

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Antinociceptive and Anti-inflammatory Activity of the Ethanolic Extract of *Cymbidium aloifolium* (L.)

Md. Amran Howlader, Mahmudul Alam, Kh. Tanvir Ahmed, Farjana Khatun and Apurba Sarker Apu
Department of Pharmacy, East West University, Dhaka, Bangladesh

Abstract: The ethanol leaf extract of *Cymbidium aloifolium* (L.) was evaluated for its analgesic and anti-inflammatory activities. The extract, at the dose of 200 and 400 mg kg⁻¹ body weight, exerted the analgesic activity by observing the number of abdominal contractions and anti-inflammatory activity against Carrageenin induced paw edema in mice by measuring the paw volume. The ethanolic extract of *Cymbidium aloifolium* (L.) showed statistically significant ($p < 0.05$) reduction of percentage of writhing of 33.57 and 61.31% at 200 and 400 mg kg⁻¹ oral dose, respectively, when compared to negative control. The Ethanolic plant extract also showed significant ($p < 0.05$) dose dependent reduction of mean increase of formation of paw edema. The results of the experiment and its statistical analysis showed that the ethanolic plant extract had shown significant ($p < 0.05$) dose dependent analgesic and anti-inflammatory activities when compared to the control.

Key words: Analgesic, anti-inflammatory, carrageenan, *Cymbidium aloifolium*, edema, writhing, antinociceptive

INTRODUCTION

Cymbidium aloifolium is an epiphytic herb found throughout Bangladesh especially in the hill tracts of Chittagong. *Cymbidium aloifolium* also has uses in the folk medicine: The root is used to cure paralysis and joining fractured bones, leaf extract for treating boils and fever, whole plant used for treating weakness of eyes, chronic illness, vertigo, burns and sores (Hossain *et al.*, 2009a). Literature review of this plant has resulted in the isolation of several Flavonoids, Reducing Sugars, Cyanogenic glycosides, Terpenoids, Tannins (Maridassa *et al.*, 2008), two substituted bibenzyls, Dihydrophenanthrene and Phenanthraquinone which are responsible for biological activity (Hossain *et al.*, 2009a). Presence of different chemical compounds of this plant may possess some pharmacological activities. However, no work has been reported on the analgesic and anti-inflammatory activities of this plant. The present study was undertaken to investigate the analgesic and anti-inflammatory activities of the crude extract of *Cymbidium aloifolium* in mice to substantiate the folklore claim.

MATERIALS AND METHODS

Plant material: The leaves of *Cymbidium aloifolium* were collected from Dhaka in December 2010 and were

identified by the experts of national herbarium, Mirpur, Dhaka, Bangladesh (Accession No. DACB-35414) and a voucher specimen was kept for future reference.

Preparation of the extract: The leaves were thoroughly washed with water. The collected leaves were cut into small pieces and dried in sun for 15 days. The dried small pieces of the plant leaves were ground into small powder by blender machine. Then the powders were preserved in separate airtight container for further use. The plant powders (100 g) were extracted by cold extraction process using ethanol (600 mL) as solvent in a bottom glass container, through occasional shaking and stirring for 7 days. After 7 days the extract filtered through the cotton at first and then through the filter paper. Then the liquid was dried with rotary evaporator to achieve a blackish mass.

Animals: Swiss albino mice (20-30 g) of either sex were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions. The animals were fed with laboratory food and water *ad libitum*. The experiments were done on an isolated and noiseless condition. The ethical clearance of the experiment was taken from the "Internal Ethical Board For Animal Research" of East West University, Dhaka, Bangladesh.

Drugs and chemicals: Acetic acid and Carrageenan were obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. Diclofenac was obtained from Square Pharmaceuticals Ltd., Bangladesh.

Experimental design: The animals were randomly divided into four groups and each group consisting of six mice. The test groups received ELECA (ethanolic leaf extract of *Cymbidium aloifolium*) at the doses of 200 and 400 mg kg⁻¹ while positive control was treated with Diclofenac Na (10 mg kg⁻¹) and control with vehicle (1% Tween 80 in water).

