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Full Length Research Paper

Anti-arthritic and cytotoxic effects of methanolic extract of *Ixora nigricans* leaf

Mohammad Nazmul Alam*, Md. Shahrear Biozid, Ahmad Ibtehaz Chowdhury, Muhammad Moin Uddin Mazumdar, Sudipta Chowdhury and Md. Irfan Amin Chowdury

[1] Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, 154/A, College Road, Chittagong-4203, Bangladesh.

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This study aimed to evaluate anti-arthritic and cytotoxic effects of methanolic extract of *Ixora nigricans* leaf. Anti-denaturation method was performed by using bovine serum albumin (BSA) to evaluate the anti-arthritic potential. The screening of cytotoxic activity was done using brine shrimp lethality bioassay. The *in vitro* study on *Ixora nigricans* leaf showed the presence of significant anti-arthritic activity. Here, the *Ixora nigricans* leaf showed 79.35% at 1000 ug/ml and 46.77% at 31.25 µg/ml concentration, whereas the standard drug showed 85.49% at 1000 ug/ml and 51.61% at 31.25 µg/ml concentration. Moderate cytotoxicity was found for methanolic extract and it was compared with the standard drug vincristine sulfate in the brine shrimp bioassay. In the present study, the LC_{50} values of methanolic crude extract of *Ixora nigricans* leaf and vincristine sulfate were 179.18 and 12.59 µg/ml, respectively. The results of this study demonstrated that methanolic extract of *I. nigricans* leaf contains significant anti-arthritic activity and moderate cytotoxic activity.

Keywords: *Ixora nigricans,* cytotoxicity, brine shrimp, anti-arthritic, inhibition, protein denaturation.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune chronic, systemic inflammatory disease predominantly affecting joins and peri-aticular tissues. RA still remains a formidable disease, being capable of producing severe crippling deformities and functional disabilities. The association of blood clotting mechanisms with inflammation is more firmly established in some types of injury. Prostaglandin E1 (PGE1) was found to act on erythrocytes in such a way that it causes phospholipids disruption. Changes in protein or lipoprotein structure might account for the development of erythrocyte membrane destabilization in polyarthritis and rheumatoid arthritis (Sadique et al., 1989). Brine shrimp lethality evaluation is a bench top bioassay method for evaluating anticancer, antimicrobial and other pharmacological activity of natural products. Natural products extracts

*Corresponding author. E-mail: Nazmul_pharmacy@yahoo.com. Tel: +8801710525784.
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fractions or pure compounds can be tested for their bioactivity by this method (Dockery et al., 2000). *Ixora nigricans* R. Br. (Rubiaceae) is a large shrub which is found throughout Bangladesh, the forests of India and Indo-malaysia (Barbhuiya et al., 2014).

In local tribes of Bangladesh, it is known as Dikranga Chuillya (Chakma, Tripura), Rongma, Frareko (Marma). Extract of root is used to treat diarrhoea and ear infections by the Chakma. A paste of the leaves is applied to affected areas for the treatment of boils, pills prepared from the paste of the leaves are taken thrice daily for dysentery by the Tanchangya. Extract prepared from leaf taken and paste prepared from root is applied in the whole body as a remedy for unconsciousness of little child and extract prepared from root, taken one cupful four times daily for two days against vomiting over bleeding by the Marma (Yusuf et al., 2009). EtOH (50%) extract of aerial parts is antiviral, spasmolytic and CNS depressant (Asolkar et al., 1992).

MATERIALS AND METHODS

Collection and proper identification of plant

Ixora nigricans R. Br. was collected from Chittagong Hill near Mimi super market area, Chittagong. The sample was identified by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong. (12th October, 2013).

Chemicals and drugs

The chemicals used were bovine serum albumin (BSA), diclofenac sodium, absolute methanol (99.50%) and vincristine sulfate (VS) were purchased from Sigma-Aldrich, Munich, Germany. All chemicals in this investigation were of analytical reagent grade.

Preparation of extract

Plant materials were dried and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40 to 80 mesh, 500 g) and soaked for 7 days with 2 to 3 days interval in 2.0 L of methanol at room temperature (23 ± 0.5°C). Filtrate obtained through cheese cloth and Whatman filter paper No. 1 was concentrated under reduced pressure at a temperature below 50°C using a rotary evaporator (RE 200, Sterling, UK). The extracts (yield 4.4 to 5.6% W/W) were all placed in glass petri dishes (90 × 15 mm, Pyrex, Germany). A 100 mg each of the extracts was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. In this way, the concentration (10 mg/ml) of extracts was prepared for screening the cytotoxic properties.

