

ANTISPASMODIC STUDIES ON LEAF AND ROOT EXTRACT OF *BLUMEA LACERA**ASHISH DIXIT, ¹PAWAN TIWARI, ¹DEEPESH PARASHER, ²SARVESH BHARGAVA¹Dept of Pharmaceutical Analysis, ¹Dept of Pharmaceutical Chemistry, ²Dept of Pharmaceutics, Shri Ram Nath Singh Institute of Pharmaceutical Science & Technology, Gwalior. Email: ashdixit2000@gmail.com

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ABSTRACT

The present study was aimed to study the effect of hexane extract of leaves of *Blumea lacera* commonly known as jungle musli belonging to family asteraceae on the guinea pig ileum. The antispasmodic activity was investigated by the contractile activity.

Control group received histamine 3×10^{-7} M, acetylcholine 10^{-8} M and barium chloride 10^{-4} M and treated group received different concentrations of 50, 100, 200 and 300 μ g/ml.

The study revealed that hexane extract of leaves of *Blumea lacera* has exhibited the antispasmodic activity by inhibiting the 50% of spasmodic response as indicated by decrease in mean inhibitory response.

The present investigation reveals that hexane extract of the plant possess the potent antispasmodic activity. Further investigation is required to isolate the active compound to see its mechanism of action and to develop an antispasmodic agent among herbal origin.

Keywords: Antispasmodic activity, *Blumea lasara*, Guinea pig, Ileum.

INTRODUCTION

Antispasmodics are muscular relaxants that are used to relieve cramps or spasms of the stomach, intestines and bladder. They are commonly used for the treatment of different gastrointestinal disorders, including diarrhea and irritable bowel syndrome, which affect millions of people. Diarrhea continues to be one of the leading causes of mortality and morbidity especially in children in developing countries [1]. Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by intestinal hurry, and an excess loss of fluid in the feces. In some diarrhoea the secretory component predominates, and other diarrhoea is characterized by hyper motility [2]. Many people nowadays turn to the use of natural product medicine for treatment of intestinal disorders. Natural products have served as a source of medicines for centuries, and about half of the pharmaceuticals in use today are derived from natural products [3]. Dependence on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health care [4]. Specifically, the aim of this study was to test the antispasmodic activity of *Blumea Lasara* leaf extract in guinea pig ileum induced by acetylcholine, histamine and barium chloride [5].

MATERIALS AND METHODS

The fresh leaves of *Blumea Lacera* were collected from the fields around the campus of Indira Gandhi Krishi Vishwavidyalaya (Agriculture University) Raipur, Chhattisgarh, India during the months of November and December 2012, identified and authenticated by Dr. Pushpa Patel Govt. PG College, Khargon, MP.

Extraction

Air dried coarsely powdered plant material was extracted with hexane for 48 hours by maceration. Thus obtained hexane extract were filtered and vacuum dried using vacuum flash evaporator to yield the solid residue of 8.8% respectively to the starting dry powder. Then the extracts were sonicated before addition to the organ bath, acetylcholine, histamine and adrenaline were prepared by adding the substance directly to Tyrode solution.

Biological experimental procedures

Animal's male guinea pigs (250 - 400 g) were used in all experiments. The animals were housed in a cage under conditions of standard light (light on from 7.0 a.m. - 7.0 p.m.), temperature ($22 \pm 1^\circ\text{C}$) and room humidity ($60 \pm 10\%$) conditions for one week before the experimental sessions. All the animals were fed with standard

animal feed (Hindustan Lever Limited) and allowed tap water *ad libitum*. The procedures involving animals and their care conformed to the international guidelines Principles of Laboratory Animals Care.

Tissue preparation

Male guinea pigs (250 - 400 g) were sacrificed by a blow to the base of the skull and cervical dislocation and 2 cm pieces of the ileum were dissected from the ileum segment 10 - 20 cm proximal to the ileocecal valve. Material was mounted for tension recording and allowed to equilibrate for 1 - 2 h in 10 ml chambers containing Tyrode solution [composition (mM): 136.0 NaCl, 5.0 KCl, 0.98 MgCl₂, 2.0 CaCl₂, = 0.36 NaH₂PO₄, 11.9 NaHCO₃, and 5.5 glucose], pH 7.4 maintained at 37 °C and bubbled with air (5% CO₂ and 95% oxygen). In

Solution with elevated [K]⁺, [Na]⁺ was simultaneously decreased to maintain isosmolarity [6]. Concentration-effect curves for extracts were performed by cumulative addition to the bath. In experiments examining the relaxation of the basal tonus of the ileum, paired Segments of ileum were set up; one piece exposed to the extract and the other receiving no treatment. Relaxation was taken to be the difference between the tonus of the control and test segments for recording the contractions using force transducers connected to a polygraph (Grass D) as previously described [7].

