, respectively, 23.1, 42.3, 53.8, and 61.5. The percent reductions were significant at the three higher doses. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of abdominal constrictions, respectively, by 38.5 and 65.4%. Thus the highest dose of the extract was nearly comparable to the highest dose of aspirin. Taken together, the extract was found to possess significant antinociceptive activity and so can be used for alleviation of pain.

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## INTRODUCTION

*Raphanus sativus* L. (Cruciferae) is a vegetable crop cultivated in many countries of the world primarily for its edible roots, which can be consumed both in the cooked or raw form. In Bangladesh, the aerial parts are also consumed along with the roots in the cooked form. The root can be of white or violet to red color depending on the absence of anthocyanins like pelargonidine and cyanidine. In Bangladesh, both the red and the white varieties are cultivated. In English the red variety is known as radish, and in Bengali it is known as 'lal mula'.

Ethnomedicinal uses have been reported for the plant. The tribals of Northeast Gujarat, India use the roots and leaves of the plant against urinary complaints (Punjani, 2010). Herbal practitioners and local people of Dharmabad Taluka of Nanded district, Maharashtra, India use leaves of the plant to treat piles (Ghorband and Biradar, 2011). The tribes of Pedabayalu Mandalam, Visakhapatnam district, Andhra Pradesh, India use roots against urinary trouble (Padal *et al.*, 2013). The natives of Bargarh district of Orissa, India use seeds against sexual debility (Sahu *et al.*, 2010).

For the last few years we had been concentrating on ethnomedicinal surveys followed by screening of the plants obtained for antihyperglycemic, antinociceptive and cytotoxic activities (Rahmatullah *et al.*, 2009a-c; Anwar *et al.*, 2010; Jahan *et al.*, 2010; Rahman *et al.*, 2010; Rahmatullah *et al.*, 2010a-h; Shoha *et al.*, 2010; Ali *et al.*, 2011; Barman *et al.*, 2011; Hossan *et al.*, 2011; Jahan *et al.*, 2011; Rahman *et al.*, 2011; Rahmatullah *et al.*, 2011a,b; Sutradhar *et al.*, 2011; Ahmed *et al.*, 2012; Arefin *et al.*, 2012; Haque *et al.*, 2012; Sathi *et al.*, 2012; Rahmatullah *et al.*, 2012a-d; Haque *et al.*, 2013). As part of the screening process to locate plants with antinociceptive properties, this study was conducted to evaluate the antinociceptive (as conducted through acetic acid induced abdominal pain) potential of methanolic extract of roots of *Raphanus sativus* (red variety) in Swiss albino mice. Notably, anti-inflammatory property has been reported for root and leaf juice of the plant (Kamble *et al.*, 2013).

## MATERIALS AND METHODS

Roots of *Raphanus sativus* were collected from a local market in Dhaka city, Bangladesh during November, 2013. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Accession

**Corresponding Author:** Professor Dr. Mohammed Rahmatullah, Pro-Vice Chancellor and Dean, Faculty of Life Sciences, University of Development Alternative, House No. 78, Road No. 11A (new), Dhanmondi, Dhaka-1205, Bangladesh  
 Tele: +88-01715032621 Fax: +88-02-815739 E-mail: rahamatm@hotmail.com

Number 38,620). The roots were air-dried in the shade, grounded into a fine powder and 150g of the powder was extracted with methanol (1:5, w/v) for 48 hours. The extract was evaporated to dryness at 40°C. The final weight of the extract was 24.3g.

#### Chemicals:

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin was obtained from Square Pharmaceuticals Ltd., Bangladesh.

#### Animals:

In the present study, Swiss albino mice (male), which weighed between 13-15g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

#### Antinociceptive activity:

Antinociceptive activity of the methanol extract of *Raphanus sativus* roots (MERSR) was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspirin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanol extract of *Raphanus sativus* roots (MERSR) at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

The following formula was used for calculation of percent inhibition of the number of writhings in aspirin and MERSR administered animals compared to control mice,

$$\text{Percent inhibition} = (1 - W_e/W_c) \times 100$$

where  $W_e$  and  $W_c$  represents the number of writhings in aspirin or MERSR administered mice (Groups 2-7), and control mice (Group 1), respectively.

