Screening of In-vitro anti-inflammatory and Antibacterial assay of Malvastrum Coromandelianum

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ABSTRACT: Inflammation is a central part of the response to injury and infection in the immune system. It is the natural way to signalize the immune system and to restore and protect itself from foreign invaders including viruses and bacteria, which are weakened tissue. It may become problematic if the inflammatory process continues for too long, or if the inflammatory response occurs in areas not required. External infections involving the skin and wound are the most frequent complications affecting humans and animals. Medicinal plants play great roles in treatment of bacterial infections. Primary aim of the present study was to investigate the possible anti-inflammatory and antibacterial mechanism of Malvastrum coromandelianum extract using in-vitro model. Ethyl acetate extract of Malvastrum coromandelianum was evaluated in vitro model by protein denaturation method for anti-inflammatory activity and disk diffusion method for antibacterial activity. The ethyl acetate extract of Malvastrum coromandelianum significantly inhibited % protein denaturation as compared to standard drug and extract shows promising antibacterial activity against different bacterial species. It can be postulated from the observed results the anti-inflammatory and antibacterial activity of Malvastrum coromandelianum could be due to its inhibition of protein denaturation and minimum inhibitory concentration.

Keywords: Malvastrum coromandelianum, protein denaturation, anti-inflammatory, antibacterial.

INTRODUCTION

Malvastrum coromandelianum (L.) Garcke (family Malvaceae), commonly known as false mallow, broom weed, and clock plant [1]. Various parts of this plant are used by numerous tribal populations throughout the world [2]. A previous study showed that the M. coromandelianum water extract exhibited a hypoglycemic effect in diabetic rabbits. M. coromandelianum is used in traditional medicine as an analgesic, antidysenteric plant and in the treatment of jaundice and ulcers [3]. Various extracts of the aerial parts of M.coromandelianum showed antinociceptive activity in the acetic acid-induced writhing test in mice [4]. This study aimed to investigate pharmacological properties of the ethyl acetate extract, including anti-inflammatory and antibacterial action [5]. Indians use the crushed leaves of this herb along with salt or alcohol to cure ringworm infection. Bhil tribes of Rajasthan use this plant in the form of decoction to cure jaundice. In Mexico leaf infusion of this plant is used to cure diabetes [6]. In traditional Indian system of medicine the plant is reported as an anti-inflammatory, analgesic, and antidysenteric [7]. Pharmacological screening showed various activities for this plant like anti-inflammatory, analgesic activity, and antimicrobial activity [8].

MATERIALS AND METHODS

Plant material

M. coromandelianum was obtained from Kasegaon, sangli, Maharashtra, India. The plant was identified and authenticated by Department of botony, Yashwantrao Chavan College of Science, Karad.

Preparation of plant extract

The collected plant materials were washed with distilled water, shadow dried, and stored in air-tight bottles separately. The aqueous extract of each plant material was prepared by soaking the powered plant parts in distilled water and maintained in incubator at 37◦C for 72 hours. The herbal extract were filtered using filter paper; marc was washed with 10 ml of distilled water and pressed [9].

Chemicals

Chemicals have been purchased from the research lab in Mumbai, India, and the solvent has been purified by distillation. DMSO as solvent, Diclofenac sodium (50 µg/ml) as a standard, 50 µg/ml, 100 µg/ml as test compound, UV spectrophotometer instrument for absorbance for anti-inflammatory activity and ciprofloxacin as a standard drug used for antibacterial activity.

Anti-inflammatory activity

Method: In vitro anti-inflammatory activity by protein denaturation method.