Measurement of contractile activity

After stabilization for 30 min, the test extracts were added to the bath. The extracts were dissolved in Dimethylsulfoxide (Merck). In control preparations of Dimethylsulfoxide, up to 100 μ l were added to the organ bath to determine whether this vehicle alone was able to induce contractions. Next, the antispasmodic effect was investigated according to the following experimental schedule. (i) Hexane extract at concentrations of 50, 100, 200 and 300 μ g/ml organ baths: 15 min contact period. (ii) When stable submaximal responses to standard agonist histamine 3×10^{-7} M, acetylcholine 10^{-8} M, and barium chloride 10^{-4} M were obtained, the extract were added into the bath [8] Percentage inhibitions of histamine, acetylcholine or barium chloride-induced contraction, in the presence of extract, were calculated for each concentration [9]. (iii) The median inhibitory concentration (IC₅₀) was determined from the graph plotted of percent inhibition versus log dose.

Data analysis

The inhibition of ileal contractions by extracts were expressed as

mean \pm standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and the differences between samples were determined by Duncan's multiple range test. P values <0.05 were regarded as significant.

RESULTS

The concentration of extracts which inhibited 50% of response (median inhibitory concentration) IC₅₀ was determined from the graph plotted of percent inhibition versus log dose. Addition of hexane extract of *Blumea Lasera* (50- 300 $\mu\text{g}/\text{mL}$) elicited a progressively increasing relaxation of the spontaneous tonus of the ileum with IC₅₀ = 83.75 $\mu\text{g}/\text{mL}$ (c.l.: 79 - 93 $\mu\text{g}/\text{mL}$, n = 6). In a preliminary screening, the histamine induced contraction in guinea pig ileum with IC₅₀ = 22 $\mu\text{g}/\text{mL}$ (c.l.: 12-28 $\mu\text{g}/\text{mL}$, n = 6), acetylcholine with IC₅₀ = 27 $\mu\text{g}/\text{mL}$ (c.l.: 15-30 $\mu\text{g}/\text{mL}$, n = 6), and

barium chloride with IC₅₀ = 48 $\mu\text{g}/\text{mL}$ (c.l.: 25-52 $\mu\text{g}/\text{mL}$, n = 6). The IC₅₀ for papaverine, used as a reference compound, were 3.4 $\mu\text{g}/\text{mL}$ (c.l.: 1.2-4.5 $\mu\text{g}/\text{mL}$, n = 6), for histamine, 3.8 $\mu\text{g}/\text{mL}$ (c.l.: 2.1-5.1 $\mu\text{g}/\text{mL}$, n = 6), for acetylcholine and 3.0 $\mu\text{g}/\text{mL}$ (c.l.: 1.8 - 4.2 $\mu\text{g}/\text{mL}$, n = 6) for barium chloride-induced contractions respectively. The antispasmodic effects of *Blumea Lasera* (50- 300 $\mu\text{g}/\text{mL}$) are shown in the **Figures 1 to 4**. The hexane extract of *Blumea Lasera* showed a concentration- dependent inhibition of tone and the amplitude of spontaneous contraction of ileum with IC₅₀= 68 $\mu\text{g}/\text{mL}$ (c.l.: 51-79 $\mu\text{g}/\text{mL}$, n = 7), acetylcholine with a IC₅₀ = 76.19 $\mu\text{g}/\text{mL}$ (c.l.: 63-86 $\mu\text{g}/\text{mL}$, n = 7), for histamine and barium chloride with a IC₅₀ = 98 $\mu\text{g}/\text{mL}$ (c.l.: 89-105 $\mu\text{g}/\text{mL}$, n = 7), IC₅₀ = 120.2 $\mu\text{g}/\text{mL}$ (c.l.: 118-132 $\mu\text{g}/\text{mL}$, n = 7) respectively. The IC₅₀ for hexane extracts are showed in **Table 1**. Hexane extracts were found to antagonize contractions of the guinea pig ileum, induced by acetylcholine, histamine and barium chloride in a concentration-dependent way.