Inhibition of protein denaturation *in vitro*

For the inhibition of protein denaturation, *in vitro* diclofenac sodium was used as standard. The test solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of methanol extract of *Ixora nigricans*. The control solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of methanolic extract of *Ixora nigricans*. Standard solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium. Various concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/ml) of methanol extract of *Ixora nigricans* (IN) and diclofenac sodium (standard) were taken, respectively.

All the solutions were adjusted to pH 6.3 using 1 N HCL. Samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the previous solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The control represents 100% protein denaturation. The results were compared with diclofenac sodium. The percentage inhibition of protein denaturation of different concentrations is tabulated in Table 1. The percentage inhibition of protein denaturation can be calculated as:

$$\% \text{ inhibition} = [100 - (\text{OD of test solution} - \text{OD of product control})] \times 100$$

Where OD = optical density.

The control represents 100% protein denaturation. The results were compared with diclofenac sodium.

Cytotoxicity screening

Cytotoxicity of the methanol extracts of *Ixora nigricans* was evaluated by the brine shrimp lethality bioassay (Figure 2), which is widely used for screening bioactive compounds (Meyer et al., 1982; Zhao et al., 1992). In this study, a simple zoological organism (*Artemiasalina*) was used as a convenient monitor for the experiment. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to develop into larval shrimp called nauplii. The cytotoxicity assay was performed on the brine shrimp nauplii using the Meyer method.

The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus seawater (3.8% NaCl in water) to attain concentrations of 10, 50, 100, 150, 200 and 300 µg/ml. A vial containing 50 µl DMSO diluted to 5 ml was used as a control. Standard vincristine sulfate was used as a positive control. Mature shrimps were placed into each of the experimental vials. After 24 h, the vials were inspected using a magnifying glass, and the number of surviving nauplii in each vial was counted. From these data, the percentage lethality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = \frac{N_t}{N_o}$$

Where N_t = Number of dead nauplii after a 24 h incubation; N_o = Number of total nauplii transferred (10).

The LC_{50} (median lethal concentration) was determined from the log concentration versus % mortality curve.

Table 1. Percentage inhibition of protein denaturation of *Ixora nigricans.*

Concentration (µg/ml)	Percentage inhibiton in protein denaturation	
	IN	Diclofenac sodium
31.25	46.77 ± 1.38*	51.61 ± 0.86
62.5	52.65 ± 1.66*	61.29 ± 0.67
125	59.43 ± 1.48	64.52 ± 0.71
250	68.52 ±1.77	74.19 ± 1.19
500	74.84 ± 1.30*	80.65 ± 0.91
1000	79.35 ± 0.81**	85.49 ± 0.86

Values are expressed as mean ± S.E.M. of three replicate (n = 3); Here, **P < 0.01, *P < 0.05.

Table 2. Percentage mortality of brine shrimp extract at six concentrations.

Concentration (µg/ml)	Log C	Percentage mortality	
		IN	Vincristine sulphate
10	1	20	40
50	1.699	30	80
100	2	50	100
200	2.301	60	100
300	2.477	70	100
500	2.699	80	100
LC_{50}	-	179.18	12.59

*IN = *Ixora nigricans.*

RESULTS AND DISCUSSION

Anti- arthritic study

Different concentrations of methanolic extract of *Ixora nigricans* and diclofenac sodium were tested for anti-arthritic activity and found significant percentage inhibition in protein denaturation (Table 1). In this study, methanolic extract of *Ixora nigricans* showed 46.77%, where the standard drug diclofenac sodium showed 51.61% of inhibition at 31.25 µg/ml. The extract of *Ixora nigricans* exhibited 79.35% inhibition, while the diclofenac sodium exhibited 85.49% inhibition of protein denaturation at 1000 ug/ml.

