

***In vivo* antinociceptive and muscle relaxant activity of leaf and bark of *Buddleja asiatica* L.**

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Abstract: The current study was designed to assess the antinociceptive and skeleton muscle relaxant effect of leaves and barks of *Buddleja asiatica* in animal models. In acetic acid induced writhing test, pretreatment of ethanolic extract of leaves and barks evoked marked dose dependent antinociceptive effect with maximum of 70% and 67% pain relief at 300mg/kg i.p. respectively. In chimney test, the ethanolic extract of leaves and barks evoked maximum of 66.66% and 53.33% muscle relaxant effect after 90min of treatment at 300mg/kg i.p respectively. In traction test, the ethanolic extract of leaves and barks caused maximum of 60% and 73.33% muscle relaxant effect after 90min of treatment at 300mg/kg i.p respectively. In short, both leaves and barks demonstrated profound antinociceptive and skeleton muscle relaxant effects and thus the study provided natural healing agents for the treatment of said disorders.

Keywords: *Buddleja asiatica* L. antinociceptive, muscle relaxant activities.

INTRODUCTION

Buddleja plants are widely distributed throughout the world and only four species are found in Pakistan, i.e., *B. asiatica*, *B. crispa*, *B. davidii* and *B. lindleyana* (Ali *et al.*, 2011). They have been used in treatment of cancer and as a cure of articular rheumatism in the Chinese traditional medicine. The whole plant *B. asiatica* has been used medicinally as an abortifacient and in skin complaints (Ali *et al.*, 2011). It is planted in gardens as an ornamental shrub, and the wood may be used for making walking sticks (Abdullah, 1972).

The current study was planned to scrutinize the antinociceptive and muscle relaxant activities of ethanolic extracts of barks and leaves of *B. asiatica* in several animal procedures.

MATERIALS AND METHODS

Plant material

The leaves and bark of *Buddleja asiatica* were collected from Botany, Department University of Peshawar (UPESH), Peshawar during the month of January. The plant was identified with the help of available literature in the herbarium, Department of Botany, University of Peshawar and was deposited for ready reference in future having voucher specimen No. 20035 (PUP).

Preparation of plant extract

The Plant materials were rinsed, cleaned and dried in shade for 15 days. After drying, these were grinded into fine powder. The powder plant materials were kept in a closed vessel free from environmental climatic alterations till the usage. The powdered materials were soaked in ethanol for successive seven days. The resulted ethanolic extracts were evaporated through rotary evaporator and then used for various biological activities (Khan *et al.*, 2013; Rauf *et al.*, 2012).

Animals

BALB/c mice (20 to 25g) of either sex were used in current tests. The animals use in the current study was bought from the Pharmacology section of the Pharmacy, Department, Peshawar University, Peshawar. The animals were preserved in standard laboratory circumstances (25°C and light/dark cycles i.e. 12/12h and were fed by standard food and water *ad libitum*.

Analgesic activity

Mice were reserved from food 2 hour before the start of experiment and were adjusted with the laboratory situation. Animals were separated into groups each of five animals each. Normal saline (10ml/kg) was administered to group I, which serve as negative control while group II was injected with diclofenac sodium (10mg/kg). Rest of the groups was injected with 100, 200 and 300mg/kg of ethanolic extract. After 30min above treatment, animals were treated with 1% acetic acid. The writhing was counted after 5min of acetic acid injection. The number of

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abdominal constrictions (writhing) was counted for 10 min (Muhammad *et al.*, 2012; Kaleem *et al.*, 2013).

$$\% \text{Activity} = 100 - \frac{\text{No. of writhing in tested animals}}{\text{No. of writhing in control animals}} \times 100$$

Muscle relaxant activity

Traction test

In this technique a metal wire which was coated with rubber and together ends of the wire were rigidly maintained with stands, about 60cm above laboratory bench. The animals were separated into eight groups ($n=5$). Groups I of animals was preserved with distilled water (10ml) attend as negative control, group II was treated with 1.0mg/kg, which was positive control. Animals of groups III to V were treated with BLE (100, 200 and 300mg/kg) respectively and groups VI to VIII were injected with BBE, 100, 200 and 300mg/kg correspondingly. The animals were uncovered to the traction test after 30, 60 and 90min of action. Each animal was suspended by their hind legs from the wire and the time of floppy was recorded for 5s. The failure to hang less than five seconds reflects the presence of muscle relaxant property and vice versa (Muhammad *et al.*, 2013a).