Procedure

Acetic acid induced writhing test in mice: To evaluate the analgesic effects of *Cymbidium aloifolium*, the method described by Dharmasiri *et al.* (2003) was used with slight modifications. Four groups of six mice each received orally normal saline solution (10 mL kg⁻¹) (i.e., control); Diclofenac (10 mg kg⁻¹) and plant extract (200 and 400 mg kg⁻¹). Forty minutes later, the mice were treated with 0.7% acetic acid (10 mL kg⁻¹) solution was injected intraperitoneally. Six minutes after acetic acid injection, mice were placed in individual cage and the number of writhes (abdominal contractions) was counted for each mouse for a period of 10 min and the percentage inhibition of writhing was calculated (Dharmasiri *et al.*, 2003).

Carrageenan-induced paw oedema: The method described by Winter *et al.* (1962) was slightly modified and adopted to determine anti-inflammatory activity in mice. The mice were divided into four groups each containing six mice each. Acute inflammation was produced by injecting 0.1 µL of carrageenan (1% w/v suspension which contained Tween 80) into plantar surface of mice hind paw. The ethanolic leaf extract (200 and 400 mg kg⁻¹), normal saline (1 mL kg⁻¹) and Diclofenac (10 mg kg⁻¹) as reference agent were given 1 h before carrageenan injection. The paw volume was measured at 30 min before and 3 h after carrageenan injection using a vernier caliper to determine the linear diameter of paw oedema. The difference between the readings at thirty mins before and three hours after the injection of carrageenan was taken as the thickness of oedema (Winter *et al.*, 1962). The percent inhibition of the inflammatory reaction was determined for each animal by comparing with control and calculated by the formula (Kouadio *et al.*, 2000).

$$I\% = \left(1 - \frac{dt}{dc}\right) \times 100$$

where, dt is the difference in paw volume of the treated group and dc is the difference in paw volume in the control group.

Statistical analysis: The data were expressed as the Mean±SEM (standard error mean). ANOVA (analysis of variance) followed by Dunnett's 't' test was performed as a post hoc test to evaluate the statistical significance while taking vehicle treated animals as control; p value of p<0.05 was considered as statistically significant.

RESULTS

Acetic acid induced writhing test in mice: In acetic acid induced paw writhing test, the results showed that the ethanolic extract of *Cymbidium aloifolium* inhibited the writhing significantly. The extract showed highest effects at dose of 400 mg kg⁻¹ where the inhibition was about 61.31% which were comparable to the standard drug Diclofenac, where the inhibition was about 66.42% at the dose of 10 mg kg⁻¹ of body weight (Table 1).

Carrageenan induced paw oedema: In Carrageenan induced paw oedema test, the results showed that the ethanolic extract of *Cymbidium aloifolium* reduced the paw volume significantly from 1 to 3 h. The extract showed highest effects at the third hour where the inhibition was about 13.33 and 32.82% at dose of 200 and 400 mg kg⁻¹, respectively which were comparable to the standard drug Diclofenac, where the inhibition was about 66.42% at the dose of 10 mg kg⁻¹ of body weight (Table 2).

Table 1: Effect of ethanolic leaf extract of *C. aloifolium* on acetic acid induced writhing in mice

Groups	No. of writhing within 15 min	Percentage inhibition
Negative control	27.4±6.18	-
Diclofenac Na	9.2±0.25*	66.42
ELECA (200 mg kg ⁻¹)	18.2±13.48*	33.57
ELECA (400 mg kg ⁻¹)	10.6±99.74*	61.31

Values are expressed as Mean±SEM (n = 6); *p<0.05, Dunnett's 't' test as compared to control. ELECA: Ethanolic leaf extract of *Cymbidium aloifolium*

Table 2: Effects of the ethanol extract of *C. aloifolium* leaf in mice by Carrageenan induced paw oedema test

Groups	Mean paw volume and (%) of inhibition	
	Paw volume (% mL)	% inhibition
Negative control	0.923±0.08	-
Diclofenac Na	0.420±0.03**	54.5
ELECA (200 mg kg ⁻¹)	0.800±0.14*	13.33
ELECA (400 mg kg ⁻¹)	0.620±0.27**	32.82

Values are expressed as Mean±SEM (n = 6); *p<0.05, **p<0.001, Dnnnett's 't' test as compared to control. ELECA: Ethanolic leaf extract of *Cymbidium aloifolium*

DISCUSSION

The ethanol extract was evaluated in the acetic acid-induced writhing test for its analgesic activity. Acetic acid-induced abdominal writhing model represents pain sensation by releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism (Adedapo *et al.*, 2009). Prostanoids such as PGE₂ and PGF_{2α} as well as lipoxygenase products have been found at higher level in the peritoneal fluid after intraperitoneal injection of acetic acid. The analgesic effect occurs due to its action on visceral receptors that is sensitive to acetic acid by inhibiting the production of algogenic substances or inhibiting the transmission of painful messages at the central level (Yerima *et al.*, 2009). The acetic acid induced writhing response is a sensitive method to evaluate peripherally acting analgesics. The response is mediated by either peritoneal mast cells or acid sensing ion channels or the prostaglandin pathways (Hossain *et al.*, 2009b).