Brine shrimp lethality bioassay

Following the procedure of Meyer, the lethality of methanol *Ixora nigricans* leaf extract was determined on *Artemia salina* after sample exposure for 24 h. The negative control (vehicle only) and vincristine sulfate (positive control) were also used to compare the toxic activities of the extracts. Percentage mortality of brine shrimp at six different concentrations (10 to 500 µg/ml) of the extracts has been presented in Table 2. From Figure 1, it is clear that the percentage mortality is directly proportional to the extract concentrations. LC_{50} values of methanol extract of *Ixora nigricans* obtained in the present experiment was 179.18 µg/ml. The LC_{50} value for the standard drug vincristine sulfate was 12.59 µg/ml. However, no mortality was obtained for the negative control group.

Conclusion

Arthritis is a type of joint disorder that involves inflammation of one or more joints, responsible for pain, swelling, stiffness and loss of function in joint. Denaturation of protein which is one of the causes of arthritis was documented. Production of auto antigen in certain arthritic disease may occur due to the denaturation of protein. The mechanism of denaturation

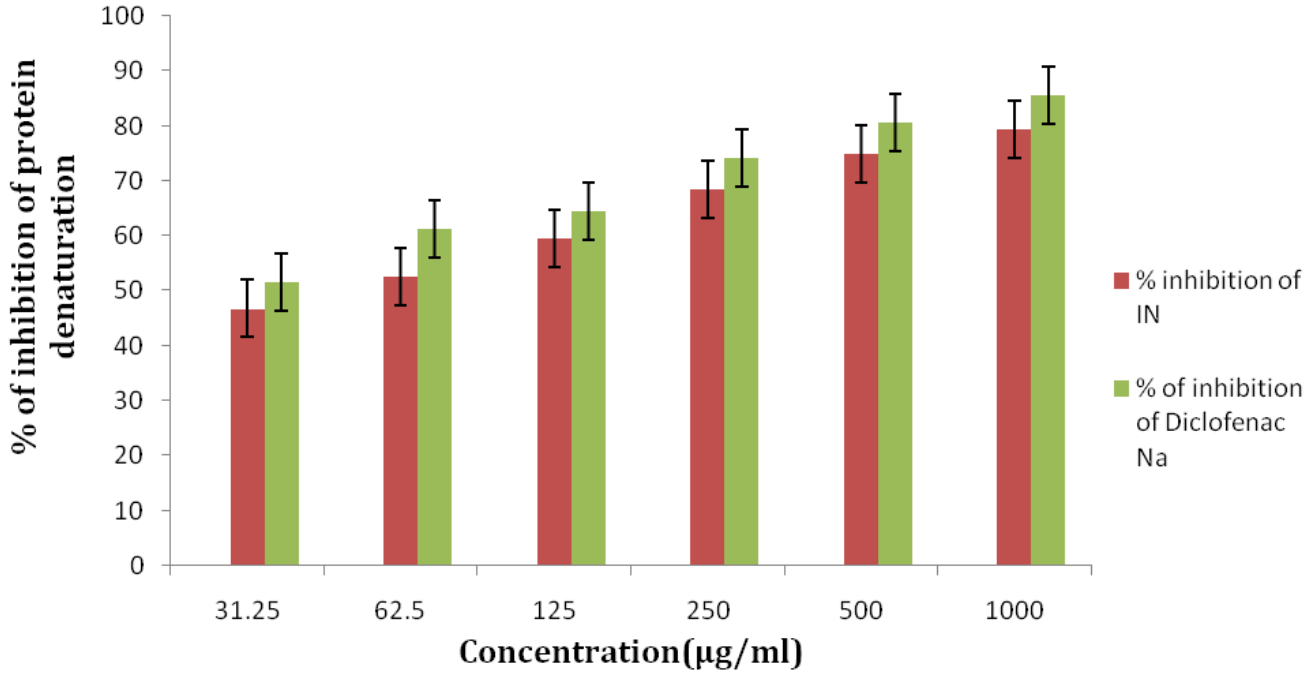

Figure 1. Percantage inhibition of **methanol**ic extract **of** *Ixora nigricans* and the standard diclofenac on protein denaturation. IN = *Ixora nigricans.*