Chimney test

This test was performed according to our already established reported protocol (Muhammad *et al.*, 2013b). A pyrex glass tube (30cm long and 3.0cm diameter) was used in this chimney test. The tube was marked at 20cm from the base and all animals were screened after 30, 60, and 90 min of treatment. The Diverse groups of five mice each were treated with diazepam (1mg/kg), distilled water (10mL/kg), and both extract of leaves and barks (100, 200 and 300mg/kg i.p.). The animal was presented at one end of the tube and allowed to move up to the mark 20cm from the base. When the animal reached the 20cm mark, the tube was moved immediately to the vertical position; the animal tried to climb the tube with a back ward movement. The mouse which failed to reach up to the mark within 30s was considered with relaxed muscles.

STATISTICAL ANALYSIS

Results are posted as the mean $\pm$ SEM of 6 independent mice. One-way ANOVA followed by a posthoc Dunnett's test was used for the determination of statistical significance for comparisons against vehicle at the level of $P < 0.05$. Graph Pad program (Graph Pad, San Diego, CA) was statistical software for analysis.

RESULT

Effect of ethanolic extract of Buddleja asiatica leaf and bark in writhing test

The analgesic effect of the ethanolic extract of *B. asiatica* leaf and bark was tested in mice with a dose of 100, 200, and 300 mg/kg body weight table 1. The diclofenac

sodium with a dose of 150mg/kg was used as standard. The dose dependent antinociceptive effect was noticed, the percent antinociceptive potential of leaves at the tested doses of 100, 200 and 300mg/kg body weight was 51, 64 and 70 % (fig. 1) and barks at the tested doses of 100, 200 and 300 mg/kg body weight was 48.3, 57.7 and 67% (fig. 2) respectively.

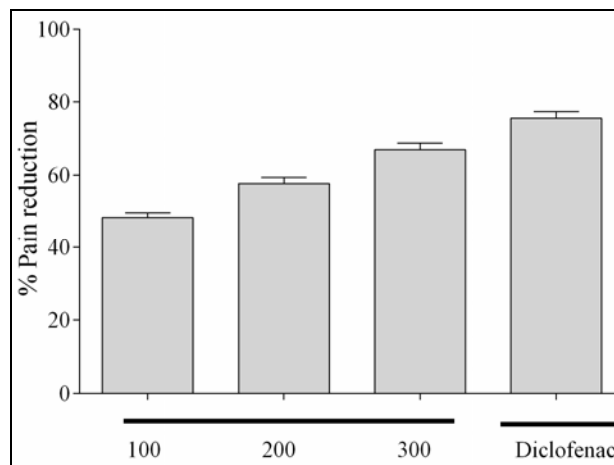


Fig. 1: Percent antinociceptive effect of crude ethanolic extract of leaves of *B. asiatica* at 100, 200 and 300mg/kg.

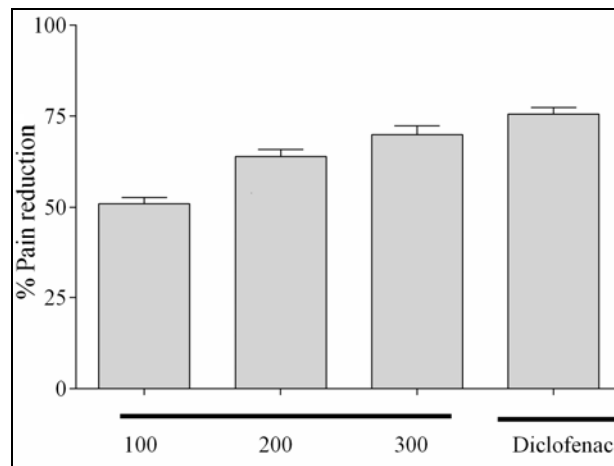


Fig. 2: Percent antinociceptive effect of crude ethanolic extract of barks of *B. asiatica* at 100, 200 and 300mg/kg.