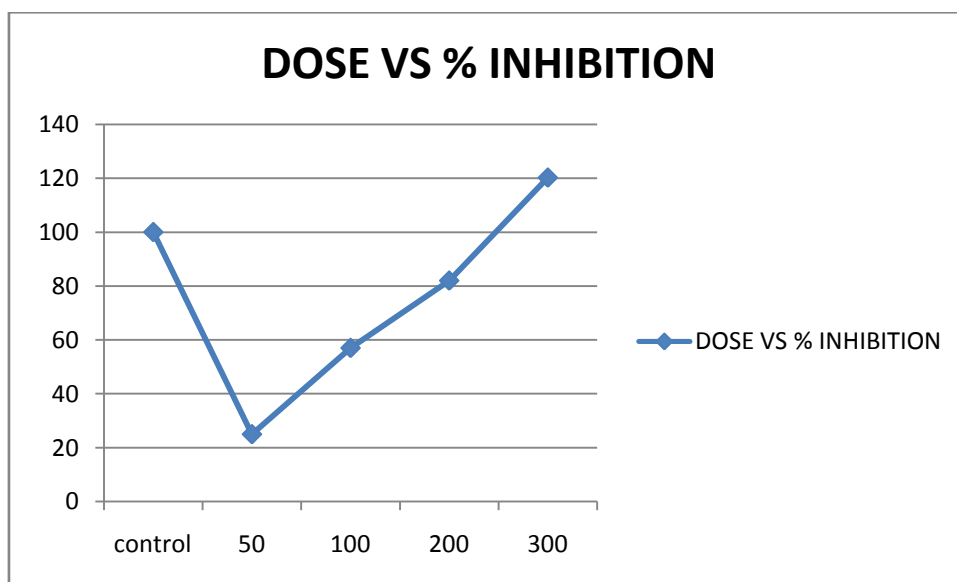


Fig. 1: The inhibitory effects of the hexane (50, 100,200,300 $\mu\text{g}/\text{ml}$) on spontaneous contraction of isolated guinea-pig ileum.

Control correspond to response to papaverine (100 μM) was set as 100% of maximum relaxation. Results are expressed as mean \pm S.E.M* $p<0.05$, ** $p<0.01$,*** $p<0.001$ (n=6)

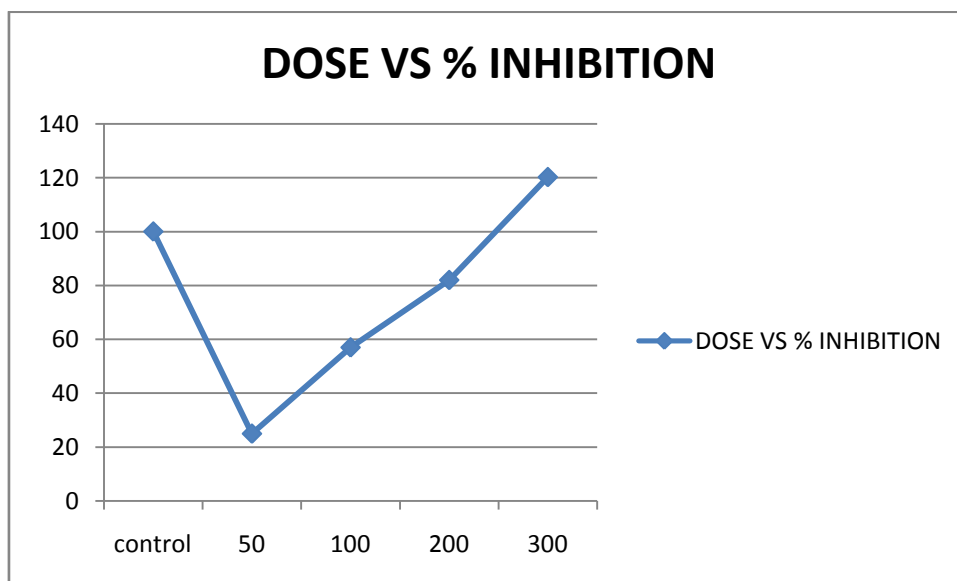


Fig. 2: The inhibitory effects of the hexane (50, 100,200,300 $\mu\text{g}/\text{ml}$) on contraction induced by acetylcholine.

Control correspond to response to papaverine (100 μM) was set as 100% of maximum relaxation. Results are expressed as mean \pm S.E.M* $p<0.05$, ** $p<0.01$,*** $p<0.001$ (n=6)