The sample mixture (10ml) consists of 0.4ml of egg albumin (from fresh egg), 0.6ml of phosphate buffered solution (pH 6.4) and 4ml of modifying test sample concentration to 50 µg / ml, 100 µg / ml of final concentration. The same DMSO volume acts as control and the sample mixtures were then incubated for 15-20 minutes at (70c ± 2). Then set at 700C for 5min. They were measured at 660 nm (JASCO UV Spectrophotometer) after cooling the sample by using the vehicle as blank. With the assistance of the Ostwald viscometer, their viscosity was determined. Diclofenac sodium was used as a standard drug at the final concentration of 50 µg/ml,100 µg/ml, and treated similarly for absorbance and viscosity determination. The percentage of protein denaturation inhibition was calculated. They were measured at 660 nm (JASCO UV Spectrophotometer) after cooling the sample by using the vehicle as blank [11].

Antibacterial activity

Bacteria used in present study were collected from Rajarambapu college of Pharmacy, Kasegaon, Maharashtra, India. The antibacterial assay is performed by the agar well diffusion method. In this method the nutrient agar media was prepared by using peptone, agar, beef extract, sodium chloride in sterilized equipment. All the equipment should be sterilized for antibacterial assay. For ethyl acetate extract different concentration 10 µg/ml, 25 µg/ml, 50 µg/ml are made. Three bacteria E.coli, S. aureus and P. aeruginosa are used. As standard ciprofloxacin is used. After making petri plate the entire plates were incubate for 35°C for 3-4 days. The zone of inhibition of bacteria growth around and each well in measured and determined [12-13].

RESULTS AND DISCUSSION

The inhibitory effect of different concentration of Malvastrum coromandelianum on protein denaturation is shown in (Table 1). M. coromandelianum (50-100µg/ml) showed significant inhibition of denaturation of egg albumin in comncentration dependent manner. M. coromandelianum at concentration of 100µg/ml and diclofenac 100µg/ml showed significant inhibition 74.17% and 76.50% respectively by protein denaturation method when compared with control.

The Antibacterial assay were performed by using agar well diffusion method is shown in (Table 2). M. coromandelianum showed maximum activity against against E.coli at 50µg/ml (6mm) by comparing with standard drug and shows significant activity against S. aureus at 25µg/ml and moderate activity against P. aeruginosa.

Table No. 1 In vitro anti-inflammatory activity of extract measuring the percentage inhibition

Sr. no	Treatment	% inhibition of protein denaturation		Viscosity(cp)	
		50µg/ml	100µg/ml	50µg/ml	100µg/ml
1	Control	-	-	-	-
2	M. coromadelianum	72.45	74.17	2.56	3.12
3	Standard (diclofenac)	74.46	76.50	2.98	3.59

Table No. 2 Antibacterial screening of extract

Treatment	concentration	Zone of inhibition		
		E. coli	S. aureus	P. aeruginosa
M. coromandelianum	10 µg/ml	4mm	5 mm	4 mm
	25 µg/ml	5 mm	10 mm	8 mm
	50 µg/ml	6 mm	10 mm	10 mm
Std.(ciprofloxacin)	10 µg/ml	4 mm	6 mm	6 mm
	25 µg/ml	8 mm	10 mm	10 mm
	50 µg/ml	6 mm	12 mm	13 mm

CONCLUSION

In current study in-vitro results confirmed the reported anti-inflammatory activity of M.coromandelianum [13]. Denaturation of proteins is a well documented cause of inflammation. Several anti-inflammatory drugs shown dose dependent ability to inhibit thermally induced protein denaturation [14]. Ability of M.coromandelianum extract is to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity [15]. The anti-inflammatory activity of extracts rich in flavonoids and rich in fractions on inflammation have previously been reported. The data of our studies suggests that M.coromandelianum shows significant anti-inflammatory activity in-vitro models tested [16]. Further studies involving the purification of the chemical

constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low-toxicity and better therapeutic index [17]. Over the last decade bacterial infections are increasing at an alarming rate [18]. This is increase increase in incidence of bacterial infections posses a great challenge to healthcare professionals [19-20]. As performing antibacterial activity on different species of bacteria found that M.coromandelianum has significant antibacterial activity [21-22].

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