Effect of ethanolic extract of Buddleja asiatica leaves and barks on skeleton muscle

The skeletal muscle relaxant effect of ethanolic extract of leaves and barks of the plant is shown in table 2. Pretreatment with extract at a dose of 100, 200 and 300mg/kg and Diazepam at dose of 1mg/kg, decreased fall off and sliding time and increase climbing time. In traction test, both ethanolic extract of leaves and barks (100, 200 and 300mg/kg) significantly decreases the muscle co-ordination activity of mice compared with control. In chimney test, the ethanolic extract of leaves and barks evoked maximum of 66.66% and 53.33% muscle relaxant effect after 90min of treatment at 300mg/kg i.p respectively. In traction test, the ethanolic

Table 1: Effect of leaves and barks of *Buddleja asiatica* in acetic acid induced writhing test.

S. No.	Group	Dose (mg/kg)	No. of writhes
1	Normal Saline	10	62.00±2.73
2	Diclofenic sodium	150	15.2±1.92***
3	Leaves	100	30.4±2.07*
		200	22.2±1.92*
		300	18.6±1.94**
4	Barks	100	32.0±2.73*
		200	26.2±1.3*
		300	20.4±1.34**

All values are given as mean ± SEM of 5 animals in comparison with control.

Table 2: Percent effect of *B. asiatica* on Muscle relaxation (Chimney and Traction).

Group	Dose	Chimney test (%)			Traction test (%)		
		30 min	60 min	90 min	30 min	60 min	90 min
Distilled water	10ml/kg	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
Diazepam	1ml/kg	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Leaves	100mg/kg	26.66	46.66	53.33	26.66	26.66	33.33
	200mg/kg	53.33	53.33	46.66	53.33	46.66	46.66
	300mg/kg	46.66	66.66	66.66	46.66	53.33	60
Barks	100mg/kg	26.66	33.33	46.66	33.33	46.66	53.33
	200mg/kg	33.33	46.66	53.33	33.33	60	53.33
	300mg/kg	53.33	46.66	53.33	46.66	66.66	73.33

Results are expressed as mean ± SEM from five observations.

extract of leaves and barks caused maximum of 60% and 73.33% muscle relaxant effect after 90 min of treatment at 300mg/kg i.p respectively.

DISCUSSION

The study revealed significant antinociceptive and muscle relaxant effects of ethanolic extracts of leaves and barks of *B. asiatica* in animal models of experiments.

The acetic acid induced writhing is frequently used as a preliminary test to scrutinize the antinociceptive effect of extract/pure compounds (Khan *et al.*, 2011; Muhammad *et al.*, 2012). The injection of acetic acid actually causes the liberation of various pain stimulating mediators that induces constriction of the abdominal muscles (Hasnain *et al.*, 2012; Rauf *et al.*, 2013; Muhammad *et al.*, 2013c). The results of our study suggested strong antinociceptive effect of leaves and barks of the plant, therefore, it is believed that the pharmacologically active constituents of these extracts inhibit or interfere with the release of pain stimulating mediators.

In vivo chimney and traction tests are the most common tools for the assessment of skeleton muscle relaxant properties (Muhammad *et al.*, 2013a,b). The current results on the leaves and barks of *B. asiatica* showed strong potential of the plant for skeleton muscle relaxant constituents.

In conclusions, the leaves and barks of *Buddleja asiatica* evoked strong antinociceptive and muscle relaxant

properties in various animal models. In this regard, further detail studies are most warrant to ascertain these effects and elucidate the mechanism (s). Additionally, isolation of pharmacology active consistent will further help in understanding the chemical background of these findings.

REFERENCES

- Abdulla P (1972). *Buddleja asiatica*. Flora of Pakistan Stewart herbarium Gordon College Rawalpindi, **56**: 3.
- Ali F, Ali I, Khan H, Khan A Gilani AH (2011). Studies on *Buddleja asiatica* antibacterial, antifungal, antispasmodic and Ca⁺⁺ antagonist activities. *Afr. J. Biotech.*, **10**: 7679-7683.
- Ali F, Iqbal M, Naz R, Malik A and Ali I (2011). Antimicrobial constituents from *Buddleja asiatica*. *J. Chem. Soc. Pak.*, **33**: 90-95.
- Anonymous (2000). Evaluation of acute systemic toxicity. Part V. Rodent and human toxicity data for the 50 reference chemicals. *ATLA*, **26**: 571-616.
- Atta-ur-Rhman Choudhary MI and Thomsen WJ (2001). Bioassay Technique for Drug Development Harwood Academic Publishers. pp.1-193.
- Barkatullah, Ibrar M and Muhammad N (2011). Evaluation of *Zanthoxylum armatum* DC for *in vitro* and *in vivo* pharmacological screening. *Afr. J. Pharm. Pharmacol.*, **5**: 1718-1723.
- Danmalam UH, Abdullahi L, Agunu A and Musa KY (2009). Acute toxicity studies and hypoglycemic activity of the methanol extract of the leaves of *Hyptis*