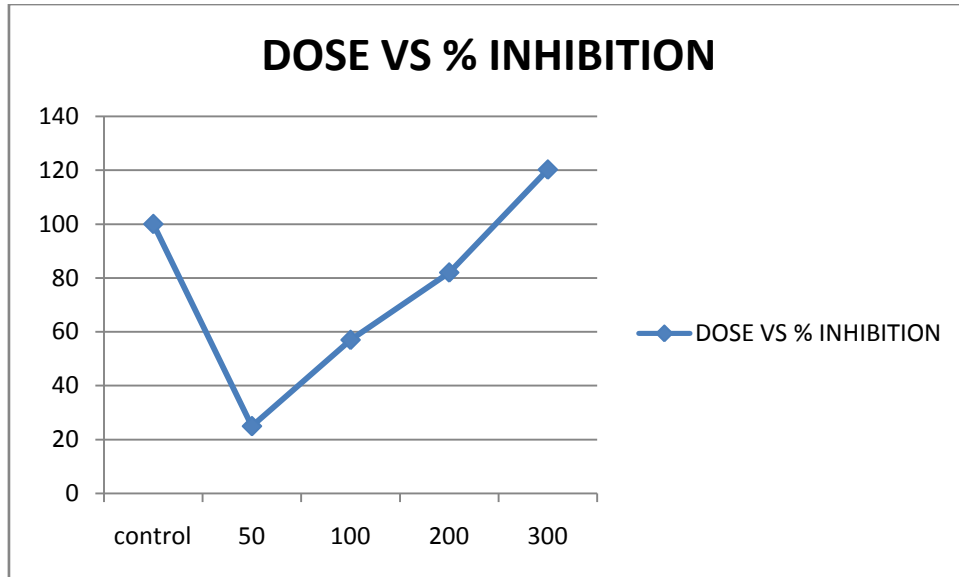


Fig. 3: The inhibitory effects of the hexane (50, 100,200,300 µg/ml) on contraction induced by histamine in isolated guinea-pig ileum. Contraction is expressed as a percentage against contraction induced by histamine in the absence of sample

Control corresponds to response to papaverine (100µM) was set as 100% of maximum relaxation. Results are expressed as mean± S.E.M*p<0.05, **p<0.01,***p<0.001 (n=6)

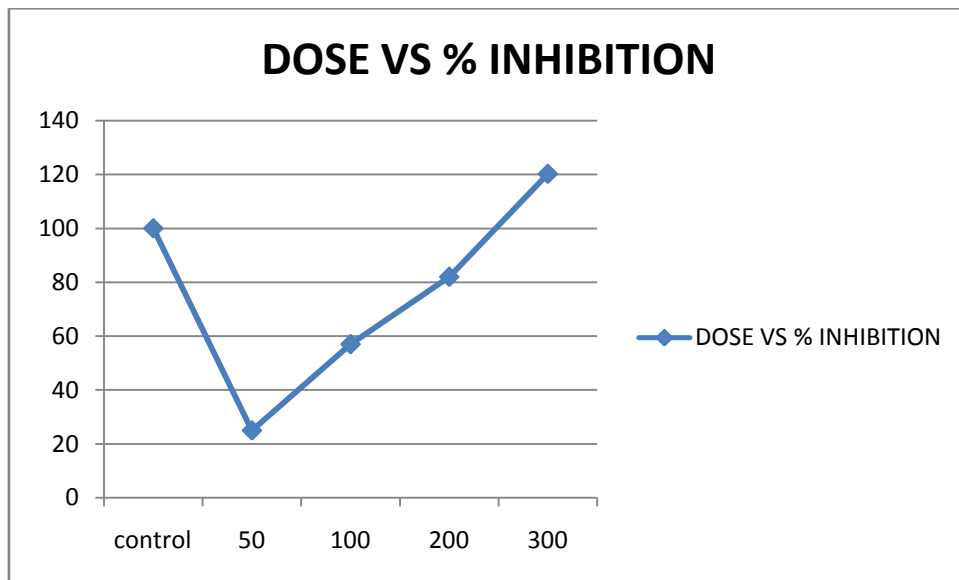


Fig. 4: The inhibitory effects of the hexane (50, 100,200,300 µg/ml) on contraction induced by barium chloride in isolated guinea-pig ileum. Contraction is expressed as a percentage of the maximum contraction obtained in the same tissue before the administration of antispasmodic.

Control correspond to response to papaverine (100µM) was set as 100% of maximum relaxation. Results are expressed as mean± S.E.M*p<0.05, **p<0.01,***p<0.001 (n=6)

Table 1: Inhibition of contraction of hexane extract of Blumea Lacera expressed as IC50.

Hexane extract	IC 50 µg/ml (Blumea Lacera)
Spontaneous Contraction	68
Contraction by Acetylcholine	76.19
Contraction by Histamine	98
Contraction by Barium chloride	120.2

DISCUSSION

The present study has shown that hexane extract from *Blumea*

Lasera exerts reversible relaxant and antispasmodic effects on guinea-pig ileum. Our current data show that extracts are also capable of inhibiting the response of a wide range of contractile stimuli, such as neurotransmitters acetylcholine and histamine, barium chloride a release bound (Ca2+) although showing no Obvious selectivity between contractile agents. Fractionation of the hexane extracts is in progress to identify the active fractions, to isolate and to characterize the actives compounds and its mechanism.

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