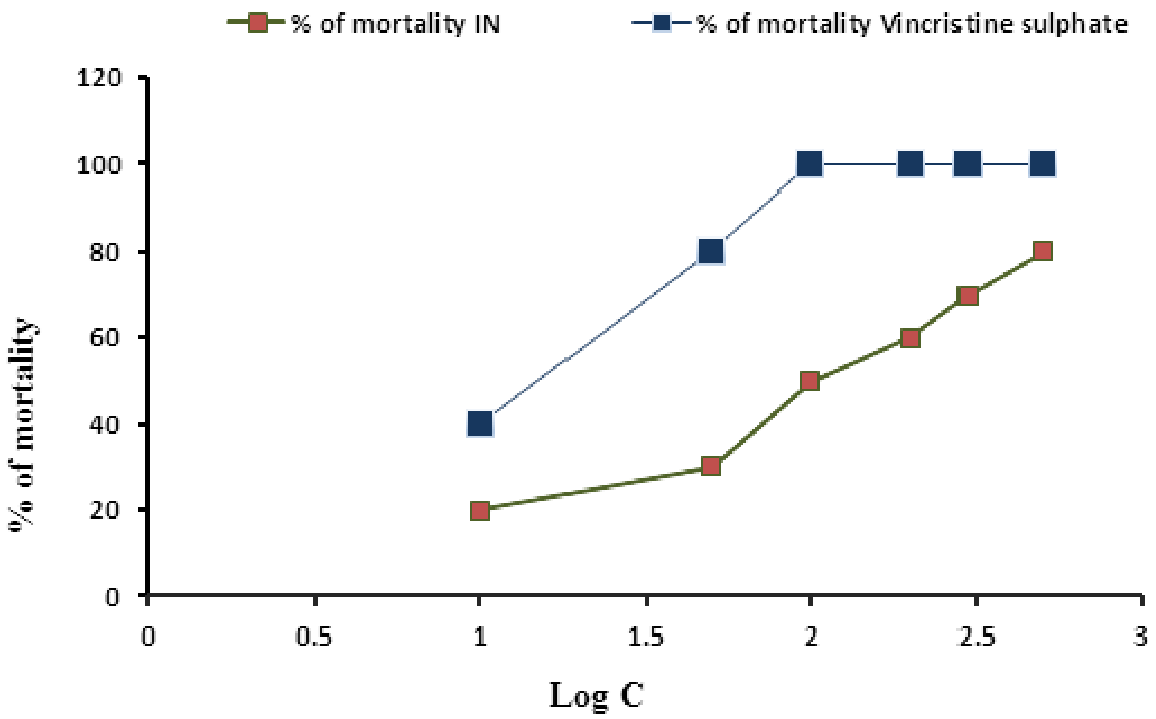

Figure 2. Brine shrimp lethality bioassay. Determination of LC50 values for methanolic extract of Ixora nigricans from a linear correlation between log concentrations versus % of mortality; *IN = *Ixora nigricans*.

probably involve alteration in electrostatic hydrogen, hydrophobic and disulphide bonding (Shravan et al., 2011). Here, the methanol extract have shown promising activity at various concentrations and the effects were compared with the standard drug diclofenac sodium.

The maximum percentage inhibition of protein denaturation of *Ixora nigricans* leaf was observed as 79.35% at 1000 µg/ml which were close to the percentage inhibition of diclofenac sodium (85.49%). From the result, it can be stated that this extract is capable of controlling the production of auto antigen to inhibit the denaturation of protein. Toxicity profile of plant materials is mainly an important criteria to experts and medical practitioners (Singh et al., 2005; Fowles et al., 2012; Okwuosa et al., 1993) and cytotoxic assay was conducted in this study to learn about the toxicity study of plant extract through the brine shrimp lethality (LC_{50}, 24 h) test. Logarto et al., (2001) showed a great correlation ($r = 0.85$; $P < 0.05$) between the LC_{50} of brine shrimp lethality test and the severe oral toxicity assay in mice. Based on that correlation, brine shrimp lethality $LC_{50} < 10$ µg/ml (LD_{50} between 100 and also 1000 mg/kg) is measured as cutoff value on cytotoxicity (Logarto et al., 2001; Chew et al., 2012).

In this present experiment, modest brine shrimp cytotoxicity was found for methanolic extract of *Ixora nigricans* compared with the standard drug vincristine sulfate. In conclusion, the present study, using in vitro experiments established that methanolic extract of *Ixora nigricans* (leaf) has moderate anti-arthritic and low cytotoxic effect.This is only a preliminary study but the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates.

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Conflicts of interest

Authors have none to declare.

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