- suaveolens* poit. (Lamiaceae). *Nig. Journ. Pharm. Sci.*, **8**: 87-92.
- Hasnain F, Janbaz K and Qureshi M (2012). Analgesic effect of ketamine and morphine after tonsillectomy in children. *Pak. J. Pharm. Sci.*, **25**: 599-606.
- Kaleem WA, Muhammad N, Qayum M, Khan H, Khan A, Aliberti L and De Feo V (2013). Antinociceptive activity of cyclopeptide alkaloids isolated from *Ziziphus oxyphylla* Edgew (Rhamnaceae). *Fitoterapia.*, **91**: 154.
- Khan H, Saeed M, Khan MA, Khan I and Ashraf N (2011). Antinociceptive activity of aerial parts of *Polygonatum verticillatum*: Attenuation of both peripheral and central pain mediators. *Phytotherapy Res.*, **25**: 1024-1030.
- Khan H, Saeed M, Muhammad N and Perviz S (2013). Phytochemical analysis, antibacterial and antifungal assessment of aerial parts of *Polygonatum verticillatum*. Toxicology and Industrial Health, DOI: 10.1177/0748233713512362.
- Kumar JP and Shankar NB (2009). Analgesic activity of *Mollugo Pentaphylla* Linn by Tail Immersion. *Asian J. Pharm. Clin. Res.*, **2**: 61-63.
- Muhammad N, Barkatullah, Ibrar M, Khan H, Saeed M, Khan AZ and Kaleem WA (2013c). *In vivo* screening of essential oils of *Skimmia laureola* leaves for antinociceptive and antipyretic activity. *Asian Pacific J. Trop. Biomed.*, **3**: 202-206.
- Muhammad N, Saeed M and Khan H (2012). Antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. *BMC. Comp. Alt. Med.*, **12**: 59.
- Muhammad N, Saeed M, Khan H and Haq I (2013b). Evaluation of n-hexane extract of *Viola betonicifolia* for its neuropharmacological properties. *J. Natural Med.*, **67**: 1-8.
- Muhammad N, Saeed M, Khan H, Adhikari A and Khan KM (2013a). Muscle Relaxant and Sedative-Hypnotic Activities of Extract of *Viola betonicifolia* in Animal Models Supported by Its Isolated Compound, 4-Hydroxy Coumarin. *J. Chem.*, **11**: 1-7.
- Nisar M, Kaleem WA, Qayum M, Hussain A, Haq MZ, Ali I and Choudhary M (2010). Biological screening of *Zizyphus oxyphylla* edgew leaves. *Pak. J. Bot.*, **42**: 4063-4069.
- Priyanka B, Sridhar Y and Shankaraiah P (2012). Antidepressant and muscle relaxant activity of *Cardiospermum halicacabum* Linn. Roots in mice. *J. Adv. Pharm. Sci.*, **2**: 193-200.
- Rauf A, Muhammad N, Barkatullah KH and Abbas H (2013). Antinociceptive, sedative and muscle relaxants activity of *Caralluma tuberculata* NE brown. *Orthopedic and Muscular System*, **2**: 2-6.
- Rauf A, Muhammad N, Khan A, Uddin N, Atif M and Barkathullah (2012). Antibacterial and Phytotoxic Profile of Selected Pakistani Medicinal Plants. *World Appl. Sci. J.*, **20**: 540-544.
- Rehman A, Mannan A, Inayatullah S, Akhtar M.Z, Qayyum M and Mirza B (2009). Biological Evaluation of Wild thyme (*Thymus serpyllum*). *Pharm. Bio.*, **47**: 628-633.
- Rehmaullah M. Ibrar, Shah S and Hameed I (2012). Phytotoxic, cytotoxic and insecticidal activities of *Calendula arvensis* L. *J. Biotech. Pharm. Res.*, **3**(6): 104-111.
- Saeed M, Khan H, Khan MA, Khan FU, Khan SA and Muhammad N (2010). Quantification of various metals and cytotoxic profile of aerial parts of *Polygonatum verticillatum*. *Pak. J. Bot.*, **42**: 3995-4002.