The development of oedema induced by carrageenan test was selected because of its sensitivity in detecting inflammatory agents especially in the acute phase of inflammation (Adedapo *et al.*, 2009). The probable mechanism of action is bi-phasic. The first phase involves the release of histamine, serotonin and kinins in the first hour; while the second phase is responsible for the release of prostaglandins and lysosome enzymes in 2 to 3 h (Yerima *et al.*, 2009).

Analgesic and anti-inflammatory effects have been found in flavonoids as well as tannins. Flavonoids such as quercetin are very much effective in acute inflammation (Yerima *et al.*, 2009). There are also reports on the role of flavonoid in analgesic activity primarily by targeting prostaglandins. Tannins are also claimed to possess analgesic activity (Hossain *et al.*, 2009b). Literature review of the plant reveals that *Cymbidium aloifolium* contains flavonoids, reducing sugar, cyanogenic glycosides, terpenoids and tannin (Maridassa *et al.*, 2008). So the results of this study are indicating that *Cymbidium aloifolium* can be effective in pain and acute inflammatory disorders.

CONCLUSION

In conclusion, this study has demonstrated that the crude ethanol extract of *Cymbidium aloifolium* exhibited anti-inflammatory and analgesic activities. So, further studies are needed to find out the active principles and evaluate these activities by using other models to confirm its pharmacological properties.

ACKNOWLEDGMENTS

The authors are thankful to Dr. Muniruddin Ahmed, Pro-vice Chancellor, East West University; Dr. Chowdhury Faiz Hossain, Dean and Professor, Department of Pharmacy, East West University; Dr. Sufia Islam, Chairperson and Associate Professor, Department of Pharmacy, East West University; for their encouragement during the research time.

REFERENCES

- Adedapo, A.A., M.O. Sofidiya and A.J. Afolayan, 2009. Anti-inflammatory and analgesic activities of the aqueous extracts of *Margaritaria discoidea* (Euphorbiaceae) stem bark in experimental animal models. *Rev. Biol. Trop.*, 57: 1193-1200.
- Dharmasiri, M.G., J.R.A.C. Jayakody, G. Galhena, S.S.P. Liyanage and W.D. Ratnasooriya, 2003. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *J. Ethnopharmacol.*, 87: 199-206.
- Hossain, M.M., I.J. Biva, R. Jahangir and M.M.I. Vhuiyan, 2009a. Central nervous system depressant and analgesic activity of *Aphanamixis polystachya* (Wall.) parker leaf extract in mice. *Afr. J. Pharm. Pharmacol.*, 3: 282-286.
- Hossain, M.M., M. Sharma and P. Pathak, 2009b. Cost effective protocol for *in vitro* mass propagation of *Cymbidium aloifolium* (L.) Sw.-a medicinally important orchid. *Eng. Life Sci.*, 6: 444-453.
- Kouadio, F., C. Kanko, M. Juge, N. Grimaud and A. Jean *et al.*, 2000. Analgesic and antiinflammatory activities of an extract from parkia biglobosa used in traditional medicine in the ivory coast. *Phytother. Res.*, 14: 635-637.
- Maridassa, M., M.I.Z. Hussain and G. Rajuc, 2008. Phytochemical survey of orchids in the tirunelveli hills of South India. *Ethnobotanical Leaflets*, 12: 705-712.
- Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin induced oedema in hind paw of the rats as an assay for anti inflammatory drugs. *Proc. Soc. Exp. Biol. Med.*, 111: 544-547.
- Yerima, M., M.G. Magaji, A.H. Yaro, Y. Tanko and M.M. Mohammed, 2009. Analgesic and anti-inflammatory activities of the methanolic leaves extract of *Securinega virosa* (euphorbiaceae). *Nig. J. Pharm. Sci.*, 8: 47-53.