REPORT

ANALGESIC ACTIVITIES OF ETHANOL EXTRACT OF LEAF, STEM AND THEIR DIFFERENT FRACTIONS OF *SWERTIA CHIRATA*

KHONDOKER DEDARUL ALAM[1]*, MOHAMMAD SHAWKAT ALI[1], SANJIDA MAHJABEEN[2], SALMA PARVIN[1], MOHAMMAD AHSANUL AKBAR[1] AND RUBINA AHAMED[1]

[1]*Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy*
[2]*Department of Pharmaceutical Technology, University of Dhaka, Bangladesh*

ABSTRACT

The purpose of the present study was to investigate the analgesic activity of ethanol extract of leaf, stem, and their different fractions i.e. pet-ether, dichloromethane, and methanol fraction of *Swertia chirata* (Family-*Gentianaceae*) on *Swiss albino* mice. Acetic acid induced writhing in mice was used as the process to evaluate the analgesic activity. The ethanol extract of leaf and stem of *Swertia chirata* showed moderate inhibition ($p<0.001$) of writhing. Among different fractions pet-ether fraction showed significant inhibition ($p<0.0001$) of writhing where as methanol fraction showed moderate inhibition ($p<0.003$) of writhing as well. The inhibition of writhing was calculated in respective to control (vehicle). The test samples were administered at a dose of 200 mg/kg body weight of experimental animals where diclofenac sodium at a dose of 25 mg/kg body weight was used as standard drug in this study.

Keywords: Analgesic activity**,** Writhing**,** *Swiss albino* mice, *Swertia chirata, Gentianaceae*.

INTRODUCTION

Though considerable progress has been achieved in medical science during the last decades, management of choric pain still remains a challenge for medical community. All the currently available analgesic drugs such as NSAIDs have more or less few adverse effects (S.M. Raquibul Hasan *et al*., 2009). As a result, more and more people are turning to herbal medicines as the alternative treatment of pain.

Herbal plants are an important source of new chemical substances with potential therapeutic uses. Approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world (Famsworth *et al*., 1985). *Swertia chirata* (Family*: Gentianaceae*) is well known for its different therapeutic uses. The plant have been reported to have anti-inflammatory (Islam *et al.*, 1995), anti-viral (Verma *et al*., 2008), anthelmintic (Iqbal *et al*., 2006), anticarcinogenic (Saha *et al*., 2004), hepatoprotectative (Mukherjee *et al*., 2006), hypoglycemic (Bajpai *et al*., 1991 and Saxena *et al*., 1993) activities. Early studies documented the presence of flavonoids, xanthones, terpenoids, iridoids, and secoiridoid glycosides in the *Swertia chirata* plant (Pant *et al*., 2000). The present study has been designed to evaluate the analgesic activity of ethanolic extract of leaf, stem, and their different fractions of *Swertia chirata***.**

**Corresponding author:* e-mail: kh.dedarul@yahoo.com

MATERIALS AND METHODS

Plant material

The plants were collected from Chawk bazar, Dhaka in November 2007. The authentication of the plant samples have been confirmed by the taxonomist of the National Herbarium of Bangladesh, (accession number - 34333). The plant was dried in shade and then leaf and stem part were ground in coarse powder. The powders were then preserved in air tight containers.

Extraction and fractionations

Coarsely powdered leaf and stem of *Swertia chirata* were extracted with ethanol by cold extraction process. The crude extracts were then filtered and the solvent were removed until solid/semisolid mass were produced. Then the crude leaf and stem extracts were dissolved in 10% water in methanol (100 ml) and partitioned between pet-ether, dichloromethane, and methanol fractions.

Experimental animals

The experiment of analgesic activity was conducted on *Swiss albino* mice of both sexes, aged 4-5 weeks, weighting about 20-25 g. Before initiating the experiment, the mice were acclimatized for few days under standard environmental conditions (12hours dark/12hours light cycle; temperature 20-22 0C; relative humidity 40-60%).

Chemicals and drugs

Diclofenac sodium from Sigma chemical co. (St. Louis, USA) is used as standard drug. 0.7 % acetic acid from

Table 1: Effect of leaf extract of *Swertia chirata* and its different fractions by acetic acid induced writhing on *Swiss albino* mice

Groups	Sample	Dose (mg kg^{-1})	No. of writhing	95% CI* value	% of writhing inhibition	T value	*p*-value
Group-I	Control	Vehicle	19.00±0.577	17.87-20.13	-		
Group-II	Standard	25	6.67±0.494	5.70-7.64	64.91	16.226	<0.0001
Group-III	Leaf extract	200	15.50±0.500	14.52-16.48	18.42	4.583	0.0010
Group-IV	Pet-ether fraction	200	10.50±0.428	9.66-11.34	44.74	11.825	<0.0001
Group-V	Dichloromethane fraction	200	16.67±0.558	15.57-17.76	12.28	2.907	0.0156
Group-VI	Methanol fraction	200	13.83±0.833	12.20-15.47	27.19	5.096	0.0005