#### Preliminary phytochemical screening:

Preliminary phytochemical analysis of MERSR for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before (Kumar *et al.*, 2013).

#### Acute toxicity test:

Acute toxicity test was conducted as previously described (Ganapaty *et al.*, 2002). Mice were divided into nine groups, each group consisting of six animals. Group 1 was given 1% Tween 80 in normal saline (2 ml per kg body weight). The other eight groups (Groups 2-9) were administered, respectively, 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg of MERSR per kg body weight. All animals were closely observed for the next 8 hours to notice any behavioral changes or mortality and were kept under close observation for the next two weeks.

#### Statistical analysis:

Experimental values are expressed as mean  $\pm$  SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value  $< 0.05$  in all cases.

## RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of saponins, alkaloids, and flavonoids in MERSR. Any signs of toxicity were not observed with the extract in mice.

Administration of methanolic extract (MERSR) to mice at doses of 50, 100, 200 and 400 mg per kg body weight lead to dose-dependent reductions in the number of abdominal constrictions (writhings) induced by acetic acid. At the afore-mentioned four doses, the percent reductions in the number of constrictions were, respectively, 23.1, 42.3, 53.8, and 61.5. The percent reductions were significant at the three higher doses. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of abdominal constrictions, respectively, by 38.5 and 65.4%. Thus the highest dose of the extract was nearly comparable to the highest dose of aspirin (400 mg), and the three highest doses of the extract demonstrated better antinociceptive activity than 200 mg aspirin. The results are shown in Table 1. Taken together, the extract was found to possess significant antinociceptive activity and so can be used for alleviation of pain.

**Table 1:** Antinociceptive effect of crude methanol extract of *Raphanus sativus* roots (MERSR) in the acetic acid-induced gastric pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control (Group 1)	10 ml	5.20 ± 0.20	-
Aspirin (Group 2)	200 mg	3.20 ± 0.58	38.5*
Aspirin (Group 3)	400 mg	1.80 ± 0.58	65.4*
MERSR	50 mg	4.00 ± 0.63	23.1
MERSR	100 mg	3.00 ± 0.45	42.3*
MERSR	200 mg	2.40 ± 0.24	53.8*
MERSR	400 mg	2.00 ± 0.32	61.5*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); \* $P < 0.05$ ; significant compared to control.

The roots are reported to contain a number of bioactive compounds including kaempferol, quercetin, myricetin, apigenin, luteolin, pelargonidine, and cyanidine (Gutiérrez and Perez, 2004). The antinociceptive action of quercetin and kaempferol, isolated from *Danae racemosa* leaves, has been described (Maleki-Dizaji *et al.*, 2007). Myricetin has also been reported to demonstrate potent analgesic and cyclooxygenase 1 inhibitory activities (Tong *et al.*, 2009). Since acetic acid-induced pain is a consequence of increased levels of prostaglandin E2 synthesis, any inhibition of cyclooxygenase(s) can lead to the observed antinociceptive effect.

Kaempferol, quercetin, and myricetin can account for the antinociceptive effects observed in the present study. However, the crude extract was also found to contain saponins, alkaloids, and flavonoids. The antinociceptive and anti-inflammatory activities of crude extract of leaves of *Ipomoea involucreta* has been reported in rats and mice. The extract contained a mixture of alkaloids, flavonoids, and saponins (Ijeoma *et al.*, 2011). Aqueous extract of leaves of *Lagenaria breviflora* has also been shown to demonstrate antinociceptive activity in acetic acid-induced writhing tests in laboratory animals (Adedapo *et al.*, 2013). The extract was reported to contain among other phytochemical compounds, alkaloids, flavonoids, and saponins. Thus other phytochemicals may be present in the extract, which can contribute to the antinociceptive effects.

Pain is a world-wide problem affecting human beings. Existing over-the-counter drugs like aspirin and paracetamol suffer from adverse side-effects like producing gastric ulceration or hepatotoxicity, when taken in overdoses or for prolonged time periods. As such, the extract can be of benefit to people, particularly who suffer from chronic pain.

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