Values in the table are presented as mean ± SEM (N = 6). The % of inhibition of writhing was calculated in respective to control (vehicle). Compared to control, *P*-values are determined by student's *t*-test.
* CI = Confidence interval

Table 2: Effect of stem extract of *Swertia chirata* and its different fractions by acetic acid induced writhing on *Swiss albino* mice

Groups	Sample	Dose (mg kg^{-1})	No. of writhing	95% CI* value	% of writhing inhibition	T value	*p*-value
Group-VII	Control	Vehicle	18.00±0.577	16.87-19.13	-		
Group-VIII	Standard	25	6.83±0.307	6.23-7.44	62.04	17.073	<0.0001
Group-IX	Stem extract	200	14.17±0.307	13.56-14.77	21.30	5.861	0.0002
Group-X	Pet-ether fraction	200	10.17±0.401	9.38-10.95	43.52	11.140	<0.0001
Group-XI	Dichloromethane fraction	200	15.17±0.601	13.99-16.34	15.74	3.400	0.0068
Group-XII	Methanol fraction	200	13.67±0.882	11.94-15.39	24.07	4.111	0.0021

Values in the table are presented as mean ± SEM (N = 6). The % of inhibition of writhing was calculated in respective to control (vehicle). Compared to control, *P*-values are determined by student's *t*-test.
* CI = Confidence interval

Merck Chemicals Ltd. (Germany) is used as writhing inducer. Tween 80 from BDH Chemicals Ltd and DMSO (Dimethyl sulphoxide) from Merck Chemicals Ltd (Germany) were used as suspending agent for extract fractions. Sterile normal saline solution (0.9% NaCl) from Beximco Infusion Ltd. (Bangladesh) is used as solvent for standard and test samples.

Preparation of the test materials and standard

50 mg crude extracts of leaf, stem, and their different fractions were triturated by the addition of small amount of suspending agent (Tween-80 and Dimethyl sulphoxide). Normal saline was slowly added to make the final volume up to 2.5 ml. To prepare the standard, diclofenac sodium 25 mg was dissolved into 0.9% normal saline and made the volume up to 10 ml. For preparing control sample, tween-80 (1%) and dimethyl sulphoxide were mixed properly in the normal saline to make the volume up to 5 ml.

Designing of the experiment

In this present study acetic acid induced writhing method (Koster *et al*., 1959 and E.M. Williamson *et al*., 1996) was used to investigate analgesic activity. The experimental animals were randomly divided into twelve groups consisting of 6 mice in each group. The groups were denoted from group-I to group-XII. Analgesic activity of leaf extract and its different fractions were carried out with group-I to group-VI where as group-VII to group-XII were employed to evaluate the analgesic activity of stem extract and its different fractions. Each group of mice received a specific treatment. Prior administering the drugs, each mouse was weighed properly and the doses were adjusted accordingly.

Experimental procedure

The test samples, control, and Standard diclofenac sodium were administered orally with the help of a feeding needle at the beginning of the experiment. After 45 minutes 0.7% acetic acid was injected intraperitoneally to each of the animals of all the groups. Approximately 10 minutes after the injection of acetic acid, a wave of contraction and elongation of abdominal musculature referred to as 'writhing' was started and the number of writhing for the next 10 minutes were counted for each mouse.

STATISTICAL ANALYSIS

Data were expressed as mean±SEM. Statistical significance was determined via Student's *t*-test. $P<0.05$ was considered as statistically significant.

RESULTS

Effect of leaf extract and its different fractions

The ethanolic crude extract of leaf showed moderate inhibition of writhing on *Swiss albino* mice. Among different fractions of leaf, pet-ether fraction showed significant inhibition of writhing. Moderate inhibition was observed for methanol fraction and Dichloromethane fraction showed mild inhibition of writhing. The results are summarized in the table 1.

Effect of stem extract and its different fractions

The ethanolic crude extract of stem also showed moderate inhibition of writhing. Pet-ether fraction of stem showed significant inhibition of writhing. Methanol fraction also showed moderate inhibition of writhing where as Dichloromethane fraction showed mild inhibition of writhing. The results are summarized in the table 2.

DISCUSSION

Intraperitoneal administration of acetic acid causes localized inflammation in mice. Such inflammatory response produces high levels of prostaglandins, PGE2 and PGF2-α in peritoneal mass (Deraedt *et al*., 1980).

In this present study, *Swertia chirata* produced significant analgesia in acetic acid induced writhing model. The leaf and stem extract of *Swertia chirata* was initially fractionated into pet-ether, dichloromethane, and methanol considering the gradual increment in their eluting power. Among these three fractions, pet-ether showed significant reduction in the number of writhing. From this experiment it may be assumed that pet-ether fractions of leaf and stem of *Swertia chirata* might have some chemical constituents that are responsible to inhibit prostaglandin synthesis or to block pain sensation or might exert other specific mechanism to counteract the pain induced by acetic acid.

Based on this present study it may be conclude that the plant *Swertia chirata* possess strong analgesic potency. However further studies are necessary to elucidate the underlying mechanisms and to isolate and characterize the active constituents responsible for the analgesic property.

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